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NEURONAL PROTECTION: THERAPEUTIC INSIGHTS FROM NERVE GROWTH FACTOR *NGF* GENE EXPRESSION ANALYSIS IN AUTISM DISEASE MANAGEMENT

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Abstract

Introduction: *Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by challenges in social interaction, communication, and behavior. Recent research has implicated nerve growth factor (NGF) in the pathogenesis of ASD, highlighting its role in neural development and connectivity. Dysregulation of NGF levels may disrupt critical processes involved in brain development, potentially contributing to the altered metabolic profiles observed in individuals with ASD.*

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

Material and Methods: *The study involved collecting EDTA blood samples (5ml each) from 100 individuals (88 Autism cases and 12 Healthy Controls) from different hospitals and clinical settings in Karachi and NGF expression analysis was performed. Statistical analysis was performed using Graph Pad Prism 9.0 to assess the strength of evidence supporting the role of NGF in ASD pathology.*

Results: *Analysis revealed significantly lower NGF expression in the Autism patients (5.97) compared to controls (12.87). Specifically, an NGF gene expression decrease correlated with cognitive impairment severity; 80% of participants scored below 9 on the MMSE with a mean age of 37 years, indicative of severe Neurodegeneration.*

Conclusion: *The strong correlation between autism severity and low NGF levels highlights the significance of metabolic variables in ASD. The potential of NGF as a biomarker creates new avenues for targeted therapies and early diagnosis.*

Keywords: *Autism, Diagnosis, Disease management, Neurodegeneration, NGF, Treatment*

INTRODUCTION

Autism, often known as Autism Spectrum Disorder (ASD), is a complicated neurological illness that begins in early childhood and lasts throughout a person's life. It can be identified by difficulties with social interaction, communication, and conduct, as well as a wide spectrum of symptoms and degrees of impairment. People on the autism spectrum may have difficulty recognizing social signs, engaging in repetitive actions, and having highly concentrated interests. Autism's causes are various, including genetic, environmental, and neurological factors (1). Individuals with autism may have unique talents, such as remarkable memory, precise perception across particular areas, and a high level of attention. Early diagnosis and personalized therapies are capable of significantly improving the quality of life for persons on the spectrum, highlighting the need to be aware of and understanding this medical condition (2).

Autism impairs social interaction and communication, and it might manifest as repetitive behaviors or restricted obsessive interests. Dementia generally impairs memory, problem-solving skills, and other

cognitive functions, which decrease over time. Neurodegeneration diseases account for approximately 70% of all instances of dementia (3). Genetic variants, such as ApoE ε4 allele, APP, and PSEN1/PSEN2, are significant risk factors. *NGF*, the protein encoded by the Brain-derived Neurotrophic Factor *NGF* gene, is associated with improved insulin sensitivity, and insulin resistance is a key factor in the development of type 2 diabetes. *NGF* also has anti-inflammatory effects, and chronic inflammation is linked to both autism and dementia (4). By lowering inflammation, *NGF* may help to lower the risk of both illnesses. Improvements in cognitive function may contribute to a decreased risk of dementia in people with autism. Lower *NGF* levels have been associated with higher levels of reactive oxygen species (ROS), glucose, Amyloid β, and Tau protein buildup, as well as an increased risk of obesity-related cardiovascular disorders such as ischemic heart disease and peripheral artery disease (PAD) (5). Genome-wide association studies identified numerous potential genes that are directly or indirectly connected to autism. Within these genes, the *NGF* gene is a novel potential gene enlisted (6).

Nerve Growth Factor, or *NGF*, is an important neurophysiological factor that affects many aspects of brain activity. It has the ability to sensitize insulin, has anti-inflammatory qualities, encourages angiogenesis, and helps vasodilate. It may also be able to pass across the blood-brain barrier and appear in the fluid surrounding the brain. *NGF* controls neurogenesis and synaptic plasticity, which is vital for preserving important brain functions including energy balance, hippocampus neurogenesis, and synaptic function. Its importance in cognitive processes is shown by AdipoR1, which is mostly located in the hippocampus. Cognitive decline and autism spectrum disorders are among the illnesses linked to disruptions in *NGF* levels or signaling pathways. Reduced levels of *NGF* are associated with increased risk, especially for women who have autism, although its neuroprotective properties hold hope for treating these disorders (7, 8).

Neurotrophins play a variety of roles in the peripheral and central nervous systems. The neurotrophin family comprises of *NGF*, NT 4/5, and NT-3, and its contribution to cell maintenance and survival in the neuronal population has been widely studied (9). TrkA is the high-affinity receptor for *NGF*, TrkB binds to both *NGF* and NT-4, and TrkC binds to NT-3. The low-affinity general receptor for neurotrphins, p75NTR, also has functional roles in both the central and peripheral nervous systems (10). Mechanismin pathways are shown in Fig. 1 (11).

