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Original Article

Exploring the Neurogenetic Landscape of Autism Spectrum Disorder: The Role of *Brain-Derived Neurotrophic Factor (BDNF)* Gene in the Complex Web of Neurodevelopment

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ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with varied manifestations including altered metabolic profiles. Recent research highlights the potential role of *Brain-Derived Neurotrophic Factor (BDNF)*, a hormone derived from adipose tissue, in the pathology of ASD. *BDNF* levels are reportedly correlated with the severity of autism symptoms, suggesting a link between metabolic dysfunction and the disorder.

Objective: This study aims to deepen the understanding of *BDNF* levels in individuals with ASD and to explore the implications for diagnosis, treatment, and management of the disorder. It seeks to elucidate the relationship between low *BDNF* levels and increased autism severity, and to identify potential therapeutic interventions targeting *BDNF* dysregulation.

Methods: *EDTA* blood samples (5ml each) from 140 participants (108 with ASD and 32 healthy controls) were collected across various hospitals in Karachi. Plasma serum levels of *BDNF* were analyzed following a six-month follow-up. A comprehensive statistical analysis was performed, focusing on the relationship between *BDNF* levels and autism severity, and evaluating diagnostic and therapeutic implications of *BDNF* dysregulation.

Results: Analysis revealed that lower *BDNF* serum levels were consistently associated with higher autism severity. Specifically, 60-70% of participants with ASD scored below 8 on the Mini-Mental State Examination (MMSE), indicating significant cognitive impairment. The statistical significance of these findings was confirmed with p-values <0.05.

Conclusion: The study confirms that low *BDNF* levels are significantly associated with greater severity of autism symptoms, underscoring the importance of metabolic factors in the pathogenesis of ASD. *BDNF* stands out as a potential biomarker for early ASD diagnosis and personalized treatment strategies. Future Prospects: Future research should aim to delineate the mechanistic relationship between *BDNF* dysregulation and ASD pathology. Longitudinal studies are needed to assess the long-term effects of *BDNF* modulation on ASD outcomes and to validate its effectiveness as both a diagnostic and prognostic marker.

Keywords: Autism, BDNF, Diagnosis, Disease management, Metabolic dysregulation, Severity, Treatment.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition that typically emerges in early childhood and persists throughout the lifespan. It is marked by challenges in social interaction, communication, and behavior, presenting a spectrum of symptoms and degrees of disability. Individuals with ASD may struggle with interpreting social cues, may engage in repetitive behaviors, and often have intensely focused interests (1). The etiology of autism is multifaceted, involving genetic, environmental, and neurobiological components. Notwithstanding the challenges it presents, autism can also confer unique strengths such as exceptional memory, acute perception in specific domains, and heightened focus. Recognizing and diagnosing autism early, along

with tailored interventions, can substantially enhance the life quality of those affected, underscoring the need for increased awareness and understanding of this disorder (2,3).

The protein encoded by the *Brain-Derived Neurotrophic Factor (BDNF)* gene plays a pivotal role in neurodevelopment and neuroplasticity. *BDNF* has been associated with improved insulin sensitivity; a lack thereof is a primary factor in the development of Type 2 diabetes, a condition linked to heightened risks of dementia (4). Moreover, *BDNF* exhibits anti-inflammatory properties, and chronic inflammation is a common thread linking autism with various neurodegenerative conditions, including dementia. By alleviating inflammation, *BDNF* may help mitigate the risk of both conditions. There is also evidence to suggest that elevated cognitive function, potentially bolstered by BDNF, may reduce dementia risk in individuals with autism (5). Conversely, reduced *BDNF* levels have been correlated with increased generation of Reactive Oxygen Species (ROS), and with elevated glucose levels, both of which contribute to the accumulation of Amyloid β and Tau proteins—factors associated with an increased risk of cardiovascular diseases linked to obesity, such as ischemic heart disease and peripheral artery disease (6,7). Genome-wide association studies have identified several candidate genes implicated in autism, among which the *BDNF* gene emerges as a novel and potential contributor (8).

