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Original Article

NeuroGenetics of Alzheimer's Disease: Crosslinking SIRT1 Gene in the Genetic Nexus of Type II Diabetes and Dementia

Muhammad Ikram Ramzan[1], Muhammad Jamal Khan Gadi[2], Hamza Shahbaz[1], Hina Ashraf[3], Mateen Liaqat[4], Sana Hafeez[5], Gufran Sajjad[4], Aqsa Yasin[6]*

[1]M. Phil Molecular Biology, Centre of Excellence in Molecular Biology (CEMB), University of the Punjab, Lahore, Pakistan.
[2]M. Phil Molecular Biology, Institute of Molecular Biology & Biotechnology (IMBB) The University of Lahore-Lahore, Pakistan.
[3]M. Phil Microbiology, Institute of Molecular Biology & Biotechnology (IMBB) The University of Lahore-Lahore, Pakistan.
[4]BS (Hons) MLT, Department of Allied Health Sciences, University of Health Sciences Lahore, Pakistan.
[5]Ph.D. Microbiology, Institute of Molecular Biology & Biotechnology (IMBB) The University of Lahore-Lahore, Pakistan
[6]M. Phil Molecular Biology, Lecturer, Department of Allied Health Sciences, University of South Asia Lahore, Pakistan.
Corresponding Author: Aqsa Yasin, Lecturer; Email: aqsa.yasin@usa.edu.pk

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ABSTRACT

Background: Background: Type II Diabetes Mellitus (DM) is a chronic condition that not only affects organ function but also contributes to the development of dementia. SIRT1, a gene implicated in the pathophysiology of Type II DM, has been associated with neurodegenerative processes and cognitive decline.

Objective: This study aimed to evaluate the expression of the SIRT1 gene in patients with Type II DM and dementia, elucidating its potential role as a biomarker for cognitive impairment and neurodegeneration in this population.

Methods: A cohort of 88 diabetic patients with dementia and 12 healthy controls were recruited, and EDTA blood samples were collected. SIRT1 serum levels were quantified using chemiluminescent immunoassay (CLIA). Genomic DNA was extracted and assessed for quality before bisulfite DNA modification and Methylation Specific PCR (MSP) to profile SIRT1 gene methylation. Real-time PCR was conducted to determine SIRT1 gene expression levels. Cognitive function was assessed using Mini-Mental State Examination (MMSE) scores.

Results: The average SIRT1 serum levels in diabetic dementia patients (4.98 ± 1.38 µg/mL) were significantly lower than those in healthy controls (14.21 ± 3.64 µg/mL). MMSE scores mirrored this trend, with patients scoring 12.26 ± 4.56, indicating more considerable cognitive impairment compared to healthy controls' score of 24.1 ± 3.12. SIRT1 gene expression was found to be underexpressed with a fold decrease of approximately 4.1 in dementia cases.

Conclusion: The study's findings highlight the downregulated expression of the SIRT1 gene in diabetic patients with dementia, confirming its potential as a biomarker for cognitive decline. Identifying SIRT1 alterations may provide a non-invasive approach for early diagnosis and management of dementia in the diabetic population.

Keywords: Type II Diabetes Mellitus, Dementia, SIRT1 Gene, Cognitive Impairment, Biomarker, Neurodegeneration, Chemiluminescent Immunoassay, DNA Methylation, Mini-Mental State Examination, Neurovascular Complications.

INTRODUCTION

Diabetes mellitus, recognized as the sixth leading cause of death globally, manifests as a complex metabolic disorder characterized by a range of microvascular and macrovascular complications. Type II Diabetes Mellitus (Type II DM), the most prevalent form of diabetes, is intricately linked with cognitive impairments that range from mild to severe, potentially escalating to dementia—a collective term for neurological disorders, among which Alzheimer's disease (AD) stands prominent, accounting for approximately 70% of dementia cases. A multitude of risk factors contributes to the onset of Alzheimer's disease, including but not limited to genetic predispositions such as the ApoE ε4 allele, APP, and PSEN1/PSEN2 mutations, alongside environmental and lifestyle factors like depression, smoking, hypertension, and notably, diabetes mellitus (1)(2)(3). The SIRT1 gene, coding for a protein belonging to the sirtuin family, plays a pivotal role in this context due to its associations with improved insulin sensitivity, a crucial aspect in the

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pathogenesis of Type II DM. The insulin-resistance pathway is a key factor in diabetes development, positioning SIRT1 as a significant gene of interest due to its potential indirect influence on diabetes risk (4).

