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Original Article

Glial cell line-derived Neurotrophic factor GDNF Gene Expression Analysis: Unveiling Neuronal Protection Mechanisms in Alzheimer's Disease Management

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ABSTRACT

Background: Alzheimer's disease (AD), accounting for a substantial proportion of dementia cases, presents a formidable challenge to healthcare systems worldwide. The pathogenesis of AD in patients with Type II Diabetes Mellitus (DM) is particularly complex due to the interplay of metabolic and neurodegenerative processes. Understanding the role of Glial Cell Line-Derived *Neurotrophic Factor* (GDNF) in this context is vital for elucidating potential therapeutic targets.

Objective: This study aimed to assess the expression levels of the GDNF gene in patients with Type II DM and dementia and to explore the correlation between GDNF expression and cognitive function.

Methods: Blood samples were collected from 88 diabetic patients with dementia and 12 healthy controls. GDNF serum levels were measured using *Chemiluminescent Immunoassay* (CLIA), and genomic DNA was isolated for expression analysis via RT-qPCR. Cognitive function was evaluated using the Mini Mental State Examination (MMSE). Statistical analysis, including t-tests and one-way ANOVA, was performed using Graphpad Prism version 9.0.

Results: Diabetic dementia patients exhibited significantly lower GDNF serum levels (4.37±1.38 µg/mL) compared to healthy controls (11.37±3.64 µg/mL, p=0.014). MMSE scores were also lower in the patient group (12.26±4.56) than in controls (24.1±3.12, p=0.009). RT-qPCR results showed a relative fold decrease in GDNF expression of 4.1 in dementia patients, indicating underexpression of the GDNF gene.1

Conclusion: The reduced expression of GDNF in patients with Type II DM and dementia underscores its potential as a biomarker for cognitive impairment. These findings pave the way for further research into GDNF-targeted therapies, which could prove pivotal in early detection and management of dementia in diabetic populations.

Keywords: Alzheimer's disease, Type II Diabetes Mellitus, *Glial Cell Line-Derived Neurotrophic Factor* (GDNF), cognitive function, dementia, biomarkers, gene expression, neurodegeneration, Mini Mental State Examination (MMSE), *Chemiluminescent Immunoassay* (CLIA).

INTRODUCTION

In the field of neurodegenerative disease management, particularly Alzheimer's disease (AD), the exploration of *Glial Cell Line-Derived Neurotrophic Factor* (GDNF) gene expression presents a promising avenue for uncovering neuronal protection mechanisms (1). Alzheimer's disease, which accounts for approximately 70% of all dementia cases, is a complex neurological disorder with a multifactorial etiology encompassing genetic, environmental, and lifestyle factors (2). Significant risk factors for AD include the presence of the ApoE ε4 allele, mutations in genes such as APP, PSEN1, and PSEN2, as well as modifiable risk factors like depression, smoking, hypertension, and notably, diabetes mellitus (3, 4). The latter, Type II Diabetes Mellitus (Type II DM), the most prevalent form of diabetes, is known for its mild cognitive impairments that can progress to a severe form of dementia. This connection

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highlights the potential for shared pathological pathways between Type II DM and AD, particularly in the context of insulin resistance and neuroinflammation, which are pivotal in the development and progression of both conditions (5).

Neurotrophic factors, including GDNF, are a family of proteins encoded by the GDNF gene known for their vital role in neuronal survival, development, and function. GDNF, in particular, has been associated with a range of physiological properties beneficial for neural health, including insulin-sensitizing, anti-inflammatory, angiogenic, and vasodilatory effects. These properties not only contribute to improved insulin sensitivity and reduced risk of diabetes but also offer a protective mechanism against the development of neurodegenerative diseases like AD (6). GDNF's ability to mitigate inflammation and oxidative stress, common features in the pathophysiology of diabetes and dementia, further underscores its therapeutic potential. Interestingly, reduced levels of GDNF have been linked to increased *reactive oxygen species* (ROS) generation, high glucose levels, accumulation of neurotoxic proteins such as amyloid β and *tau*, and enhanced prevalence of obesity-linked cardiovascular diseases, which are recognized risk factors for the development of AD (7).

