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NEURONAL PROTECTION IN AUTISM: *SIRT1* GENE EXPRESSION ANALYSIS AND ITS IMPLICATIONS

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Abstract

Introduction: Autism spectrum disorders (ASD) are a diverse group of conditions. They are characterized by some degree of difficulty with social interaction and communication. Recent studies have suggested that sirtuin 1 (SIRT1) has a role in brain development and connectivity, which may be relevant to the pathophysiology of ASD. SIRT1 dysregulation may interfere with important brain development processes, which may explain the altered metabolic profiles seen in ASD patients.

***Objective:** To examine the current understanding of SIRT1 levels in autism patients and their implications for diagnosis, treatment, and disease management.*

***Material and Methods:** This inter-collaborative study involves collecting EDTA blood samples (5ml each) from 100 individuals (68 Autism cases and 32 Healthy Controls) from different hospitals and clinical settings in the regions of Hazara, Islamabad and Karachi and SIRT1 expression analysis was performed. Minimal Mental State Examination MMSE score statistical analysis was performed using Graph Pad Prism 9.0 to assess the strength of evidence supporting the role of SIRT1 in ASD pathology.*

***Results:** Analysis revealed significantly lower SIRT1 expression in the Autism patients (6.94) as compared to controls (14.32). Specifically, an SIRT1 gene expression decrease correlated with cognitive impairment severity; 80% of participants scored below 9 on the MMSE score with mean age 37 years, indicative of severe Neurodegeneration.*

Conclusion: Low SIRT1 levels appear to be intricately linked to autism severity, highlighting the importance of considering a neuronal protection and a metabolic factor in ASD pathogenesis.

Keywords: *Autism, Diagnosis, Disease management, Severity, SIRT1, Treatment*

INTRODUCTION

Autism spectrum disorders (ASD) are a diverse group of conditions. They are characterized by some degree of difficulty with social interaction and communication. ASD first appears in early childhood and lasts the entirety of a person's life. It presents a wide variety of symptoms and levels of disability and is characterized by difficulties with behaviour, social interaction, and communication. Individuals on the autism spectrum may exhibit extremely concentrated interactions, experience difficulty interpreting social signs, and repeat repetitive behaviours (1). There are several variables that contribute to autism, including environmental, genetic, and neurological ones. Despite their difficulties, people with autism may also possess some strengths, such as exceptional memory, highly focused attention, and detailed observation in particular domains. The quality of life for those on the spectrum may be greatly enhanced by early diagnosis and customized therapies, highlighting the significance of knowledge and comprehension of this illness (2).

Autism has an impact on communication and social interaction. It can also cause restricted, obsessive interests or repetitive behaviours. The acronym *SIRT1* is an acronym for sirtuin 1. A member of the sirtuin family of proteins is the protein *SIRT1* (3). A class of proteins known as auxins has been demonstrated to regulate aging, transcription, stress tolerance, energy reserves, and attentiveness in low-calorie

environments, among other cellular functions (4). Sirtuins (*SIRT1*) are a significant protein that is now known to be relevant in a number of neurological illnesses, particularly those caused by oxidative stress-induced neurodegenerative disorders. It is found that TREM2 has a potentially significant role in Alzheimer's disease (AD), the most prevalent kind of dementia that accounts for four out of every five instances of memory loss and confused thinking. Prior research has also demonstrated the impact of *SIRT1* on pathways associated with AD, including reducing reactive oxygen species (ROS) and inhibiting the inclusion of proteins such as amyloid β (Aβ) and Tau clumps, which are indicative of AD pathology (5).

SIRT1 has also been linked to autism spectrum disorders (ASD) in addition to AD. Research also points to the production of oxidative stress and inflammation, both of which may be mediated by pathways involving this gene's downstream dysregulation, as contributing factors to the pathophysiology of ASD. *SIRT1* has been postulated as the gene that regulates both processes. These protein-modulated pathways suggest a possible link between *SIRT1* malfunction and the onset of ASD (6).

Furthermore, *SIRT1* is involved in the control of angiogenesis and oxidative stress in a number of different disease states (not just neurological ones), such as cardiovascular diseases including ischemic heart disease and peripheral arterial illness (7). *SIRT1* is a new candidate gene that has been directly linked to Alzheimer's dementia through genome-wide association studies (GWAS), highlighting its significance in neurodegenerative disorders as well as other medical problems affected by inflammatory and cellular stress responses (8).