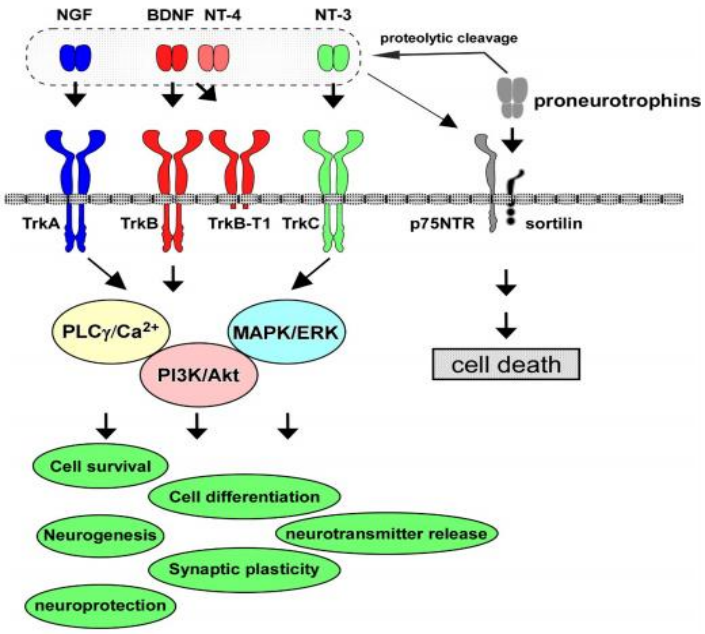

Fig. 1. Neurptrophins and Receptors in CNS

Hence *NGF* gene is not only linked to diabetes in individuals but also cognitive impairments in diabetic individuals which were the focus of our study, dealing with events like hyperglycemia or insulin resistance. The following study aims to analyze *NGF* gene, which is the focus of the study may be involved in the cross linkage of Type II diabetes disease and dementia. We investigated the expression of *NGF* 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 88 EDTA blood samples (5ml each) of patients of Autism and 12 healthy controls were collected after written informed consent and according to predefined inclusion and exclusion criteria, from clinically diagnosed cases of diabetes and dementia from diabetic clinics and Neurology OPDs of Jinnah Hospital Lahore and Shaikh Zayed Hospital Lahore**.** Inclusion criteria were: age of patients (>40y), genders both males and females, healthy controls without cognitive impairment or neurodegeneration, Type II DM, confirmed cases of 30 months disease course neurocognitive impairment patients with Autism Spectrum Disorders ASD. Exclusion criteria were: no clinical history and diagnostic tests record, refuse informed consent along with secondary diabetics.

NGF DETECTION BY ELISA AND PCR TECHNIQUES

NGF serum levels in the samples was measured by ELISA using a kit for the quantitative determination of serum *NGF* in plasma (IHUADPNKTC # IH0556) by manufacturer's protocol. QIAgen blood kit (QIAamp#56604) was used to isolate genomic DNA. Techniques such as UV spectrophotometry, fluorometry, and gel electrophoresis were used to assess the concentration, purity and integrity. Optimal absorbance 260/280 and 260/230 ratios, visualizing intact bands, and confirming amplifiability through PCR ensure high-quality DNA as shown in Fig. 2.

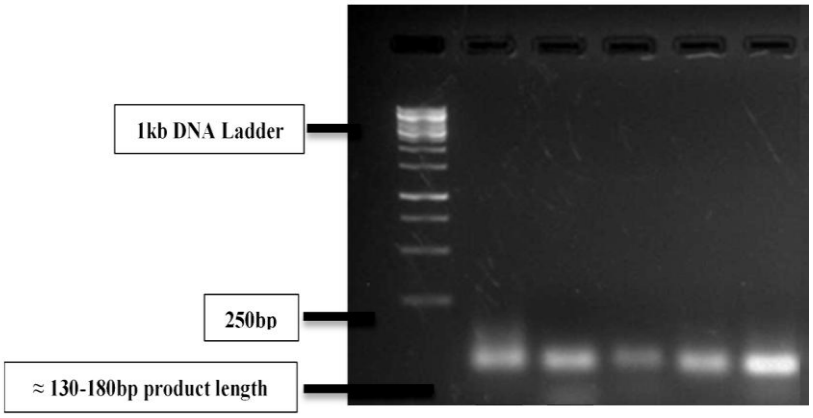

Fig. 2. NGF gene Gel Bands under UV

The primers set (forward and Reverse) 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 a shown in Table I.