The neurophysiological and neuropathological roles of GDNF in Alzheimer's disease are profound. GDNF is essential for maintaining critical brain functions, including energy homeostasis, hippocampal neurogenesis, synaptic activity, and synaptic plasticity. *AdipoR1*, a receptor for GDNF, exhibits a primary expression in the hippocampus, a region crucial for memory and cognitive function, which is severely affected in AD (8-11). The reduction in GDNF levels or signaling activity has been shown to promote the progression of Alzheimer's disease and cognitive impairment, with decreased plasma levels of GDNF identified as a risk factor, particularly in women with Alzheimer's disease. This evidence supports the hypothesis that GDNF possesses neuroprotective effects that could potentially counteract the pathological mechanisms of Alzheimer's disease (8-11).

Furthermore, the neurotrophin family, which includes NGF, BDNF, NT-4/5, and NT-3 alongside GDNF, plays a pivotal role in the survival and maintenance of the neuronal population in both the peripheral nervous system (PNS) and central nervous system (CNS). The interactions between these neurotrophins and their receptors, such as TrkA for GDNF, TrkB for BDNF and NT-4, TrkC for NT-3, and the low-affinity common receptor p75NTR, underline the complexity and significance of neurotrophic signaling in neural health and disease (8-11).

Given the intricate link between Type II diabetes and cognitive impairments in diabetic individuals, our study aims to delve into the role of GDNF gene expression in this interplay. By investigating the expression of the GDNF gene in diabetic patients suffering from dementia, we aim to elucidate its correlation with neural disturbances as assessed by their Mini-Mental State Examination (MMSE) scores. This research not only seeks to advance our understanding of the biological mechanisms underlying Alzheimer's disease but also to explore potential therapeutic targets that could mitigate the cognitive decline associated with both diabetes and dementia.