SIRT1 regulates chromatin remodelling and gene transcription by deacetylating histones, including H1 lysine 26, H3 lysine 9, H3 lysine 56, and H4 lysine 16. It has also been shown that the homolog expresses conflicting reactions to both tumor development and suppression. According to studies, *SIRT1* may have anti-inflammatory qualities via up regulating autophagy and blocking the effects of NF-kB. Additionally, *SIRT1* affects neurodevelopment and neuroplasticity, two processes that are essential to the human brain's early stages of development. It is believed that abnormalities in these mechanisms have a major role in the pathophysiology of autism. *SIRT1* plays a part in epigenetic modification, which includes histone modification and DNA methylation. These modifications are crucial for controlling gene expression throughout brain development. Autism has been linked to aberrant epigenetic regulation, indicating a possible, albeit indirect, and linkage through common molecular pathways as shown in Fig. 1 (9).

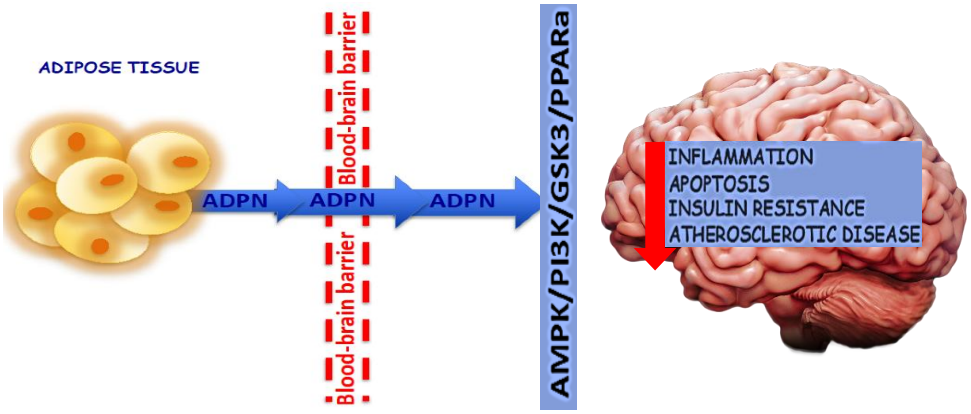

Fig. 1. *SIRT1*, a molecular neuroprotective target for dementia

Hence, the *SIRT1* gene is not only linked to diabetes in individuals but also to cognitive impairments in those with diabetes, particularly in the context of hyperglycemia or insulin resistance. Therefore, our study focuses on the association between the *SIRT1* gene, diabetes, and cognitive deficits under conditions like insulin resistance or hyperglycemia (10). The objective of this research was to examine the role of the *SIRT1* gene in the relationship between dementia and Type II diabetes. The Mini-Mental State Examination (MMSE), also known as the Folstein test, is a widely used 30-point questionnaire in clinical and research settings to measure cognitive impairment. It is commonly employed in medicine and allied health fields to screen for neurodegenerative disorders such as autism and dementia. The present study focused to analyze *SIRT1* gene, which was the spotlight of the study may be involved in the cross linkage of Type II

diabetes disease and dementia. We investigated the expression of *SIRT1* gene in diabetic patients suffering from dementia and its correlation with neural disturbances in assessment to their MMSE scores.

MATERIALS AND METHODS

A total of 68 EDTA blood samples (5ml each) of patients of Autism and 32 healthy controls were collected after written informed consent and according to predefined inclusion and exclusion criteria, from clinically diagnosed cases of Autism and cognitive impairment with a disease course on 30 months from various clinical settings of Hazara, Islamabad and Karachi. Inclusion criteria of study consisted of the patients with the age >40 years, gender (male and females), healthy controls without any neurodegenerative disorders, Patients with Type II DM, confirmed cases dementia. Exclusion criteria were the patients without clinical history, patients with brain cancer; no previous diagnostic tests record and refuse informed consent.

SIRT1 DETECTION BY ELISA AND PCR TECHNIQUES

SIRT1 serum levels in the samples were measured by Enzyme Linked Immunosorbent Assay ELISA using a kit for the quantitative determination of serum *SIRT1* in plasma (IHUADPNKTC # IH0514) by manufacturer's protocol. QIAgen blood kit (QIAamp#51103) was used to isolate genomic DNA. UV spectrophotometry and Gel Electrophoresis were performed for quality check to primers. Optimal absorbance at 260/280 and 260/230 ratios for visualizing the intact bands were adjusted, confirming amplifiability through PCR ensuring high-quality DNA as shown in Fig. 2.

