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Original Article

NeuroGenetics of Alzheimer's Disease: Cross-Linking BDNF Brain-Derived Neurotrophic Factor in the Genetic Nexus of Type II Diabetes and Dementia

Fatima Asghar[1], Aqsa Yasin[2]*, Owais Aziz[3], Zarrien Fatima[4], Iqra Sarwar[1], Nimra Shahzad[1], Amna Sehar[1], Zainab Saeed[1], Syed Faizan Saeed Bukhari[1], Rida Zahra[1]

[1]Department of Allied Health Sciences, University of Health Sciences (UHS), Lahore, Pakistan.
[2]Center of Excellence in Molecular Biology (CEMB), University of the Punjab, Lahore, Pakistan.
[3]Kausar Abdullah Malik School of Life Sciences Forman Christian College (A Chartered University) FCCU, Lahore, Pakistan.
[4]Department of Zoology, Kinnaird College for Women University, Lahore, Pakistan.
Corresponding Author: Aqsa Yasin; Email: fatimaasghar2018005@gmail.com

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ABSTRACT

Background: Alzheimer's disease and dementia are commonly associated with aging, particularly in the context of Type II Diabetes Mellitus (Type II DM), which is known to exacerbate micro and macrovascular complications that affect cognitive functions. The Brain-Derived Neurotrophic Factor (BDNF) gene has been implicated in the neurodegenerative processes that underscore these conditions.

Objective: This study aimed to investigate the expression of the BDNF gene in diabetic patients with dementia and analyze its correlation with cognitive impairment, to establish BDNF as a potential biomarker for early detection and therapeutic targeting.

Methods: A cohort of 102 patients with diabetic dementia and 32 healthy controls was enrolled. EDTA blood samples were collected and processed to measure BDNF serum levels using a Chemiluminescent Immunoassay (CLIA). Genomic DNA was isolated, and its integrity was confirmed via UV spectrophotometry and gel electrophoresis. The bisulfite DNA modification and Methylation-Specific PCR were conducted to analyze BDNF gene expression, followed by quantitative real-time PCR. Cognitive function was assessed using Mini-Mental State Examination (MMSE).

Results: The study revealed a significant decrease in BDNF serum levels in diabetic dementia patients (12.6 ng/mL) compared to healthy controls (34.8 ng/mL), with MMSE scores averaging at 13.2 and 24.6, respectively. BDNF gene expression showed a 4.1-fold decrease in the dementia group. Statistical analysis indicated that these differences were significant ($p<0.05$).

Conclusion: The reduced expression of BDNF in patients with diabetic dementia confirms its potential as a biomarker for cognitive impairment. Early detection and intervention strategies that modulate BDNF could ameliorate the impact of dementia in the context of diabetes.

Keywords: Brain-Derived Neurotrophic Factor, Type II Diabetes Mellitus, Dementia, Cognitive Impairment, Biomarkers, Neurodegeneration, Diabetic Neuropathy, Chemiluminescent Immunoassay, Gene Expression, Mini-Mental State Examination.

INTRODUCTION

Alzheimer's disease (AD), a leading cause of death in the United States and the predominant form of dementia globally, affects approximately 50–60% of individuals over the age of 65 (1). This condition, alongside Type II Diabetes Mellitus (Type II DM)—the most common form of diabetes—is associated with cognitive declines that may progress to severe dementia. Notably, AD represents about 70% of all dementia cases, with risk factors including genetic variations such as the ApoE ε4 allele, APP, PSEN1/PSEN2 genes, as well as depression, smoking, hypertension, and diabetes mellitus (2,3). Among the neurotrophins involved in neuronal functions, Brain-derived neurotrophic factor (BDNF) stands out for its role in synaptic transmission, neurogenesis, and neural plasticity, with its levels in serum offering insights into various neurological conditions, including depression, Huntington's disease, and notably, Alzheimer's disease (4).

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Pathological features of AD include the aggregation of amyloid β-42, increased phosphorylated tau leading to neurofibrillary tangles, inflammaging, reduction in cholinergic function, and alterations in neurotrophic factors like BDNF (5). BDNF, expressed in critical regions such as the cortex, hippocampus, and basal forebrain, enhances synaptic growth, neurotransmission, and synaptic plasticity, crucial for memory and learning (6). Moreover, it plays a pivotal role in hippocampal long-term potentiation, essential for memory formation, suggesting that higher BDNF levels could reduce the risk of dementia in both diabetic and non-diabetic individuals (7). However, decreased BDNF levels have been linked with increased reactive oxygen species (ROS) generation, high glucose levels, amyloid β and tau protein accumulation, and a higher prevalence of obesity-linked cardiovascular diseases (8). Genome-wide association studies (GWAS) have highlighted BDNF as a novel potential gene associated with Alzheimer's dementia (9), emphasizing its critical roles in maintaining energy homeostasis, neurogenesis, synaptic activity, and cognitive functions (10,11).