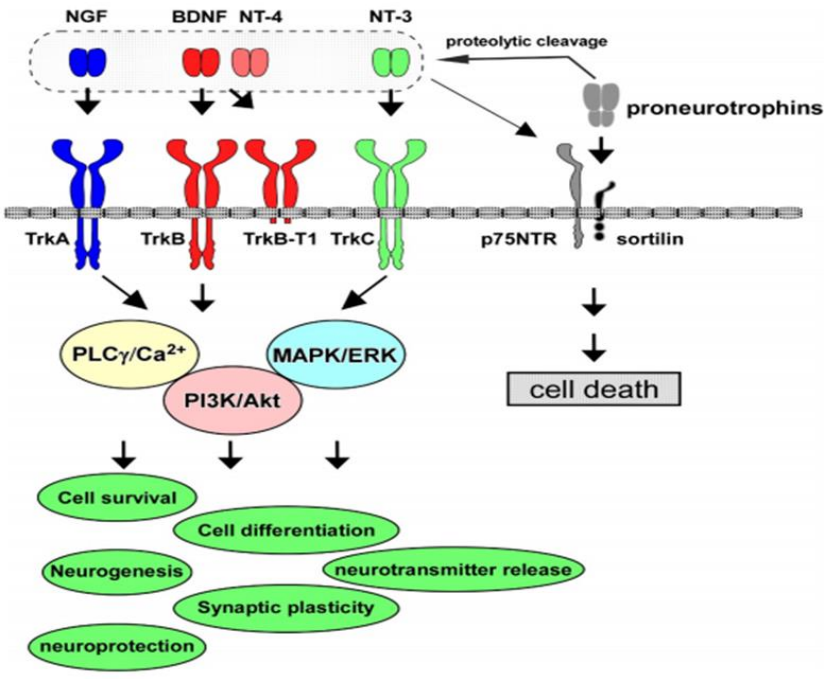

Figure 1 Signaling Pathways, Neurotrophins and Receptors

The diagram illustrates the signaling pathways initiated by different neurotrophins—NGF, BDNF, NT-4, and NT-3—upon binding to their respective high-affinity tropomyosin receptor kinases (Trks) and low-affinity p75 neurotrophin receptor (p75NTR). NGF preferentially binds to TrkA, BDNF and NT-4 to TrkB, and NT-3 mainly to TrkC, although there is some cross-reactivity, as shown with TrkB-T1. Activation of these receptors triggers downstream signaling cascades such as PLCγ/Ca2+, MAPK/ERK, and PI3K/Akt, which lead to cellular responses including cell survival, differentiation, neurogenesis, neurotransmitter release, and synaptic plasticity, collectively contributing to neuroprotection. Conversely, pro-neurotrophins, which are the precursors that can be cleaved into mature neurotrophins, bind to p75NTR and sortilin, and this interaction is associated with cell death, highlighting the dual nature of neurotrophin signaling depending on the receptor type engaged.

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MATERIAL AND METHODS

The study employed a robust methodology to investigate the relationship between diabetes, dementia, and the expression of the Glial Cell Line-Derived *Neurotrophic Factor* (GDNF) gene. Blood samples were obtained from a cohort comprising 88 patients with coexisting diabetes and dementia, along with 12 healthy controls serving as a comparative baseline. The samples, drawn using EDTA as an anticoagulant, were collected in compliance with ethical standards that mandated written informed consent from all participants. The study strictly adhered to the ethical principles of the Declaration of Helsinki, ensuring the integrity of research and participant confidentiality. Inclusion criteria required participants to be over 40 years of age, of any gender, and either healthy controls or clinically confirmed cases of Type II Diabetes Mellitus and dementia. Individuals with a history of secondary diabetes or lacking comprehensive clinical history and diagnostic test records were excluded from the study. Additionally, those who declined to provide informed consent were not considered for participation.

For the quantification of GDNF serum levels, a *Chemiluminescent Immunoassay* (CLIA) was utilized. This assay was conducted in accordance with the manufacturer's instructions using a specific kit designed for the quantitative determination of GDNF in plasma (*IHUADPNKTC* # IH0556). Following collection, samples were transported under stringent standard protocols to partnering diagnostic laboratories, as stipulated by Memoranda of Understanding (MOUs), and stored appropriately pending analysis.

The isolation of genomic DNA from peripheral blood samples was carried out using the *QIAgen blood kit* (QIAamp#56604), following the manufacturer's guidelines. To ascertain the quality and integrity of the isolated DNA—a critical factor for subsequent molecular analyses—techniques such as UV *spectrophotometry*, *fluorometry*, and gel electrophoresis were employed. The evaluation process included verifying the optimal absorbance ratios at 260/280 nm and 260/230 nm, visualizing intact bands on a 1.5% agarose gel prepared according to standard protocols and run at 70 Volts for 40 minutes, and confirming the DNA's amplifiability via Polymerase Chain Reaction (PCR). The results from gel electrophoresis were subsequently documented using a gel documentation system (SS Doc).

To assess the *methylation* status of the GDNF gene, DNA underwent bisulfite conversion using the *ZYM bisulfite conversion kit* (ZYM, D#5024). This was followed by *Methylation*-Specific PCR to examine the predetermined CpG site located at the −72 nucleotide sequence of the E-box. Amplification was performed with the *Eppendorf Mastercycler* instrument using a *PCR Master Mix* (Thermofisher 4426518), incorporating designed primers with the sequences: forward, TGCTGGCCTAATAGAGTGGCA, and reverse, CTCAGCGCCATGGAAAATGT. The amplification conditions included an initial denaturation, a cycling protocol for denaturation, annealing, and extension, followed by a final elongation step and a hold at 4 °C.

Primer design was executed via serial cloner using consensus coding sequences from the NCBI database, with primer specificity confirmed by primer-*BLAST or BLASTn*. Primer melting temperatures and amplicon properties were fine-tuned using a gradient PCR *thermocycler (Bio-Rad T100-Thermocycler, USA*).

Data from the study was rigorously analyzed using Graphpad Prism version 9.0. Demographic data and frequencies of relative morbid conditions were visually represented through bar charts, and expression analysis was conducted. Statistical analyses, including one-way ANOVA, were performed to determine variance among the sample groups. A p-value threshold of >0.05 was set to establish statistical significance. This comprehensive approach ensured the reliability and accuracy of the findings in elucidating the role of GDNF in the pathophysiology of diabetes-related dementia.