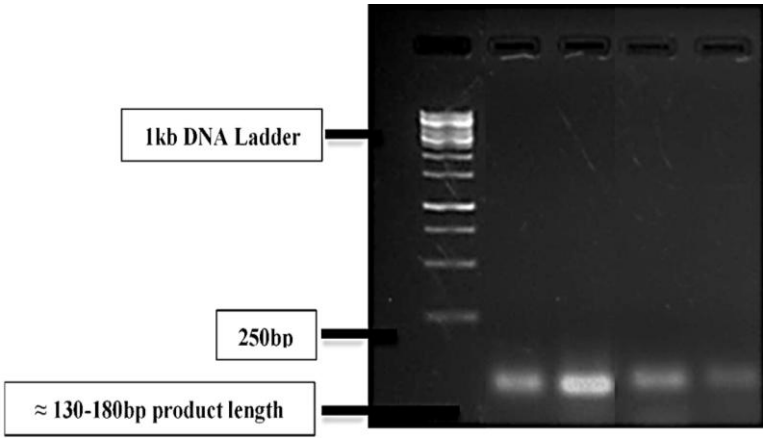

Fig. 2. *SIRT1* gene Gel Bands under UV

The primers were designed on a serial cloner by using the consensus CDS sequence of specific genes from the NCBI database and then primer specificity or universality was checked by primer-BLAST or BLASTn respectively as shown in Table I.

Table I. Sequences of designed primers

***SIRT1* Primers Pair**	***SIRT1* sequence (5/ to 3/) , −74 nt sequence**
Froward	TGCTTGCCTAAGTAGAGTGC
Reverse	CGTGAGATGAACTCGTTCTG

Primers were optimized using a gradient PCR thermocycler (Bio-Rad T100-Thermocycler, USA) to get the best optimal temperature. Their melting temperatures (Tm) and amplicon properties were optimized. Amplification was performed on Mastercycler instrument (Eppendorf) using PCR Master Mix (Thermofisher 442-6024) in 40 µL of RNAse-free water containing 0.35 µM primers. PCR conditions were as: 4 min of initial denaturation at 95°C, 1 min of denaturation at 94 °C, 15 s of annealing at 53°C, and 1 min of extension at 72°C. Te PCR cycle was repeated 40 times with a final extension at 72 °C for 10 min, followed by cooling to 4 °C.

STATISTICAL ANALYSIS

The data was analyzed by GraphPad Prism 9.0. Demographic data was plotted through bar charts and frequencies of relative morbid conditions were plotted as bar charts and expression analysis was

performed. Relative gene fold was measured. One way ANOVA t-test was done to find variance amongst the samples. Statistical significance of $p>0.05$ is considered.

MMSE SCORING

MMSE stands for Mini-Mental State Examination, a short standardized test that evaluates the patient's ability to carry out basic mathematical calculations, ability to recall events and other aspects that pertain to the patient's orientation. In mentally retarded patients, the test supports the assessment of functioning status and changes in the degree of cognitive dysfunction using the MMSE score. This test includes the questionnaire part and the tasks part, and the total possible points for this evaluation is 30 points, while the lower points show the higher degree of cognitive impairment.

RESULTS

ANALYTICAL PARAMETERS OF ENROLLED SUBJECTS

In this study, 100 blood samples were taken. The demographical features are given in Table II. The Clinical parameters are given in Table III.

Table II. Demographical summary of confirmed cases of Autism patients (n=100)

Gender	Cases (%)	Average Age (x̄)	Standard Deviation (S)/σ
Male	41	55.4	±8.5
Female	59	59.1	±4.6

Table III. Biochemical parameters of healthy and Autism patients (n=100)

Clinical Parameters/Variables	Healthy Controls (n=32)	Autism Patients (68)	t-test P value
SIRT1 (µg/mL)	16.17±3.14	3.57±1.09	0.011*
MMSE Scores	22.1±2.12	13.26±3.19	0.007*

*Statistically Significant

SIRT1 LEVELS AND COGNITIVE IMPAIRMENT ASSESSMENT VIA MINI MENTAL STATE EXAMINATION (MMSE) SCORE

The mental status of the patients was evaluated using MMSE questionnaire. The examination conducted via this questionnaire gave us an idea regarding the severity of the disease within the aforementioned study groups.