Moreover, SIRT1 is noted for its anti-inflammatory properties, an attribute of considerable interest given the chronic inflammation observed in both diabetes and dementia. This anti-inflammatory action of SIRT1 suggests a protective role, possibly mitigating the risks associated with both conditions (5). Additionally, reduced levels of SIRT1 have been correlated with increased generation of reactive oxygen species (ROS), elevated glucose levels, and the accumulation of amyloid β and tau proteins, factors known to contribute to the pathophysiology of Alzheimer's disease. Such observations further extend to associations with obesity-linked cardiovascular diseases, emphasizing the wide-reaching impact of SIRT1 on health conditions related to diabetes and cognitive functions (6)(7).

Genome-wide association studies (GWAS) have underscored the significance of the SIRT1 gene by listing it among potential candidates linked to Alzheimer's dementia, highlighting its importance in the genetic landscape of this neurological disorder (8). The physiological functions of SIRT1, encompassing insulin sensitization, anti-inflammatory actions, and its role in angiogenesis and vasodilation, underline its contribution to crucial brain functions such as neurogenesis, synaptic activity, and plasticity. Notably, the expression of AdipoR1, primarily in the hippocampus, and its significant effect on cognitive functions, align with the neuroprotective potential of SIRT1 in combating Alzheimer's disease (9). The reduction in SIRT1 levels or signaling activity is associated with the progression of Alzheimer's disease and cognitive impairments, with decreased plasma levels of SIRT1 being identified as a risk factor, particularly in women with Alzheimer's disease (10).

The mechanism through which SIRT1 exerts its effects includes the deacetylation of histones—H1 lysine 26, H3 lysine 9, H3 lysine 56, and H4 lysine 16—thereby regulating chromatin remodeling and gene transcription. This action facilitates contradictory responses, ranging from tumor suppression to promotion. The anti-inflammatory properties of SIRT1, mediated through the inhibition of NF-κB effects and the stimulation of autophagy, further illustrate its multifaceted role in health and disease (11). Activators of SIRT1, such as resveratrol and cilostazol, have been shown to improve cognitive performance measured by the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), suggesting a promising avenue for therapeutic strategies aimed at mitigating cognitive decline in Alzheimer's disease (12)(13)(14)(15).

The present study delves into the intricate relationship between the SIRT1 gene, Type II diabetes, and dementia, focusing on the cross-linkages that might exist among these conditions. By investigating the expression of the SIRT1 gene in diabetic patients suffering from dementia and analyzing its correlation with neural disturbances and Mini-Mental State Examination (MMSE) scores, this research aims to elucidate the complex interplay between genetic factors and the manifestation of cognitive impairments in individuals with diabetes, shedding light on potential therapeutic targets and interventions.

MATERIAL AND METHODS

In this study, the collection and analysis of samples were meticulously planned and executed following the principles laid out in the Declaration of Helsinki to ensure ethical standards and participant safety. A total of 100 EDTA blood samples, each comprising 5ml, were obtained from individuals after receiving written informed consent. The participant cohort included 88 patients diagnosed with both diabetes and dementia, alongside 12 healthy controls, recruited from the diabetic clinics and Neurology Outpatient Departments of Jinnah Hospital Lahore and Shaikh Zayed Hospital Lahore. The inclusion criteria for this study were individuals over the age of 40, of any gender, exhibiting confirmed cases of Type II Diabetes Mellitus and dementia, as well as healthy controls. Individuals with no clinical history or diagnostic records, those who refused informed consent, and cases of secondary diabetes were excluded from participation.

The chemiluminescent immunoassay (CLIA) was employed to measure SIRT1 serum levels in the collected samples, utilizing a specific ELISA kit (IHUADPNKTC # IH0556) for the quantitative determination of SIRT1 in plasma, strictly adhering to the manufacturer's instructions. The isolation of genomic DNA from the peripheral blood samples was achieved using the QIAgen blood kit (QIAamp#56604), following the manufacturer's protocol to ensure consistency and reliability in DNA quality. The assessment of DNA purity and concentration was conducted through UV spectrophotometry, fluorometry, and gel electrophoresis, with optimal absorbance ratios at 260/280 and 260/230 being indicative of high-quality DNA. The integrity of the DNA was further validated by visualizing intact bands via gel electrophoresis, conducted using a 1.5% gel under specific apparatus conditions of 70 volts for approximately 40 minutes, and the results were analyzed on a SS Doc system.

