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Original Article

Tracing Neurogenetic Pathways: *SIRT1* Gene's Influence on Autism, Alzheimer's, Type II Diabetes, Dementia, and its role in Neurodevelopmental Dynamics

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ABSTRACT

Background: Autism Spectrum Disorder (ASD) encompasses a range of neurodevelopmental conditions characterized by challenges in social interaction, communication, and repetitive behaviors. Concurrent conditions such as Type II Diabetes Mellitus (DM) and dementia are prevalent comorbidities that exacerbate the cognitive impairments associated with ASD. The *SIRT1* gene, known for its role in neuroprotection and longevity, has been implicated in the pathophysiology of these disorders.

Objective: To determine the expression of the *SIRT1* gene in patients with diabetes and dementia and to investigate its potential as a biomarker for cognitive impairment in these populations.

Methods: A cohort of 108 diabetic dementia patients and 32 healthy controls were enrolled. *SIRT1* expression levels were quantified using *chemiluminescent immunoassay (CLIA)* and methylation-specific PCR after *bisulfite DNA* modification. Cognitive function was assessed using the Mini-Mental State Examination (MMSE), and demographic data, along with biochemical parameters such as fasting glucose and *HbA1c* levels, were analyzed using SPSS version 25.

Results: Diabetic dementia patients exhibited significantly lower *SIRT1* levels (4.37 ± 1.38 µg/mL) and MMSE scores (12.26 ± 4.56) compared to healthy controls (*SIRT1*: 11.37 ± 3.64 µg/mL, MMSE: 24.1 ± 3.12). The relative gene expression of *SIRT1* in diabetic dementia patients showed a 6.7-fold decrease compared to healthy individuals. Statistical analysis revealed a significant difference in both *SIRT1* levels and MMSE scores between the two groups ($P<0.05$).

Conclusion: The underexpression of *SIRT1* in diabetic dementia patients and its correlation with lower MMSE scores suggest that *SIRT1* could serve as a biomarker for cognitive impairment in this population. The findings advocate for further exploration into *SIRT1*-targeted interventions, which may improve diagnostics and therapeutic approaches for neurodegenerative conditions associated with ASD.

Keywords: Autism Spectrum Disorder, *SIRT1* Gene Expression, Diabetic Dementia, Cognitive Impairment, Biomarker, Neurodegeneration, *Chemiluminescent Immunoassay*, Methylation-Specific PCR.

INTRODUCTION

Autism Spectrum Disorder (ASD) represents a complex neurodevelopmental condition that typically emerges in early childhood and persists throughout an individual's life. It is marked by difficulties in social interaction, communication, and often characterized by repetitive behaviors and narrowly focused interests. Despite these challenges, individuals with autism may exhibit unique strengths such as exceptional memory and intense concentration in specific domains (1). The multifactorial etiology of autism encompasses genetic, environmental, and neurobiological factors. Early diagnosis and tailored interventions are crucial, as they substantially enhance the quality of life for those affected (1,2).

Emerging research has begun to explore the potential links between the *SIRT1* gene and various neurological conditions, including autism. *SIRT1*, which encodes a protein belonging to the sirtuin family, is involved in critical cellular processes such as DNA repair,

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inflammation, and oxidative stress—all of which are implicated in neurodevelopmental and neurodegenerative disorders (4). Notably, oxidative stress and inflammation are believed to play roles in the development of ASD. Given that *SIRT1* regulates these processes, it may influence autism-related pathways. Diminished levels of *SIRT1* have been associated with increased generation of Reactive Oxygen Species (ROS) and enhanced prevalence of conditions such as obesity-linked cardiovascular diseases (6,7). Additionally, genome-wide association studies have identified the *SIRT1* gene as a novel potential candidate linked to Alzheimer's disease (8).

SIRT1 also impacts insulin sensitivity and exhibits anti-inflammatory properties, which are significant because insulin resistance is a key factor in type 2 diabetes, and chronic inflammation is associated with both diabetes and dementia. By enhancing insulin sensitivity and mitigating inflammation, *SIRT1* could indirectly influence the risk of these conditions. There is evidence that improved cognitive function, potentially influenced by *SIRT1* activity, could reduce the risk of dementia in individuals with or without diabetes (4,5).