Table I. Sequences of designed primers

NGF Primers Pair	NGF sequence (5/ to 3/) , −74 nt sequence
Froward	TGCTGGCCTAATAGAGTGGC
Reverse	CTCAGCGCCATGGAAAATGT

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 4426518) 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. The 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. The use of MMSE offers significant benefit to the clinicians when it comes to understanding the extent of cognitive impairment and hence, the interventions to be taken.

RESULTS

ANALYTICAL PARAMETERS OF ENROLLED SUBJECTS

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

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

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

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

Clinical Parameters/Variables	Healthy Controls (n=12)	Autism Patients (88)	t-test P value
NGF (µg/mL)	13.99±4.14	5.02±1.04	0.014*
MMSE Scores	27.56±3.12	11.56±2.56	0.006*

*Statistically Significant

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

The mental status of the patients was assessed using the Mini-Mental State Examination (MMSE) questionnaire. This evaluation provided insights into the severity of the disease among the study groups. Based on their MMSE scores, individuals were categorized into two groups: severe cognitive impairment (scores of 0-15) and no/less cognitive impairment (scores of 24 or higher). The scoring was performed according to the criteria established by the medical professionals who developed the MMSE. Fig. 3 and Fig. 4 below illustrate the interpretation of the scores based on severity.

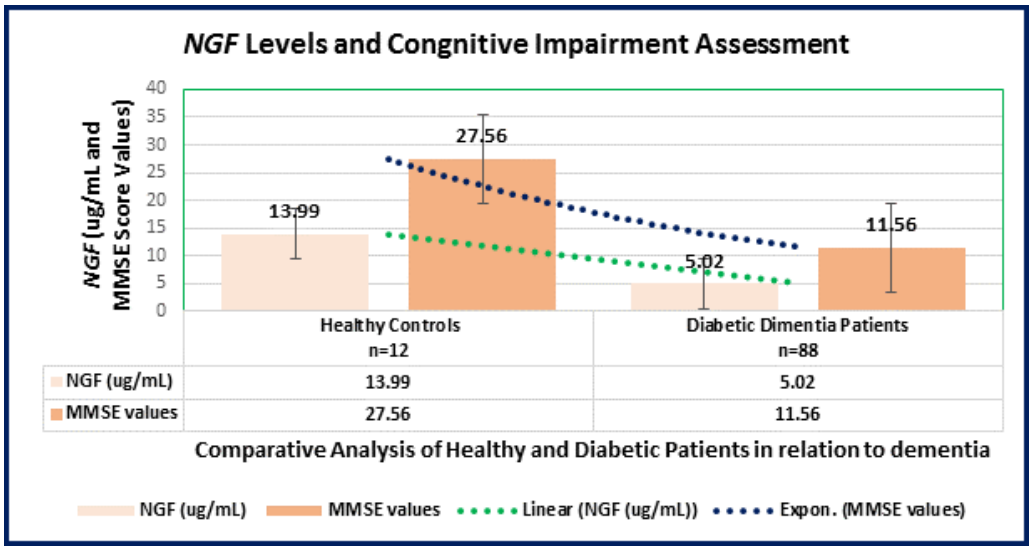

Fig. 3. *NGF* assessment for diabetic dementia and MMSE analysis for cognitive impairment

Compared to healthy individuals with *NGF* levels of 13.99 µl, diabetic patients exhibited reduced *NGF* levels (5.02 µl) and lower MMSE scores (11.56), confirming the presence of cognitive impairment in diabetic dementia patients.

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

Our data demonstrates a significant difference in the expression levels of Nerve Growth Factor (NGF) between healthy individuals and diabetic dementia patients. Specifically, the relative fold change of NGF in healthy controls was notably higher, with an expression level of 12.87, compared to 5.97 in diabetic dementia patients. This indicates a marked decrease in NGF levels as cognitive impairment increases in the context of diabetes. The reduced NGF expression in diabetic dementia patients suggests a potential link between decreased NGF levels and the severity of cognitive deficits associated with diabetes.