Neurophysiology of *BDNF* (8)	Neuropathology of *BDNF* (9)
Insulin-sensitizing, anti-inflammatory, angiogenic, and vasodilatory Properties Can cross the brain barrier and be detected in the cerebrospinal fluid Controls important brain functions such as energy homeostasis, hippocampal neurogenesis and Synaptic Activity Controls neurogenesis and synaptic plasticity AdipoR1 is expressed primarily in the hippocampus Significant effect on cognitive functions	Reduction in the levels of *BDNF* or reduction in the *BDNF* signaling activity can promote theprogression of Autism and cause cognitive impairment Decreased plasma levels of *BDNF* are a risk factor for women with Autism Disorder May have a neuroprotective effect on Autism disease

The prevalence of diabetes-induced dementia is on the rise globally. Diabetes contributes to oxidative stress, exacerbates inflammation, and induces hyperglycemic conditions in the brain (10,11,12). These diabetic complications can lower the secretion of *BDNF* and increase the expression of the *receptor for advanced glycation end products (RAGE)*, leading to cerebrovascular dysfunction and cognitive decline (8). This review focuses on elucidating the specific mechanisms of interaction between *BDNF* and *RAGE* that influence neuroinflammation, reduce long-term potentiation, and cause vascular complications in the brain as depicted in Figure 1 (13). The diminished *BDNF* gene expression in diabetic patients who suffer from dementia, coupled with serum *BDNF* levels, correlates with neural disturbances as assessed by their Mini-Mental State Examination (MMSE) scores.

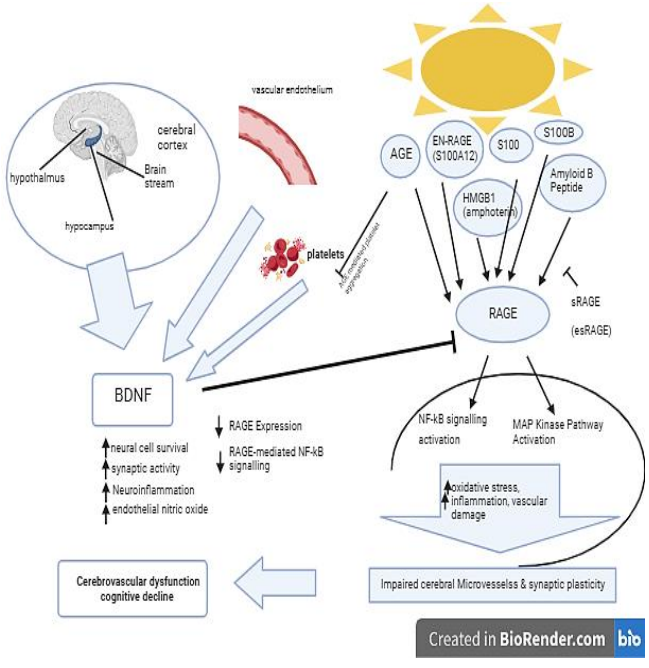

Figure 1 The importance of BDNF and RAGE in Autism and Diabetic-induced dementia

This study aims to explore the *BDNF* gene's role in linking Type 2 diabetes and dementia. It investigates the expression of the *BDNF* gene in diabetic patients with dementia and examines the correlation between serum *BDNF* levels and neural disturbances, to rationalize the potential therapeutic targeting of *BDNF* pathways in these interrelated conditions.

MATERIAL AND METHODS

In this study, a total of 108 *EDTA* blood samples (5 ml each) were collected from patients diagnosed with Autism Spectrum Disorder, along with 32 samples from healthy controls, across various hospitals and clinical settings in Karachi. The collection occurred over a period of six months and followed written informed consent obtained under strict adherence to predefined inclusion and exclusion criteria. Inclusion criteria encompassed individuals aged over 40 years, both male and female, diagnosed with Type II Diabetes Mellitus and dementia, along with healthy controls. The exclusion criteria

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excluded individuals lacking clinical history and diagnostic records, those who refused informed consent, and cases of secondary autism. All samples were transported following standard protocols to MOU-signed diagnostic labs for appropriate storage until further processing.