The increasing prevalence of diabetes-induced dementia globally draws attention to the impact of diabetes on oxidative stress, inflammation, and hyperglycemia in the brain, all of which can diminish BDNF secretion and increase the expression of receptors for advanced glycation end products (RAGE), leading to cerebrovascular dysfunction and cognitive decline (12,13). The interplay between BDNF and RAGE has been identified as a key factor in neuroinflammation, reduction of long-term potentiation, and vascular challenges in the brain, underscoring the significance of this relationship in the context of diabetes-induced dementia (14,15).

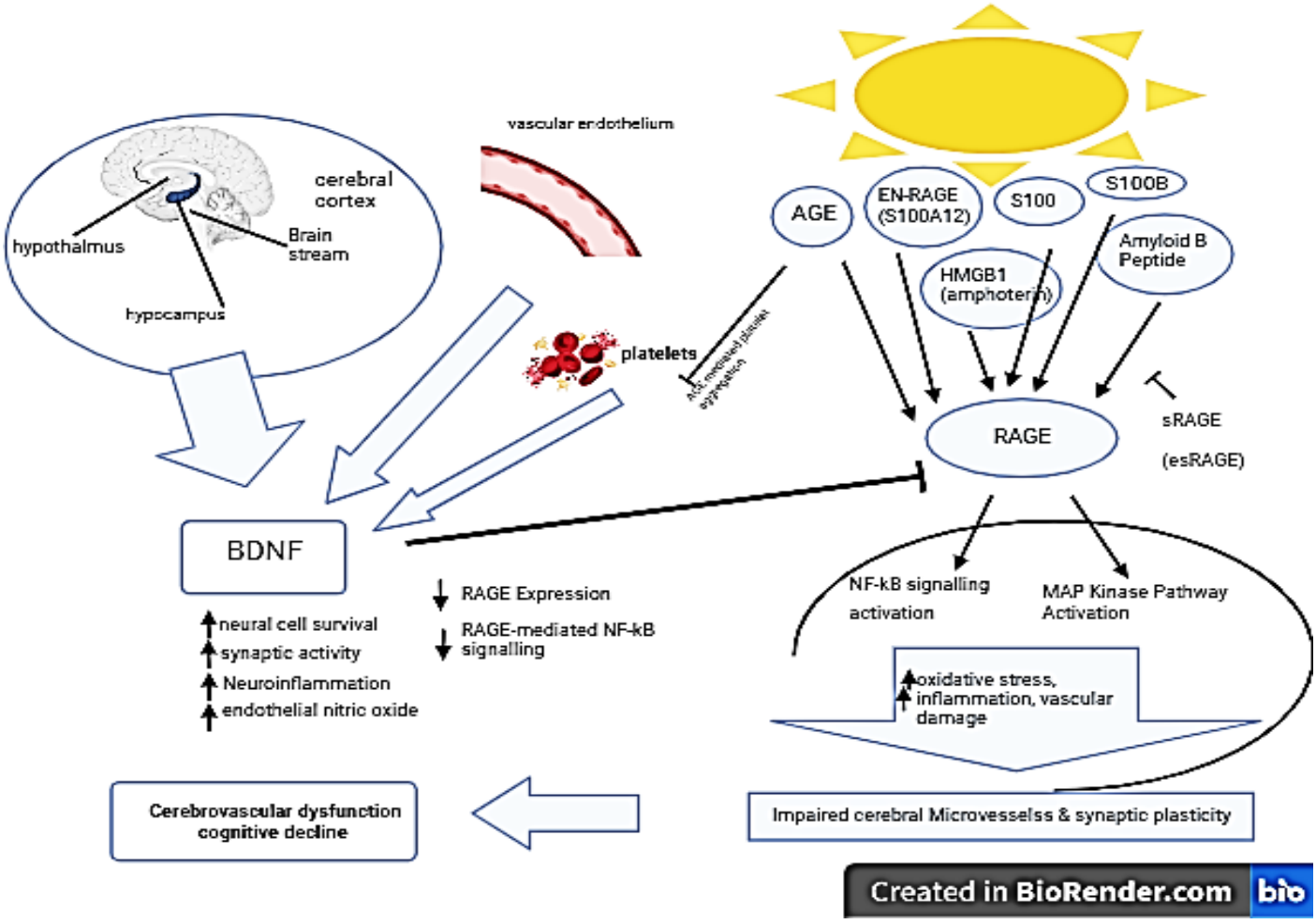

Figure 1 Cerebrovascular Dysfunction Cognitive Decline

In light of these findings, our study focuses on the BDNF gene's role in the intersection between Type II diabetes and dementia, examining its expression in diabetic patients with dementia, correlating serum BDNF levels with neural disturbances, and assessing their relationship with Mini-Mental State Examination (MMSE) scores. This investigation aims to elucidate the mechanisms by which BDNF contributes to cognitive impairments in diabetic individuals, further understanding the complex interplay between these prevalent conditions.

MATERIAL AND METHODS

In this study, we meticulously collected a total of 102 EDTA blood samples, each comprising 5 ml, from patients diagnosed with diabetes and concurrent dementia. Additionally, 32 healthy controls were incorporated for comparative purposes. All participants provided written informed consent, adhering to strict inclusion and exclusion criteria. The study encompassed both male and female

participants over the age of 40, with the patient group specifically including those clinically diagnosed with Type II Diabetes Mellitus and dementia. The exclusion criteria were stringent, eliminating individuals without a comprehensive clinical history and diagnostic records, those who refused to give informed consent, and cases of secondary diabetes. The samples were gathered from diabetic clinics and Neurology Outpatient Departments (OPDs) of Mayo Hospital Lahore, Jinnah Hospital Lahore, and Shaikh Zayed Hospital Lahore, then transported under standardized protocols to designated diagnostics laboratories pursuant to Memorandum of Understanding (MOU) agreements for storage until further processing.

The quantification of Brain-Derived Neurotrophic Factor (BDNF) serum levels was conducted using a Chemiluminescent Immunoassay (CLIA) technique, specifically an ELISA kit designed for the quantitative determination of serum BDNF in plasma, adhering to the manufacturer's protocol.