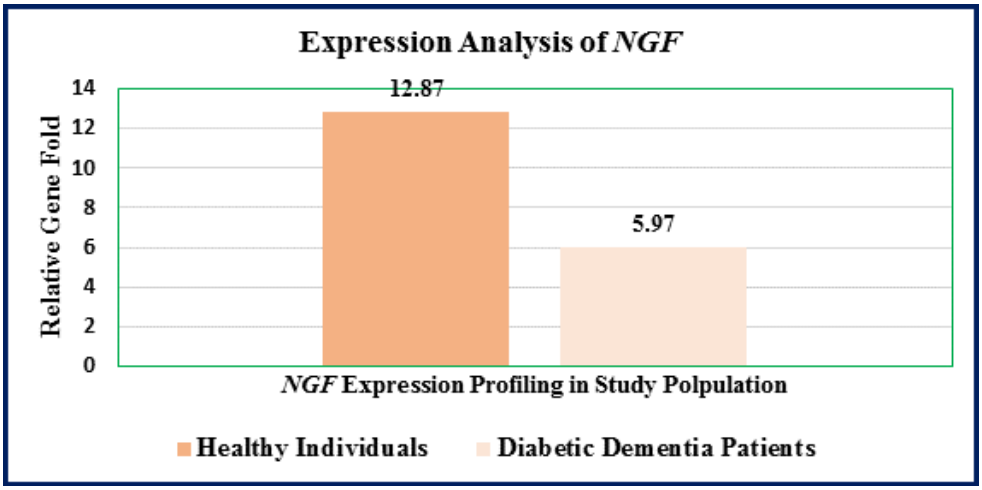

Fig. 4. A fold change in healthy controls and diabetic dementia cases

DISCUSSION

Autism spectrum disorder is a complex neurodevelopmental illness marked by a variety of problems, including altered metabolic profiles (11). Recent research has shown the potential significance of *NGF*, an adipose tissue-derived hormone, in ASD pathogenesis. *NGF* levels have been related to autism severity, indicating a probable association between metabolic dysregulation and autism spectrum disorders (12). Hyperglycemia, genetics insights and insulin resistance increase an individual's chances of developing cognitive impairment (13, 21). The gene *NGF*, which is the central focus of our study, has been implicated in worsening/accelerating these states, thereby altering an individual's mental functional capacities. Our findings also show that aberrant expression of these genes promotes accelerated hypoglycemia and cognitive impairment. Autistic individuals with a history of high HbA1c levels may have poor body compliance due to increased expression of the *NGF* gene. This can lead to the accumulation of AβPP in the Golgi, preventing it from being sorted to late endosomal compartments where Aβ is produced (14). Overexpression leads to Aβ buildup and higher levels compared to controls, according to another research. Our findings were similar to a study that found that reduced expression of the *NGF* gene in mice resulted in the production of amyloid beta metabolism (15).

Our investigation found that people with dementia had reduced levels of *NGF* expression. An MMSE score of less than 10 indicates significant cognitive impairment. The relative fold change when measured using real-time PCR indicated a fold drop of roughly 4.1 in our dementia patients, indicating underexpression of the *NGF* gene (16). Furthermore, a 4.9-fold drop is seen in diabetics with dementia, which indicates reduced expression of the related gene (17). These findings were consistent with the findings of another study group, which revealed a reduction in *NGF* activity in AD participants in comparison to non-autistic subjects (18). The reduction in brain-derived neurotrophic factor (*NGF*) appears as a significant feature. There is abundant evidence linking lower *NGF* levels to cognitive impairment. Patients with dementia have decreased *NGF* expression in their brains, which contributes to synaptic dysfunction, neuronal atrophy, and reduced neuroplasticity. This drop in *NGF* is accompanied by a

downregulation of its precursor, pro-*NGF*, which further impairs neurotropic support (15). Furthermore, peripheral signs such as reduced *NGF* serum levels and decreased *NGF* gene expression in peripheral blood mononuclear cells have been identified as possible diagnostic markers in the studies before. In a study, the function of neurotrophins—including *NGF*—in ASD was investigated. It outlined putative biomarkers and *NGF*-related neurodevelopmental pathways that may be involved in the etiology of ASD (22, 24). The link between cognitive decline and decreased *NGF* levels in serum emphasizes its usefulness as a peripheral biomarker for dementia (18, 23).

Understanding the complex interplay between *NGF*, dementia, and peripheral markers not only provides insight on the underlying pathophysiology, but also opens up new opportunities for diagnostic advances and therapeutic intervention (19). Targeting *NGF* dysregulation may be a potential technique for slowing cognitive decline and improving the chances of early identification and intervention in dementia-related illnesses (20).

CONCLUSION

This study found that the selected gene, *NGF*, might serve as possible biomarkers in identifying or diagnosing neurodegenerative illnesses, such as autism. Studies have provided insights into neurodevelopmental processes and potential biomarkers associated with ASD, emphasizing the complexity of interactions involving *NGF* and other neurotrophic factors in the context of autism spectrum disorders. Furthermore, the downregulation of *NGF* mRNA in PBMCs indicates a systematic component, suggesting the potential value of peripheral markers in reflecting CNS alterations.

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