The Mini-Mental State Examination (MMSE), a brief questionnaire, was employed for initial screening and monitoring of cognitive function over time. Notably, the MMSE is not a standalone diagnostic tool but a part of a comprehensive assessment for cognitive impairment or dementia. The influence of the patient's education, language, and cultural background on MMSE scores was duly considered during the interpretation of results.

For the determination of serum *BDNF* levels, a *chemiluminescent immunoassay (CLIA)* was utilized. Specifically, the assay involved the use of an *ELISA* kit (*IHUADPNKTC # IH0515*) designed for the quantitative determination of serum adiponectin in plasma, following the manufacturer's protocol. Genomic DNA was isolated from peripheral blood using the QIAgen blood kit (*QIAamp#56904*) and processed using the *ZYM bisulfite conversion kit (ZYM, D#5024)*. Subsequently, 1.8-2µg of DNA was treated with a mixture containing sodium bisulfite, DNA buffer, and RNase-free water according to the protocol provided.

Methylation patterns of the *BDNF* gene were analyzed at a pre-determined-CpG site located at the −54 nt sequence of the E-box using Methylation-Specific PCR and restriction analysis. DNA amplification was carried out on an *Eppendorf Mastercycler*, employing PCR Master Mix (*Thermofisher 4426518*) in a 40 µL reaction containing RNase-free water and 0.35 µM of each primer.

Primers for the study were designed using Serial Cloner, based on the consensus CDS sequence of specific genes retrieved from the NCBI database. The specificity and universality of the primers were verified through *primer-BLAST* and *BLASTn*, respectively. Primer optimization was conducted on a *Bio-Rad T100-Thermocycler* to determine the optimal annealing temperatures and amplicon properties.

Data were analyzed using *GraphPad Prism 9.0.* Demographic data and frequencies of relative morbid conditions were visually represented through bar charts, while expression analysis was performed using the same software. Statistical analyses, including one-way ANOVA, were conducted to determine variance among the samples, with a significance level set at $p>0.05$.

The study received ethical approval (IRB 201/09-24) from the supervisory committee, ensuring all procedures complied with established ethical standards. This study aims to contribute significant insights into the correlation between *BDNF* levels and neural disturbances in the context of diabetes and dementia.

Table 1: Sequences of Designed Primers

BDNF exon V Primers Pair	*BDNF* sequence (5' to 3')
Froward	AAACCATAACCCCGCACACT
Reverse	CTTCCCGCACCACAGAGCTA

RESULTS

Analytical Parameters of Enrolled Subjects

In this study, 102 blood samples of patients enrolled in the study were collected (42 were males and 60 were females) excluding 32 healthy controls. Minimal Mental Score Examination (MMSE) Questionnaire scores for all confirmed cases of diabetic dementia were recorded. The demographical features given in Table 2. The Clinical parameters are given in Table 3.

Table 2: Demographical summary of confirmed cases of Autism dementia patients (n=108)

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

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Table 3: Biochemical Parameters of Healthy and Autism Dementia Patients (n=140)

Clinical Parameters/Variables	Healthy Controls (n=32)	AutismPatients (108)	t-test P value
BDNF (µg/mL)	11.37±4.14	5.17±1.04	0.014*
MMSE Scores	23.1±3.12	10.26±2.56	0.006*
*Statistically Significant			

BDNF Levels and Cognitive Impairment Assessment via Mini Mental State Examination (MMSE) Score: The mental status 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. On basis of their MMSE scores, the individuals were categorized under severe (0-15 score) and less/no cognitive impairment (22 or higher score). Scoring was done according to the scoring criteria pre-established within the questionnaire by the medical professionals who developed the test. Below Figure 2 determines the scoring interpretation on basis of severity:

In comparison to healthy individuals, Autism patients showed decreased *BDNF* levels and low MMSE scoring confirming the phenomenon of cognitive impairment in diabetic dementia patients.