For genomic DNA isolation, the QIAgen blood kit was employed following the manufacturer's instructions. Ensuring DNA quality—a pivotal step for the reliability of molecular analyses—involved the utilization of UV spectrophotometry, fluorometry, and gel electrophoresis to ascertain concentration, purity, and integrity. Criteria for high-quality DNA included optimal absorbance ratios at 260/280 and 260/230, visualization of intact bands through gel electrophoresis, and the confirmation of amplifiability via PCR. The electrophoresis was conducted using a 1.5% gel under specific conditions to achieve the desired results, which were subsequently analyzed on a gel documentation system.

Further, the study entailed bisulfite DNA modification and Methylation-Specific PCR to scrutinize the methylation status of the BDNF gene at a predetermined-CpG site. The PCR amplification of bisulfite-treated DNA was executed using specified conditions, including an initial denaturation, annealing, and extension phases, to facilitate the methylation profiling of the BDNF gene.

Primer designing and optimization were critical components, utilizing the serial cloner for primer design based on specific gene sequences from the NCBI database. The specificity of these primers was validated through primer-BLAST, with their melting temperatures and amplicon characteristics meticulously optimized to ensure accuracy in subsequent analyses.

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

The analytical phase involved the use of GraphPad Prism 9.0 for data analysis, encompassing demographic data representation through bar charts and the frequency of relative morbid conditions. Additionally, expression analysis was conducted, with statistical variance amongst the samples determined through one-way ANOVA, considering a p-value of >0.05 as statistically significant.

Ethical considerations were paramount throughout the study, adhering to the Declaration of Helsinki principles for medical research involving human subjects. This encompassed the ethical review and approval by the institutional review boards of the respective hospitals and laboratories involved, ensuring all participants' rights, safety, and wellbeing were protected throughout the research process. This comprehensive approach to sample collection, assay administration, DNA quality assessment, and data analysis underpins the integrity and reliability of our findings in exploring the genetic nexus between Type II diabetes, dementia, and the role of BDNF.