In the brain, *SIRT1* contributes to important functions such as energy homeostasis, hippocampal neurogenesis, and synaptic activity. It can cross the brain barrier, being detectable in cerebrospinal fluid, and has significant effects on cognitive functions. Reductions in *SIRT1* levels or signaling activity have been linked to the progression of autism and cognitive impairment. In females, decreased plasma levels of *SIRT1* have been identified as a risk factor for autism (9,10).

On a molecular level, *SIRT1* deacetylates histones to regulate chromatin remodeling and gene transcription, processes essential for brain development and function. These epigenetic modifications are crucial during neurodevelopment, and their dysregulation has been implicated in autism, suggesting a possible indirect connection through shared biochemical pathways (12,13). Compounds such as resveratrol (found in red wine) and cilostazol (an antiplatelet drug) have shown potential in enhancing cognitive outcomes, possibly through *SIRT1* activation. These substances promote neuronal survival, reduce inflammation, and enhance cellular resilience, indicating that *SIRT1* activation might be a promising therapeutic avenue for addressing cognitive decline in Alzheimer's disease (14,15).

In summary, the *SIRT1* gene not only influences diabetes through effects on insulin sensitivity but also plays a role in cognitive impairments in individuals with diabetes. Our study focuses on investigating the expression of the *SIRT1* gene in diabetic patients suffering from dementia, examining its correlation with neural disturbances and their assessment through Mini-Mental State Examination (MMSE) scores, which could provide insights into the interconnectedness of these conditions.

MATERIAL AND METHODS

In this study, a total of 140 participants were enrolled, comprising 108 patients diagnosed with diabetes and dementia, and 32 healthy controls. *EDTA* blood samples (5ml each) were collected from the participants after obtaining written informed consent, in accordance with the ethical standards of the Declaration of Helsinki and the predefined inclusion and exclusion criteria. The inclusion criteria were adults over the age of 40, both male and female, diagnosed with Type II Diabetes Mellitus and confirmed cases of dementia. The exclusion criteria included absence of comprehensive clinical history, refusal of consent, and secondary diabetes. The samples were collected from clinically diagnosed cases at the diabetic clinics and neurology outpatient departments of Jinnah Hospital Lahore and Shaikh Zayed Hospital Lahore. Following collection, samples were transported under standard protocols to MOU-signed diagnostic laboratories for storage and further processing.

For the measurement of *SIRT1* serum levels, a *chemiluminescent immunoassay (CLIA)* was utilized, specifically an *ELISA* kit designed for the quantitative determination of serum *SIRT1* in plasma (IHUADPNKTC # IH0556), following the manufacturer's instructions. DNA was isolated from peripheral blood using the *QIAgen* blood kit (*QIAamp*#56604), and its quality was assessed via UV spectrophotometry, fluorometry, and gel electrophoresis. The DNA's purity and integrity were confirmed by optimal absorbance ratios (260/280 and 260/230) and visualization of intact bands on a 1.5% gel, run at 70 volts for approximately 40 minutes, with results analyzed on a SS Doc system.

The bisulfite modification of DNA was conducted using the ZYM bisulfite conversion kit (ZYM, D#5024), adding 1.8-2µg of DNA to a mixture containing sodium bisulfite, DNA buffer, and *RNase*-free water. Methylation-specific PCR targeted the CpG site located at the −58 nt sequence of the E-box of the *SIRT1* gene. The PCR was performed using a *Mastercycler* (Eppendorf) with specific primers designed via Serial Cloner and checked for specificity using BLAST. The cycling conditions included an initial denaturation at 95°C, followed by 40 cycles of denaturation, annealing at 53°C, and extension at 72°C, with a final extension at 72°C for 10 minutes.

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The primer sequences used were as follows: forward TGCTGGCCTAATAGAGTGGCA and reverse *CTCAGCGCCATGGAAAATGT*. These were optimized for their melting temperatures and amplicon properties using a gradient PCR *thermocycler* (Bio-Rad T100-*Thermocycler*, USA).