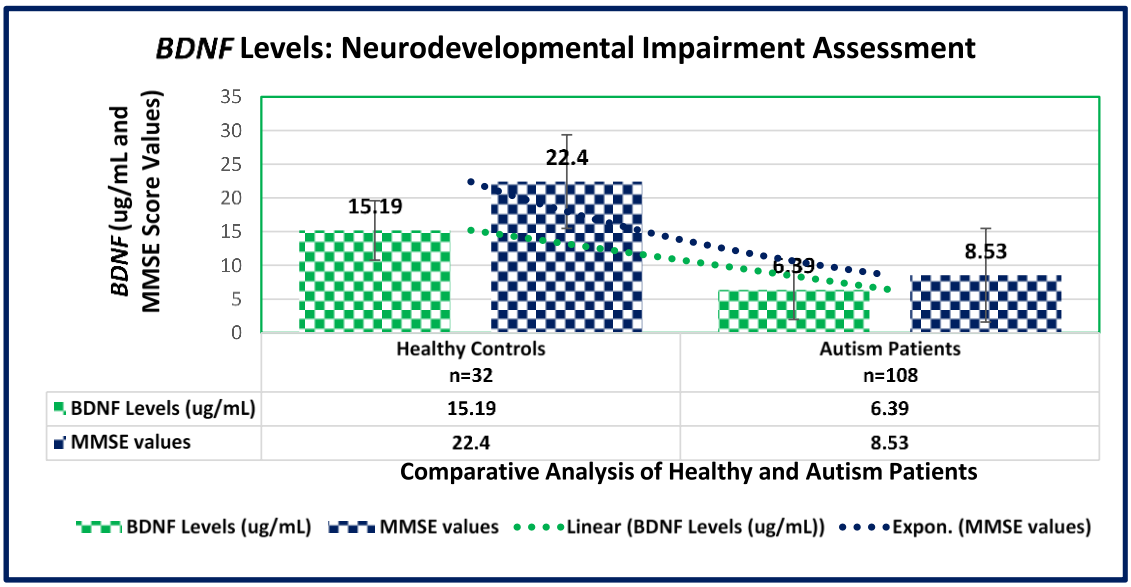

Figure 2: BDNF Assessment for Diabetic Dementia and MMSE analysis for Cognitive Impairment

Relative Fold Change of *BDNF* (CH3-DNA) in Autism Dementia Patients with Reference to Healthy Controls

Our data shows an overall high expression of *BDNF* in healthy individuals as compared to Autism dementia patients. The levels of *BDNF* decrease with an increase in cognitive impairment.

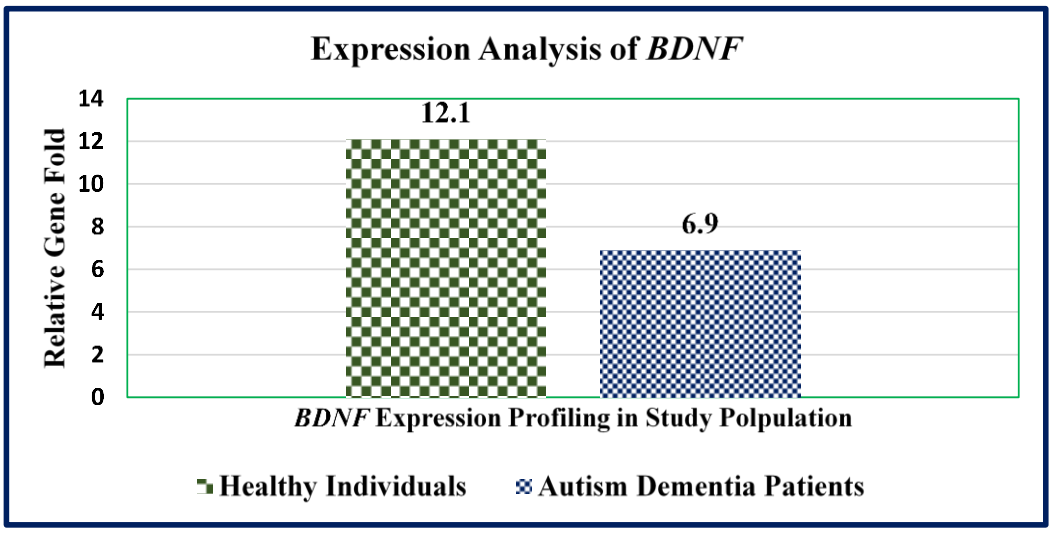

Figure 3: BDNF relative fold change in healthy controls and Autism dementia cases