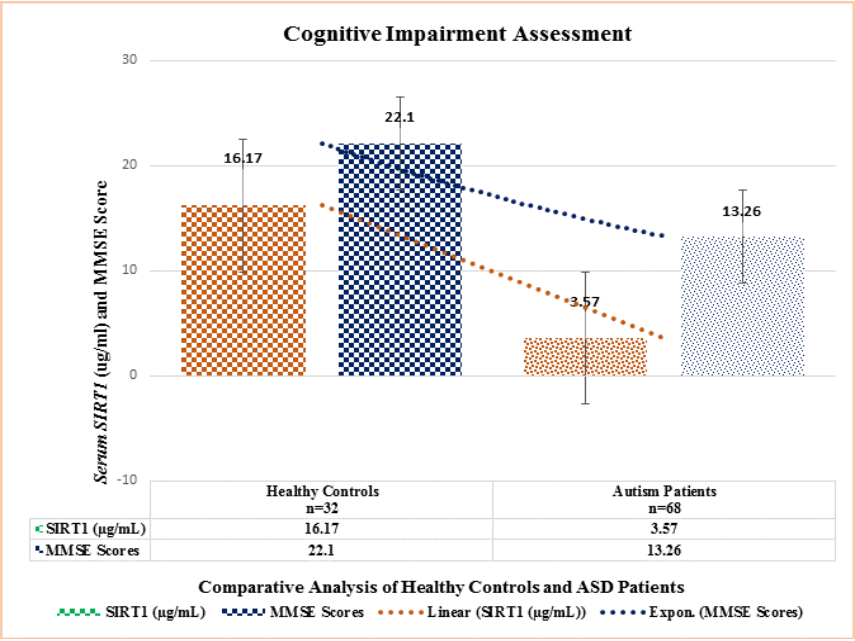

Fig. 2. *SIRT1* assessment for Autism Patients with respect to MMSE score analysis for Cognitive Impairment

On basis of their MMSE scores, the individuals were categorized under severe (0-15 score) and less/no cognitive impairment (24 or higher score). Scoring was done according to the scoring criteria pre-established within the questionnaire by the medical professionals who developed the test. The scoring interpretations on basis of severity are shown in Fig. 2.

In comparison to healthy individuals, Autism patients showed decreased *SIRT1* levels (3.57ug/ml) and low MMSE scores ((13.26) confirming the phenomenon of cognitive impairment in Autism patients.

RELATIVE FOLD CHANGE OF *SIRT1* (CH3-DNA) IN AUTISM PATIENTS WITH REFERENCE TO HEALTHY CONTROLS VIA RT-QPCR

Our data shows an overall high expression of *SIRT1* (14.32) in healthy individuals as compared to Autism patients. The levels of *SIRT1* decrease (6.94) with the increase in cognitive impairment (Fig. 3).

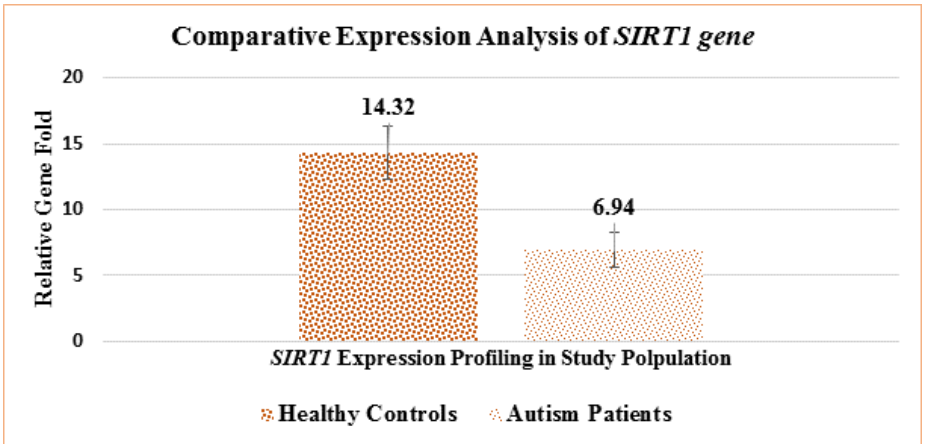

Fig. 3. Relative fold change in healthy controls and Autism cases

DISCUSSION

Autism spectrum disorders (ASD) are a diverse group of conditions. They are characterized by some degree of difficulty with social interaction and communication. Conditions such as insulin resistance or hyperglycaemia raise an individual's risk of developing cognitive impairment. The main target of our investigation, the gene i-e *SIRT1*, has been linked to aggravating these conditions and subsequently changing an individual's capacity for mental function (11). Additionally, our research showed that abnormal expression of these genes speeds up the development of autism and diabetes (12).