Data were analyzed using SPSS version 25. Demographic data and frequencies of relative morbid conditions were presented in bar charts, and expression analysis was conducted. Statistical analysis included one-way ANOVA to determine variance among the sample groups, with a significance threshold set at $p<0.05$. The MMSE was administered as part of the comprehensive assessment to monitor cognitive function, taking into account the influence of educational background, language, and cultural factors on the scores. This methodological approach ensured rigorous analysis and adherence to ethical guidelines throughout the study.

The ethical aspects of this study were reviewed and approved by the Ethical Review Board under the reference ERC 321/11-12. Additionally, the study received an Approval Certificate from the Institutional Review Board, with the reference IRB 201/09-24, issued by the Supervisory Committee.

RESULTS

In the exploration of *SIRT1* gene expression and its association with diabetic dementia, the study delineated a marked contrast between patients and healthy controls. Delving into the demographic breakdown, Table 2 unveils that among the 88 confirmed cases of diabetic dementia, the distribution of male to female was 41 to 59 with an average age of 55.4 (±8.5) and 59.1 (±4.6) years respectively, signaling a slight female predominance and a higher average age in females.

The biochemical parameters further corroborate the differential impact of diabetic dementia on the study population. As documented in Table 3, fasting glucose levels and *HbA1c* percentages in diabetic dementia patients were significantly elevated at 9.01 (±0.86) mmol/L and 10.59% (±1.62), respectively, when juxtaposed with healthy controls who exhibited 5.01 (±0.56) mmol/L for glucose and 4.31% (±0.67) for *HbA1c*. This stark difference was statistically significant ($P<0.05$), underscoring the biochemical disparity between the cohorts. *SIRT1* levels in the patient group were depressed at 4.37 (±1.38) µg/mL compared to the healthy controls who maintained a level of 11.37 (±3.64) µg/mL, reiterating the inverse relationship between *SIRT1* expression and diabetic dementia.

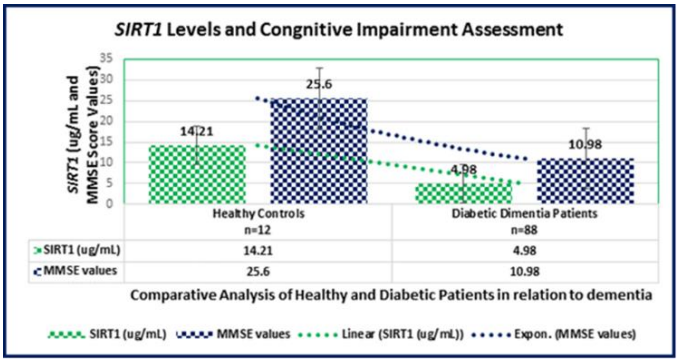
Cognitive function, as gauged by MMSE scores, painted a similar picture of decline in the patient group. The average MMSE score for diabetic dementia patients was substantially lower at 12.26 (±4.56), significantly diverging from the healthy controls' average score of 24.1 (±3.12), with the t-test yielding a P value of 0.009, further emphasizing the cognitive decrement associated with diabetic dementia.

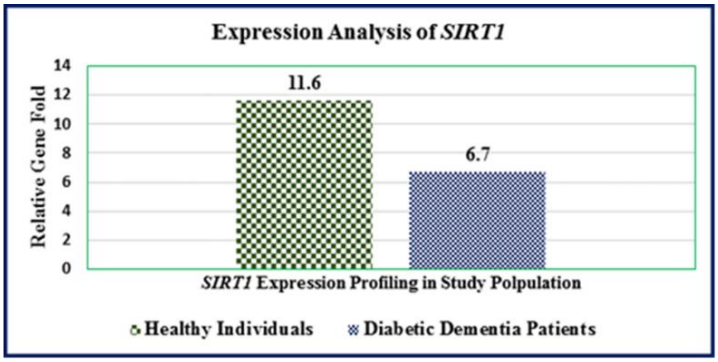
Table 1: Demographical Summary of Confirmed Cases of Diabetic Dementia Patients (n=88)

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

Table 2: Biochemical Parameters of Healthy Controls and Diabetic Dementia Patients

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*





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DISCUSSION

In the present study, the exploration of the *SIRT1* gene's involvement in the progression of cognitive impairments in diabetic dementia has unveiled significant insights. It was observed that patients with Type II Diabetes Mellitus (DM) displayed exacerbated microvascular and macrovascular complications that potentially disrupt neurovascular coupling, culminating in cognitive irregularities, with dementia being particularly prevalent (16). The adverse states such as hyperglycemia or insulin resistance were found to elevate the risk of cognitive deficits, corroborating earlier findings that implicate these conditions in cognitive decline (16,17).