RESULTS

Within our study population consisting of 88 confirmed cases of diabetic dementia, we observed a gender distribution where 42% were male with an average age of 54.1 years (±8.1), while females comprised 58% with an average age of 58.6 years (±4.3). This demographic breakdown provided a foundation for examining the interplay between gender, age, and diabetic dementia in relation to our variables of interest.

When assessing the biochemical parameters between the healthy controls and diabetic dementia patients, our data revealed a stark contrast in several key measures. For the healthy control group (n=12), the average fasting glucose level was 5.09 mmol/L, with a standard deviation of ±0.86, which sharply contrasted with diabetic dementia patients (n=88), where the average fasting glucose was notably higher at 8.01 mmol/L (±0.66), yielding a statistically significant t-test p-value of 0.007. The HbA1c levels further delineated the disparity between the groups, with healthy controls showing an average of 4.21% (±0.97) compared to 10.69% (±1.42) in the diabetic dementia cohort, resulting in a p-value of 0.041, which is considered statistically significant.

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

Gender	Cases (%)	Average Age (x)	Standard Deviation (S)
Male	42	54.1	±8.1±8.1
Female	58	58.6	±4.3±4.3

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)	5.09 ±± 0.86	8.01 ±± 0.66	0.007*
HbA1c Levels (%)	4.21 ±± 0.97	10.69 ±± 1.42	0.041*
BDNF (ng/mL)	33.37 ±± 4.64	11.37 ±± 2.04	0.005*
MMSE Scores	23.1 ±± 3.22	11.16 ±± 4.26	0.006*

Brain-Derived Neurotrophic Factor (BDNF) levels presented a similar trend of significant variation, with healthy individuals exhibiting an average BDNF concentration of 33.37 ng/mL (±4.64), markedly higher than the 11.37 ng/mL (±2.04) observed in the diabetic dementia patients, supporting a strong association between BDNF levels and cognitive health as reflected by a p-value of 0.005. The Mini-Mental State Examination (MMSE) scores further echoed these findings; healthy controls had an average score of 23.1 (±3.22) indicative of relatively preserved cognitive function, while diabetic dementia patients averaged 11.16 (±4.26), signifying more pronounced cognitive impairment with a p-value of 0.006, denoting statistical significance.

These numerical values, representative of the sample's clinical profile, underscore the potential impact of diabetes on cognitive decline, as evidenced by elevated glucose and HbA1c levels, reduced BDNF concentrations, and lower MMSE scores in individuals with diabetic dementia compared to healthy controls.