Patients with a history of autism spectrum disorder typically have problems with metabolism. For example, greater HbA1c values (> 6.8%) indicate more challenges with glucose regulation. Numerous studies suggest that *SIRT1*, a protein involved in lifespan and the cellular stress response, could also be involved in the dysregulations of metabolism. Specifically, proper *SIRT1* activation may improve the low glucose compliance that is frequently seen in these individuals (13). The case study highlights how accumulation of *SIRT1* in the Golgi apparatus has been linked to incorrect sorting of ABPP, which may cause the organelle to misform. Abnormal processing of AB, a crucial component of Alzheimer's disease, is the outcome (14). Furthermore, it is plausible that *SIRT1* potentially has a neuroprotective role.

According to recent research, *SIRT1* activity may be implicated in the interference with processes that result in the production of NFTs and NSPs (15). Remarkably, compounds that are known to activate *SIRT1*, including cilostazol and resveratrol, have demonstrated neuroprotective properties in models of Alzheimer's disease. Utilizing tools such as the Alzheimer's disease Assessment Scale-Cognitive Subscale (ADAS-Cog), these *SIRT1* activators have also been linked to enhanced cognitive function. This opens up exciting avenues for investigating their therapeutic potential in treating cognitive difficulties in people with ASD, possibly through modifying *SIRT1*-mediated pathways involving neurotransmitter release and synaptic plasticity, which are essential for normal brain development and function (16). The production of amyloid protein synthesis is facilitated by the high activation of the *SIRT1* gene. In severe cases, this process causes amyloid plaques to form in people's brains, which exacerbates neurological diseases like autism and dementia (17,23,25). In our dementia patients, the relative fold change, as determined by real-time PCR,

showed a fold drop of around 4.1, indicating under expression of the *SIRT1* gene. Additionally, a factor drop (4.9) was noted in diabetics with diabetes mellitus, indicating a decreased expression of the corresponding gene (18). These findings were consistent with those of another research group that found that ASD participants had lower *SIRT1* activity than non-ASD patients (19). One important factor in ASD, a complex illness that includes conditions such as Alzheimer's disease, is the decrease of *SIRT1*.There is a wealth of data that links lower *SIRT1* levels to cognitive impairment (20). Reduced *SIRT1* expression is seen in the brains of dementia patients, which may be linked to neuronal shrinkage, poor neuroplasticity, and synaptic dysfunction (21,24).

SIRT1 decrease results from pro*SIRT1*, its precursor, being down regulated, which results in a loss of neuroprotective properties. Furthermore, peripheral markers have emerged as prospective diagnostic markers. These indications include reduced serum levels of *SIRT1* and decreased *SIRT1* gene expression in peripheral blood mononuclear cells (PBMCs). Reduced serum *SIRT1* levels are correlated with cognitive deterioration, which emphasizes the significance of *SIRT1* as a peripheral biomarker for dementia (22).

CONCLUSION

According to the study, *SIRT1* may be a useful biomarker for the early diagnosis of neurodegenerative conditions like dementia in individuals with autism, thereby increasing survival and enabling more focused treatments. It emphasizes the significance of peripheral *SIRT1* mRNA levels in Peripheral Bloood Mononuclear Cells PBMCs as markers of alterations in the central nervous system.

Future prospects:

Investigations into *SIRT1* in autism spectrum disorder (ASD) and associated neurodegenerative disorders have potential for novel therapeutic approaches and diagnostic instruments. Knowing its molecular function may help researchers find targets for therapeutic treatments, such as gene and pharmacological ones, to slow down neurodegeneration and enhance cognitive functions. By incorporating peripheral *SIRT1* mRNA levels into clinical practice, it may be possible to improve early diagnosis and monitoring, which might benefit patients with ASD and improve their prognosis and quality of life.

Conflict of Interest:

Authors have no conflict of interest.

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