Analyses of the patients' historical diabetic profiles revealed *HbA1c* levels exceeding 6.8%, indicative of suboptimal glucose regulation—a finding that aligns with the increased *SIRT1* expression and suggests a possible mechanistic linkage between *SIRT1* gene expression and glucose dysregulation. The accumulation of Amyloid Beta Precursor Protein (*AβPP*) in the Golgi apparatus, inhibited from reaching endosomal compartments where Aβ is produced, may be attributed to altered *SIRT1* expression (17). Echoing the literature, activators of *SIRT1* such as resveratrol and cilostazol were shown to ameliorate cognitive scores in Alzheimer's disease, evaluated using the *Alzheimer's disease Assessment Scale-Cognitive Subscale (ADAS-Cog),* providing a tangible link between *SIRT1* activation and cognitive enhancement (12,18).

In contrast, overexpression of *SIRT1* was associated with an increase in Aβ levels, indicating a complex role of *SIRT1* in Alzheimer's pathology (20). Diminished *SIRT1* expression, observed in a mouse model, pointed to an accelerated amyloid beta metabolism, hinting at a contributory role of *SIRT1* underexpression in Alzheimer's disease-like pathology. These findings resonate with our study's results, which identified an underexpression of *SIRT1* in individuals diagnosed with dementia, with MMSE scores indicating severe cognitive impairment. The relative fold decrease in *SIRT1* expression, as determined by real-time PCR, was substantial, mirroring the reduced *SIRT1* activity reported in Alzheimer's patients in comparison to non-Alzheimer's individuals (21,23).

Further investigations into the *SIRT1* gene's high activity unveiled its pivotal role in amyloidogenesis, potentially leading to the formation of neurodegenerative-inducing amyloid plaques (22). This study presented lower *SIRT1* expression levels in subjects diagnosed with dementia, aligning with previous reports of synaptic dysfunction and neuronal atrophy associated with reduced *SIRT1* (24). Peripheral indicators such as decreased serum levels and diminished gene expression in peripheral blood mononuclear cells (PBMCs) have emerged as promising diagnostic markers, reflecting cognitive decline and further endorsing the pertinence of *SIRT1* as a peripheral biomarker for dementia (24).

The study's findings posited that *SIRT1* could serve as a biomarker for early detection and diagnosis of neurodegenerative diseases like dementia in Autism Spectrum Disorder (ASD) patients. Early detection could pave the way for extending patient longevity and the development of targeted therapeutics to manage diseases that impose significant burdens on healthcare systems. Furthermore, the study underlined the downregulation of *SIRT1* mRNA in PBMCs as a systemic reflection of central nervous system changes, highlighting the potential of peripheral markers in diagnosing neurodegenerative alterations (7, 23).

Looking ahead, research into *SIRT1*'s role in ASD and neurodegenerative diseases presents promising avenues for novel diagnostics and therapies. Understanding *SIRT1* dysregulation could lead to targeted treatment strategies and the development of interventions to curb neurodegeneration and enhance cognitive function in ASD patients. Moreover, incorporating peripheral markers such as *SIRT1* mRNA levels from PBMCs into clinical practice could improve early detection and monitoring, offering a more optimistic prognosis for individuals afflicted with neurodevelopmental disorders (24).

CONCLUSION

This study concluded that the *SIRT1* gene potentially serves as a biomarker for early detection of dementia in diabetic patients, offering a window for timely intervention that could improve patient outcomes and alleviate the healthcare burden posed by these neurodegenerative diseases. By identifying the systemic underexpression of *SIRT1* mRNA in peripheral blood mononuclear cells, the study points to novel avenues for the development of targeted therapies that could not only extend the lifespan of affected individuals but also enhance the quality of life for those on the autism spectrum facing an elevated risk of cognitive decline.

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