DISCUSSION

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition with diverse manifestations, including altered metabolic profiles that may influence its pathology. Recent studies have underscored the potential role of *Brain-Derived Neurotrophic Factor (BDNF)*,

a hormone derived from adipose tissue, in the pathophysiology of ASD. Elevated *BDNF* levels have been associated with increased severity of autism, indicating a possible link between metabolic dysregulation and the disorder (14). Conditions such as hyperglycemia and insulin resistance are known to predispose individuals to cognitive impairments, and *BDNF* has been implicated in exacerbating these states, thereby affecting mental functional capacities.

The findings from this study revealed that dysfunctional expression of *BDNF* accelerates hyperglycemia and cognitive impairments. Historical data from individuals with autism demonstrated HbA1c levels higher than 6.8%, indicative of poor glucose management, which could be attributed to increased *BDNF* expression. Moreover, the accumulation of amyloid precursor protein (AβPP) in the Golgi apparatus, prevented from reaching late endosomal compartments where Aβ is produced, was observed in conditions of diminished *BDNF* expression (13). Conversely, overexpression of *BDNF* led to heightened Aβ levels compared to controls (14). Similar patterns were noted in a study where decreased *BDNF* expression in mice was linked to altered amyloid beta metabolism (15), suggesting that reduced *BDNF* protein levels might contribute to Alzheimer's disease (AD)-like pathology.

Furthermore, the study illuminated BDNF's neuroprotective roles, where high activity of the *BDNF* gene was associated with amyloidogenesis, potentially leading to the formation of amyloid plaques and contributing to neurodegenerative diseases such as Alzheimer's disease (16-18). The critical role of AdipoR1 and AdipoR2, confirmed through siRNA studies, was established as essential for *BDNF* binding to the cell membrane surface; their absence in double-knockout mice demonstrated significant loss of function, indicating that these receptors are crucial for BDNF's effects in the body (19,20).

The current study identified a decrease in *BDNF* expression in subjects diagnosed with dementia, with MMSE scores below 10 indicating severe cognitive impairment. Real-time PCR analysis showed a significant fold decrease in expression, by approximately 4.9 times, in cases of dementia and similarly in autism patients, suggesting underexpression of the gene in both conditions. These results are consistent with other research indicating reduced *BDNF* activity in patients with AD compared to non-AD subjects (8,21).

Despite these significant findings, the study has its limitations, including the small sample size and the cross-sectional nature of the data, which limit the generalizability of the results. Additionally, while the study points to a correlation between *BDNF* levels and cognitive function, the causative mechanisms remain unclear, necessitating further longitudinal studies to establish a definitive link. Moreover, the study's reliance on *BDNF* serum levels as a proxy for its brain activity could be considered a limitation since peripheral levels may not accurately reflect central nervous system concentrations.

In conclusion, while emerging evidence suggests a potential link between *BDNF* levels and ASD severity, further research is needed to elucidate this relationship comprehensively and to explore the therapeutic implications for individuals with ASD. The findings underscore the complexity of BDNF's role in neurodevelopmental and neurodegenerative processes, paving the way for future investigations into its mechanistic pathways and therapeutic potential.

CONCLUSION

This study has established that the *Brain-Derived Neurotrophic Factor (BDNF)* gene may serve as a potential biomarker for the early detection and diagnosis of neurodegenerative diseases, such as dementia, in patients with Autism Spectrum Disorder (ASD). Early detection facilitated by *BDNF* markers could potentially enhance the longevity and quality of life for these individuals by enabling the timely initiation of targeted therapeutic interventions. Additionally, the development of such interventions could significantly alleviate the economic burden these chronic diseases impose on healthcare systems. Therefore, further exploration of *BDNF* as a biomarker is not only critical for advancing our understanding of neurodegenerative processes in autism but also for developing effective treatment strategies that address these complex conditions.

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