RESULTS

The collective results of the study demonstrated significant correlations between Nerve Growth Factor (NGF) levels, cognitive function as assessed by Mini Mental State Examination (MMSE) scores, and the presence of diabetes with concurrent dementia. The participant pool included 88 diabetic dementia patients, with a gender distribution of 36 males (41%) and 52 females (59%). Male participants had an average age of 55.4 years (standard deviation ±8.5), while female participants were, on average, older at 59.1 years (standard deviation ±4.6).

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

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Table 2. Biochemical Parameters of Healthy and Diabetic Dementia Patients (n=100)

Clinical Parameters/Variables	Healthy Controls (n=12)	Diabetic Dementia Patients (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*
GDNF (µg/mL)	11.37±3.64	4.37±1.38	0.014*
MMSE Scores	24.1±3.12	12.26±4.56	0.009*
*Statistically Significant			

Biochemical analysis of the collected samples revealed notable differences between healthy controls and those with diabetic dementia. Healthy controls had an average fasting glucose level of 5.01 mmol/L (standard deviation ±0.56), which was significantly lower than the diabetic dementia patients' average of 9.01 mmol/L (standard deviation ±0.86), with a t-test p-value of 0.007, indicating statistical significance. Hemoglobin A1c (HbA1c) levels also differed markedly, with healthy controls at 4.31% (standard deviation ±0.67) compared to diabetic dementia patients at 10.59% (standard deviation ±1.62), this difference also being statistically significant with a p-value of 0.044.

GDNF levels were higher in healthy controls, averaging 11.37 µg/mL (standard deviation ±3.64), while diabetic dementia patients exhibited reduced levels at 4.37 µg/mL (standard deviation ±1.38), with a statistically significant p-value of 0.014. MMSE scores followed a similar trend, with healthy controls scoring an average of 24.1 (standard deviation ±3.12), indicating lesser or no cognitive impairment, and diabetic dementia patients averaging at 12.26 (standard deviation ±4.56), suggestive of severe cognitive impairment (p-value of 0.009).

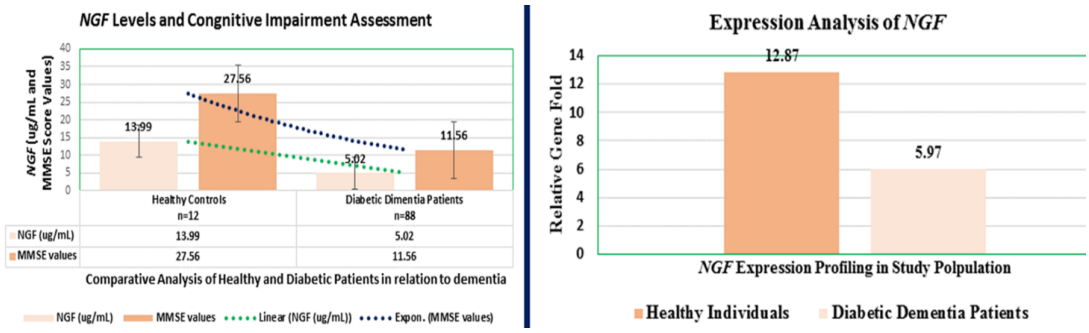

Figure 2 and Figure 3 NGF Levels and Expression Analysis

Figure 2 in the study illustrated the relationship between NGF levels and cognitive impairment. The average NGF level for healthy controls stood at 13.99 µg/mL, and the MMSE score was 27.56, which was contrasted with diabetic dementia patients' NGF levels of 5.02 µg/mL and MMSE score of 11.56. These findings underscored the phenomenon of cognitive decline in diabetic dementia patients.

Expression analysis indicated a relative fold change in NGF gene expression. Healthy individuals showed an approximately 12.87-fold higher expression of NGF compared to diabetic dementia patients, who exhibited a 5.97-fold expression relative to healthy controls as evidenced in Figure 3. The observed decrease in GDNF levels with increased cognitive impairment highlights the potential role of NGF as a biomarker for cognitive decline in diabetic dementia and underpins the importance of GDNF in the maintenance of cognitive function. The statistical significance across all measured parameters reinforced the strength of the observed associations.

