Journal of Health and Rehabilitation Research 2791-156X JHRR

For contributions to JHRR, contact at email: editor@jhrlmc.com

Original Article

Guardians of Neurons: The CNTF Gene and Ciliary Neurotrophic Factor (CNTF) in the Genetic Landscape of Alzheimer ’s disease Neuroprotection

Pirya Nangdev[1], Shamshad Bano[2], Gulzar Fatima[3], Hina Ayub[4]*

[1]M.Phil (Anatomy), MBBS, Lecturer, Department of Anatomy, Bilawal Medical College, Liaquat University of Medical and Health Sciences (LUMHS) Jamshoro Sindh, Pakistan.

[2]MBBS M.Phil Anatomy, Department of Anatomy, Bilawal Medical College, Liaquat University of Medical and Health Sciences (LUMHS) Jamshoro Sindh, Pakistan.

[3]M.Phil in Molecular pathology and genomics, Kauser Abdullah Malik School of Life Sciences, Forman Christian College (A Chartered University) Lahore, Pakistan.

[4]BS Zoology, MS in Advance Convergence Science and Technology PhD*, Department of Advanced Convergence Technology and Science, Jeju National University South Korea

Corresponding Author: Hina Ayub; Email: hinarajpoot1112@gmail.com

Conflict of Interest: None.

Nangdev P., et al. (2024). 4(1): DOI: https://doi.org/10.61919/jhrr.v4i1.717

ABSTRACT

Background: Type II Diabetes Mellitus (Type II DM) is a complex systemic disorder that affects various organs and has been strongly associated with an increased risk of cognitive impairments, including dementia. The Ciliary Neurotrophic Factor (CNTF) gene is implicated in the pathophysiology of diabetes-induced cognitive decline, suggesting its potential as a biomarker for neurodegenerative diseases.

Objective: The primary objective of this study was to investigate the expression of the CNTF gene in diabetic patients with dementia and to evaluate its correlation with cognitive dysfunction.

Methods: A cohort of 88 diabetic dementia patients and 12 healthy controls was selected based on inclusion criteria. CNTF serum levels were measured using Chemiluminescent immunoassay (CLIA), and CNTF gene expression was assessed through real-time PCR. DNA was isolated using the QIAgen blood kit, followed by bisulfite DNA modification and Methylation Specific PCR for methylation profiling. Data analysis included comparative studies using GraphPad Prism 9.0 with statistical significance set at a p-value of <0.05.

Results: The study found that CNTF serum levels in diabetic dementia patients (n=88) were significantly lower at 4.98 µg/mL compared to 14.21 µg/mL in healthy controls (n=12). MMSE scores were also reduced in the patient cohort, averaging 10.98 versus 25.6 in controls. A notable decrease in CNTF gene expression was observed with a fold change of approximately 4.1 in diabetic dementia patients, indicating underexpression in this group.

Conclusion: CNTF gene expression is inversely correlated with cognitive function in diabetic patients, suggesting that CNTF could serve as a useful biomarker for the early detection of dementia in this population. The identification of this gene's role opens new avenues for targeted therapeutic interventions and enhances the understanding of dementia's etiology in diabetic patients.

Keywords: Type II Diabetes Mellitus, Dementia, Ciliary Neurotrophic Factor, CNTF Gene Expression, Cognitive Impairment, Biomarkers, Neurodegenerative Diseases, Chemiluminescent Immunoassay, Methylation Specific PCR, Gene Expression Profiling.

INTRODUCTION

Diabetes, recognized as the sixth leading cause of mortality globally, is a complex metabolic disorder associated with various microvascular and macrovascular complications (1). Among its forms, Type II Diabetes Mellitus (Type II DM) is the most prevalent, characterized by its link to mild cognitive declines that may escalate to severe forms of dementia, including Alzheimer's disease (AD), which accounts for approximately 70% of all dementia cases (2). The interplay of genetic variants, notably the ApoE ε4 allele, APP, and PSEN1/PSEN2, alongside factors such as depression, smoking, hypertension, and diabetes mellitus, emerges as significant contributors to the risk of AD (3). In this context, the CNTF gene, encoding the Sirtuin protein family, plays a crucial role due to its association with improved insulin sensitivity, a key factor in Type II DM development (4). CNTF's anti-inflammatory properties also suggest its potential in mitigating chronic inflammation, a commonality between diabetes and dementia, thereby possibly lowering

the risk for both conditions by enhancing cognitive functions (5). Diminished levels of CNTF have been correlated with increased Reactive Oxygen Species (ROS) production, accumulation of high glucose, Amyloid β, and Tau protein, alongside an elevated prevalence of obesity-linked cardiovascular diseases (6,7). Recent Genome-Wide Association Studies (GWAS) have identified the CNTF gene as a novel candidate linked with Alzheimer's dementia (8).

The neurophysiology of CNTF encompasses a spectrum of functions, including insulin sensitization, anti-inflammatory actions, angiogenesis, and vasodilation, crucially influencing brain functions such as energy homeostasis, hippocampal neurogenesis, and synaptic activity (9). It facilitates neurogenesis and synaptic plasticity, with a significant expression of AdipoR1 primarily in the hippocampus, impacting cognitive functions markedly. On the other hand, neuropathological perspectives reveal that reductions in CNTF levels or its signaling activity can exacerbate Alzheimer's disease progression and cognitive impairment (10). Specifically, decreased plasma levels of CNTF have been identified as a risk factor in women with Alzheimer's disease, although its neuroprotective effects on the disease are evident.