For the bisulfite DNA modification and Methylation Specific PCR (MSP), the DNA underwent processing with the ZYM bisulfite conversion kit (ZYM, D#5024). The procedure included the addition of 1.8-2µg of DNA to a mixture containing sodium bisulfite, DNA buffer, and protected RNase-free water, following the manufacturer's guidelines. The methylation status of the SIRT1 gene at a pre-determined CpG site was examined using Methylation-Specific PCR, with the amplification carried out on a Mastercycler (Eppendorf)

using PCR Master Mix (Thermofisher 4426518). The PCR conditions comprised an initial denaturation, followed by cycles of denaturation, annealing, and extension, with the specifics outlined in the methodology.

Primer design was conducted using serial cloner software, referencing the consensus CDS sequence of the SIRT1 gene from the NCBI database. Primer specificity was confirmed via primer-BLAST, with the designed primers optimized for their melting temperatures (Tm) and amplicon properties through gradient PCR thermocycling. The sequences of the designed primers are as follows: SIRT1 Primers Pair with the forward sequence TGCTGGCCTAATAGAGTGGCA and the reverse sequence CTCAGCGCCATGGAAAATGT.

Data analysis was conducted using SPSS version 25.0, where demographic data and frequencies of relative morbid conditions were presented through bar charts. Expression analysis was performed, and the statistical significance was determined using one-way ANOVA, with a p-value of less than 0.05 considered significant. This comprehensive approach to sample collection, DNA isolation, quality check, and subsequent analyses underpins the study's commitment to rigor and ethical consideration in exploring the genetic nexus between Type II Diabetes Mellitus and dementia through the lens of the SIRT1 gene.

RESULTS

In the exploration of the relationship between SIRT1 levels and cognitive function in diabetic dementia, the demographic summary revealed a gender distribution among the confirmed cases, with 41% being male and 59% female. The average age for males was reported as 55.4 years with a standard deviation of 8.5, while females had an average age of 59.1 years with a narrower standard deviation of 4.6 (Table 2). The biochemical parameters across the study population demonstrated significant differences between healthy controls and diabetic dementia patients (Table 3). Healthy controls had an average fasting glucose level of 5.01 mmol/L with a standard deviation of 0.56, contrasting with diabetic dementia patients who had a markedly elevated average of 9.01 mmol/L with a standard deviation of 0.86, yielding a statistically significant t-test p-value of 0.007. Similarly, HbA1c levels differed significantly between the two groups, with healthy controls at 4.31% (±0.67) compared to diabetic dementia patients at 10.59% (±1.62), with a t-test p-value of 0.044. SIRT1 levels also varied substantially, being higher in healthy controls at 11.37 µg/mL (±3.64) relative to 4.37 µg/mL (±1.38) in diabetic dementia patients, with this difference achieving a t-test p-value of 0.014. MMSE scores further emphasized the disparity in cognitive function between the groups, with healthy controls scoring an average of 24.1 (±3.12) and diabetic dementia patients averaging 12.26 (±4.56), again statistically significant with a p-value of 0.009.

Table 1: Demographic Summary of Confirmed Cases of Diabetic Dementia Patients (n=88)

Gender	Cases (%)	Average Age ($\bar{x}$)	Standard Deviation (σ)
Male	41%	55.4 years	±8.5
Female	59%	59.1 years	±4.6

Table 2: Biochemical Parameters of Healthy Controls and Diabetic Dementia Patients (n=100)

Clinical Parameters/Variables	Healthy Controls (n=12)	Diabetic Dementia Patients (n=88)	t-test P value
Fasting Glucose (mmol/L)	5.01 ± 0.56	9.01 ± 0.86	0.007*
HbA1c Levels (%)	4.31 ± 0.67	10.59 ± 1.62	0.044*
SIRT1 (µg/mL)	11.37 ± 3.64	4.37 ± 1.38	0.014*
MMSE Scores	24.1 ± 3.12	12.26 ± 4.56	0.009*

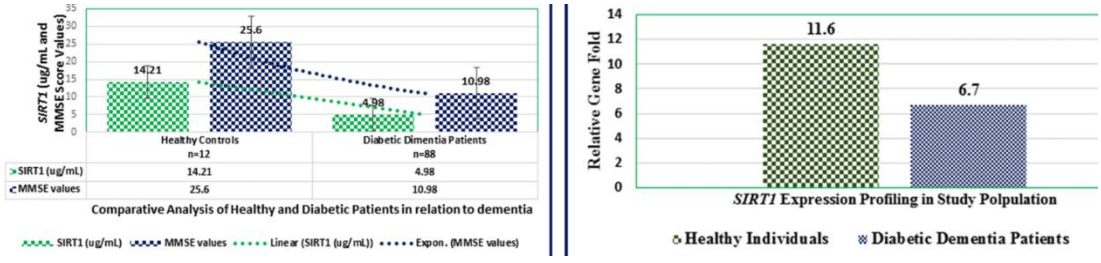

Figure 1 SIRT1 Levels and Cognitive Impairment Assessment; Expression Analysis of SIRT1