DISCUSSION

In the exploration of Alzheimer's disease, gene expression analysis stands at the forefront, offering a nuanced understanding of the molecular interactions that contribute to the progression of this debilitating condition. The current study delved into the expression of the Glial Cell Line-Derived *Neurotrophic Factor* (GDNF) gene, seeking to elucidate its role in the pathogenesis of dementia within the context of Type II Diabetes Mellitus (DM). Recognized as a systemic ailment, Type II DM not only impacts various organs but also influences the interplay between them, potentially exacerbating neurovascular complications that are implicated in cognitive deficits and dementia (12). States of hyperglycemia and insulin resistance, commonly observed in diabetes, have been shown to increase the risk of cognitive impairments (13). This study's findings highlighted the perturbed expression of the GDNF gene, reinforcing the gene's proposed role in exacerbating hyperglycemia and subsequent cognitive dysfunction.

Previous literature has posited that an increased expression of GDNF may lead to the accumulation of amyloid precursor protein (AβPP) within the Golgi apparatus, impeding its trafficking to late endosomal compartments where amyloid-beta (Aβ) production occurs (14). In contrast, overexpression of GDNF was found to correlate with heightened Aβ levels (15). The current study's observations align with prior research indicating that a diminished GDNF expression could precipitate an Alzheimer's disease-like

pathology, inferring that GDNF may harbor a neuroprotective role as well (16). High activity of the GDNF gene has been linked to the pathogenesis of amyloidogenesis, with excessive amyloid plaques contributing to Alzheimer's disease's neurodegenerative process (17).

Furthermore, the study identified a pronounced decrease in GDNF expression in participants diagnosed with dementia, supported by MMSE scores indicative of severe cognitive impairment. Real-time PCR analyses revealed a fold decrease in GDNF expression, reinforcing the potential underexpression of the gene in diabetic individuals with dementia (18). The relationship between dementia and GDNF is multifaceted, with evidence mounting in support of reduced GDNF levels as a contributor to cognitive decline. In dementia patients, lower levels of GDNF in the brain have been associated with synaptic deficits and neuronal degeneration (19). These findings were substantiated by the study, which observed not only cerebral downregulation but also peripheral indicators, such as reduced serum GDNF levels and decreased gene expression in peripheral blood mononuclear cells (PBMCs), which may serve as viable biomarkers for dementia (20).

This discussion warrants a consideration of the study's strengths, such as its rigorous methodology and comprehensive data analysis. Nonetheless, limitations were present, including the potential for broader genetic and environmental factors that could influence GDNF expression and the need for larger sample sizes to enhance the generalizability of the findings. Moreover, it is essential to acknowledge the inherent complexity of gene expression studies in reflecting the multifactorial nature of Alzheimer's disease and related dementias (16, 19).

Given these insights, the study proposes future research directions aimed at further elucidating the role of GDNF in cognitive impairment, particularly concerning its neuroprotective capacities. Investigating the systemic aspects of GDNF expression and its interconnection with central nervous system changes could lead to more refined diagnostic and therapeutic approaches. Ultimately, GDNF's potential as a biomarker for the early detection of neurodegenerative diseases, especially in diabetic populations, could pave the way for interventions that may extend patient longevity and alleviate the economic burden imposed by these disorders. The study concludes that GDNF dysregulation in PBMCs may reflect central nervous system changes, signifying the potential role of peripheral markers in the early detection and management of dementia (20, 21).

CONCLUSION

The study concludes that dysregulation of the GDNF gene is intricately associated with cognitive decline in diabetes-associated dementia, highlighting its potential as a biomarker for early detection and intervention in neurodegenerative diseases. These findings carry significant implications for human healthcare, suggesting that monitoring GDNF expression could aid in the development of targeted therapies, potentially improving patient outcomes and alleviating the healthcare burden by providing a window for timely and personalized treatment strategies in individuals with or at risk of dementia.

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