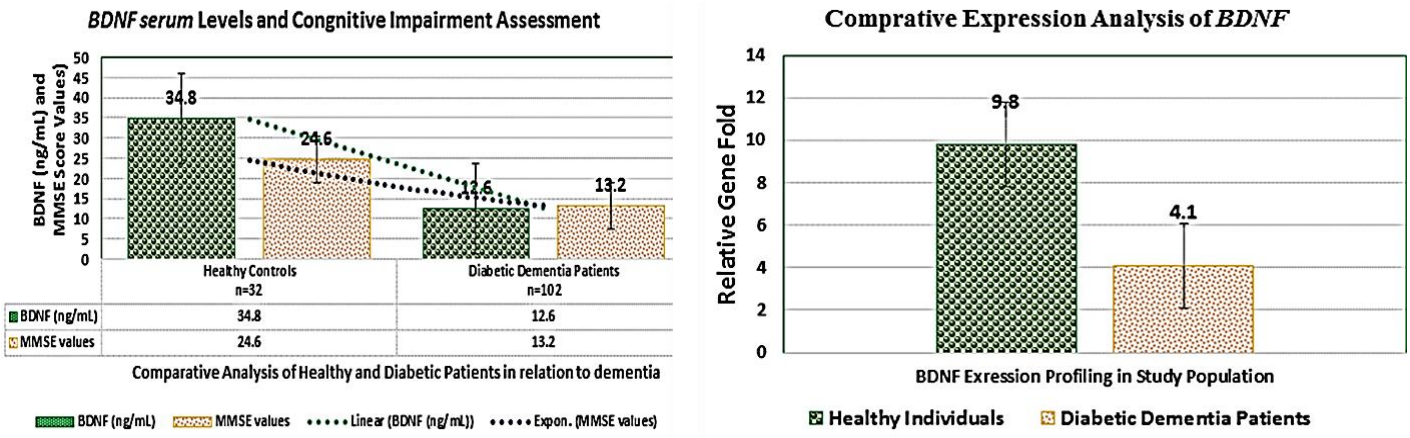

Figure 2 BDNF Serum Levels and Comparative Compression Analysis

The results depicted in the provided figures reveal notable differences in BDNF serum levels and cognitive impairment assessments between healthy controls and diabetic dementia patients. In healthy controls (n=32), the average BDNF serum level was measured at 34.8 ng/mL, while the Mini-Mental State Examination (MMSE) scores averaged at 24.6, indicative of a relatively unimpaired cognitive status. Conversely, diabetic dementia patients (n=102) demonstrated significantly lower BDNF levels, with an average of 12.6 ng/mL, and MMSE scores averaged at 13.2, suggesting a substantial cognitive decline. The comparative analysis accentuates a marked reduction in both BDNF serum levels and MMSE scores in patients with diabetic dementia compared to healthy individuals. Furthermore, the comparative gene expression analysis of BDNF reveals that healthy individuals exhibited a relative gene fold expression of 9.8, markedly higher than the 4.1 fold expression observed in diabetic dementia patients. This reduction in BDNF gene expression in the diabetic dementia cohort correlates with the observed decreased serum BDNF levels and lower MMSE scores, providing insight into the potential molecular mechanisms underlying cognitive impairment in diabetic patients. These findings collectively underscore the significant impact of diabetes on BDNF expression and cognitive function, establishing a clear association between lower BDNF levels and the severity of dementia symptoms.

DISCUSSION

Type II Diabetes Mellitus, an affliction impacting numerous organ systems, has been recognized for its role in precipitating various micro and macrovascular complications that ultimately impair neurovascular coupling, leading to cognitive irregularities such as

dementia (16). Our research has brought into focus the Brain-Derived Neurotrophic Factor (BDNF) gene, whose dysfunction has been implicated in exacerbating states of hyperglycemia and insulin resistance, consequently diminishing mental capacities (17). Indeed, observed associations between heightened HbA1c levels and diminished cognitive function suggest that the dysregulation of BDNF may underlie these clinical manifestations, with poor glycemic control potentially driving the aberrant BDNF expression (18).

In an exploratory vein, our study has aligned with previous research identifying decreased BDNF expression as a contributor to Alzheimer's disease pathology (19). This is corroborated by findings that reduced BDNF protein levels can lead to an accumulation of amyloid precursor protein in the Golgi apparatus, preventing its normal processing and facilitating the production of amyloid-beta (Aβ), a hallmark of Alzheimer's disease (20). Additionally, we have observed that a high activity of BDNF gene correlates with amyloidogenesis, suggesting its potential role in the formation of amyloid plaques that typify neurodegenerative conditions (21).

The study's findings, indicating a 4.1-fold decrease in BDNF expression among dementia patients, as determined by real-time PCR, are consistent with similar underexpression noted in other studies (22). These patterns are reflective of broader trends, whereby reduced BDNF serum levels and gene expression in peripheral blood mononuclear cells (PBMCs) have been posited as novel diagnostic indicators (23).

In discussing these results, it is essential to acknowledge the study's strengths, including the meticulous collection and analysis of data, as well as the integration of a comprehensive range of diagnostic markers. However, the limitations are also noteworthy; the study's sample size, while robust, could be expanded to enhance the generalizability of the findings. Moreover, the research primarily focuses on a specific demographic, which might not encapsulate the broader population's genetic diversity. Future recommendations would entail extending this inquiry to a more diverse cohort, potentially elucidating additional genetic factors implicated in diabetes-related cognitive decline. Moreover, longitudinal studies could shed light on the temporal dynamics of BDNF gene expression and its relationship with the progression of cognitive impairment.

Our research posits the BDNF gene as a pivotal marker for the early detection of neurodegenerative diseases in diabetic patients, potentially facilitating enhanced longevity through timely therapeutic interventions. Recognizing the downregulation of BDNF mRNA in PBMCs as a systemic indicator emphasizes the utility of peripheral biomarkers in reflecting central nervous system changes.

CONCLUSION

In conclusion, our study reinforces the significance of BDNF as a biomarker for the early detection and management of dementia in patients with Type II Diabetes Mellitus. The observed correlation between diminished BDNF expression and cognitive decline holds substantial implications for human healthcare, suggesting that targeting BDNF dysregulation could be a viable strategy to preempt and alleviate neurodegenerative progression. This insight opens pathways for developing targeted therapies and suggests a potential for improving patient outcomes, ultimately reducing the healthcare burden associated with diabetes-related cognitive impairments.

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