The graphical representation of SIRT1 levels and cognitive function distinctly highlighted these differences. The bar graph on SIRT1 levels and cognitive impairment assessment (Figure 1, left graph) indicated that healthy controls not only had higher SIRT1 serum levels with an average of 14.21 µg/mL but also scored better on the MMSE with an average of 25.6, compared to diabetic dementia patients whose SIRT1 levels and MMSE scores were significantly lower, at 4.98 µg/mL and 10.98, respectively. The visual distinction between the SIRT1 levels and MMSE values was

effectively made using different patterns within the bars on the graph. On the expression analysis of SIRT1 (Figure 1, right graph), a pronounced difference was observed in the gene expression profiles. Healthy individuals exhibited a relative gene fold expression of 11.6, a considerable difference when juxtaposed with the 6.7-fold expression seen in diabetic dementia patients. This comparative analysis underscores the potential link between decreased SIRT1 expression and cognitive decline in diabetic dementia.

DISCUSSION

The intersection of Type II Diabetes Mellitus (DM) and cognitive disorders, particularly dementia, presents a clinical landscape where SIRT1 emerges as a molecule of considerable significance. The propensity of diabetes to engender microvascular and macrovascular complications is well-documented, with implications for neurovascular coupling and subsequent cognitive decrements (16). In the milieu of diabetes, conditions such as hyperglycemia and insulin resistance are not merely metabolic disturbances but serve as harbingers of cognitive decline, with dementia as the predominant sequel (17).

Our investigation into the SIRT1 gene reflects a paradigm wherein its dysregulation correlates with exacerbation of hyperglycemic states and cognitive deficits. This is substantiated by findings within the diabetic cohort, where HbA1c levels exceeded 6.8%, indicative of suboptimal glycemic control and possibly implicating SIRT1 in this dysregulation (18). The nexus between SIRT1 expression and Alzheimer's disease (AD) pathology is particularly compelling. Reduced expression of SIRT1 has been associated with a propensity for amyloid beta (Aβ) accumulation, a hallmark of AD. Contrasting evidence suggests that overexpression of SIRT1 may also potentiate Aβ levels (19), though our data align more closely with studies where a decrease in SIRT1 expression is paralleled by increased amyloid beta metabolism, thereby contributing to AD pathology (20).

The neuroprotective potential of SIRT1 is an aspect that merits further exploration. Emerging literature posits SIRT1's involvement in modulating neurodegenerative processes, with calorie restriction and consequent SIRT1 activation proposing a protective mechanism against AD through transcription factor modulation (21). In parallel, the high activity of SIRT1 has been implicated in amyloidogenesis, with an extreme sequela resulting in amyloid plaque formation characteristic of neurodegenerative conditions such as AD (22). Within our study, the expression levels of SIRT1 were notably diminished among subjects with dementia. Severely impaired cognitive function, as evidenced by MMSE scores below 10, corroborated a significant fold decrease in SIRT1 expression, echoing the results of studies reporting reduced SIRT1 activity in AD (23).

Brain-Derived Neurotrophic Factor (BDNF), often linked to cognitive functions, parallels SIRT1 in its significance. Decreased SIRT1 levels in dementia are symptomatic of synaptic dysfunction and impaired neuroplasticity (24). The downregulation of its precursor, proSIRT1, and the subsequent loss of neuroprotective functions, positions peripheral SIRT1 serum levels and gene expression as promising diagnostic biomarkers, offering a non-invasive window into the central nervous system's state (24).

This research is not without limitations. The modest sample size and singular geographic focus may limit the generalizability of the findings. Further, the study design precludes causal inferences, and longitudinal studies could more effectively elucidate the trajectory of SIRT1's role in cognitive decline. In terms of recommendations, it would be prudent to extend this research to a broader, more diverse population. Moreover, the pursuit of potential interventions targeting SIRT1 could yield therapeutic avenues to mitigate the progression of neurodegenerative diseases.

CONCLUSION

Conclusively, the study underscores the importance of SIRT1 as a potential biomarker for early detection and progression monitoring of dementia in diabetic patients, offering avenues for targeted interventions that could improve patient outcomes and alleviate the healthcare burden. The downregulation of SIRT1 mRNA in peripheral blood mononuclear cells presents a novel, non-invasive biomarker that could reflect central nervous system changes and guide therapeutic strategies, thereby holding significant implications for the management and treatment of neurodegenerative diseases within the sphere of human healthcare.

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