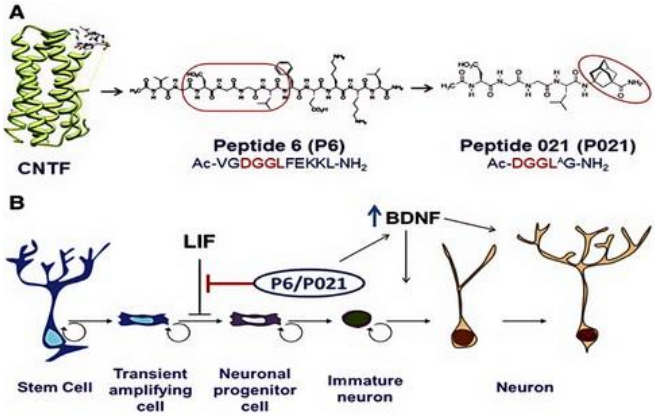

Figure 1 Design and structures of CNTF small-molecule mimetics and mechanism of action.

Furthermore, the structural explanation of CNTF, illustrated by the Protein Data Base rendering of a truncated human CNTF segment (Residues 2–187), highlights its functional intricacies. This depiction aids in understanding the design and function of CNTF small-molecule mimetics, such as the neurogenic 11-mer, Ac-VGDGGLFEKKL-NH2 (P6), and a neurogenic pentamer, Ac-DGGLAG-NH2 (P021), which promote the formation and maturation of neural progenitor cells (NPCs) and integration of new-born neurons by competitively inhibiting LIF signaling and increasing BDNF expression, respectively (11).

Thus, the CNTF gene's linkage to diabetes and cognitive impairments in diabetic individuals underscores the significance of hyperglycemia and insulin resistance in this context. This study aims to delve into the CNTF gene's role at the intersection of Type II diabetes and dementia, investigating its expression in diabetic patients with dementia and correlating it with neural disturbances as assessed by their Mini-Mental State Examination (MMSE) scores. This approach seeks to illuminate the complex interrelations between these conditions, contributing to a nuanced understanding of their interconnected pathologies.

MATERIAL AND METHODS

In adherence to the Declaration of Helsinki and following approval by the institutional review board, this study methodically collected 88 EDTA blood samples (5ml each) from patients diagnosed with diabetes and dementia, in addition to 12 healthy controls, at the diabetic clinics and Neurology Outpatient Departments of Jinnah Hospital Lahore and Shaikh Zayed Hospital Lahore. Ensuring ethical rigor, written informed consent was obtained from all participants, aligning with predefined inclusion and exclusion criteria. Specifically, the study targeted individuals over 40 years of age, encompassing both males and females, spanning healthy controls to confirmed cases of Type II DM and dementia. Exclusion criteria encompassed the absence of clinical history and diagnostic records, refusal to provide informed consent, or the presence of secondary diabetes.

Following their collection, samples were transported in accordance with standard protocols to partnered diagnostics labs for secure storage pending further analysis. Genomic DNA was isolated from peripheral blood using the QIAgen blood kit (QIAamp#56604), with its quality assured through methods including UV spectrophotometry, fluorometry, and gel electrophoresis. These assessments ensured the DNA's integrity, as indicated by optimal absorbance ratios (260/280 and 260/230) and the visual confirmation of intact bands suitable for PCR amplification.

The CNTF serum levels were quantitatively determined using a Chemiluminescent immunoassay (CLIA) based on an ELISA kit (IHUADPNKTC # IH0556), following the manufacturer's protocols. Additionally, the DNA underwent bisulfite modification using the ZYM bisulfite conversion kit (ZYM, D#5024) for methylation profiling of the CNTF gene at a specific CpG site. The subsequent Methylation-Specific PCR and restriction analysis were facilitated by primers designed via serial cloner, based on the consensus CDS sequence from the NCBI database. The specificity and universality of these primers were validated through primer-BLAST or BLASTn, ensuring precise amplification under defined PCR conditions.

The designed primer sequences targeted the −74 nt sequence of the CNTF gene, with the forward primer sequence being TGCTGGCCTAATAGAGTGGCA, and the reverse, CTCAGCGCCATGGAAAATGT. For the data analysis, GraphPad Prism 9.0 was utilized,

marking a departure from earlier mentions of SPSS version 25. This approach facilitated the plotting of demographic data and the frequencies of relative morbid conditions through bar charts, alongside performing expression analysis. To assess the variance among the samples, one-way ANOVA was employed, setting a threshold of statistical significance at a p-value less than 0.05. Study Approval Certificate (IRB 201/09-24) was issued by Supervisory committee.

RESULTS

The first figure presents a comparative analysis of CNTF levels and cognitive impairment assessment between healthy controls and diabetic dementia patients. In the healthy control group (n=12), the CNTF levels are indicated with a mean value of 14.21 µg/mL, while the mean MMSE (Mini-Mental State Examination) score is relatively high at 25.6, suggesting good cognitive function. In contrast, diabetic dementia patients (n=88) exhibit significantly lower CNTF levels with a mean of 4.98 µg/mL and MMSE scores with a mean of 10.98, illustrating a pronounced decline in cognitive function. The graphical representation clearly shows the inverse relationship between CNTF levels and cognitive impairment, with higher CNTF concentrations being associated with better cognitive outcomes.

Table 2: Demographical Summary of Confirmed Cases of Diabetic Dementia Patients (n=88)

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

Table 3: Biochemical Parameters of Healthy 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)	5.01 ± 0.56	9.01 ± 0.86	0.007*
HbA1c Levels (%)	4.31 ± 0.67	10.59 ± 1.62	0.044*
CNTF (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

The second figure delineates the expression analysis of the CNTF gene. Healthy individuals show a higher relative gene expression with a fold change of 11.6, compared to diabetic dementia patients who have a fold change of 6.7. This indicates that the expression of the CNTF gene is almost halved in diabetic dementia patients compared to healthy individuals. The data, presumably normalized against a control gene, underscore the diminished expression of the CNTF gene in the patient group.

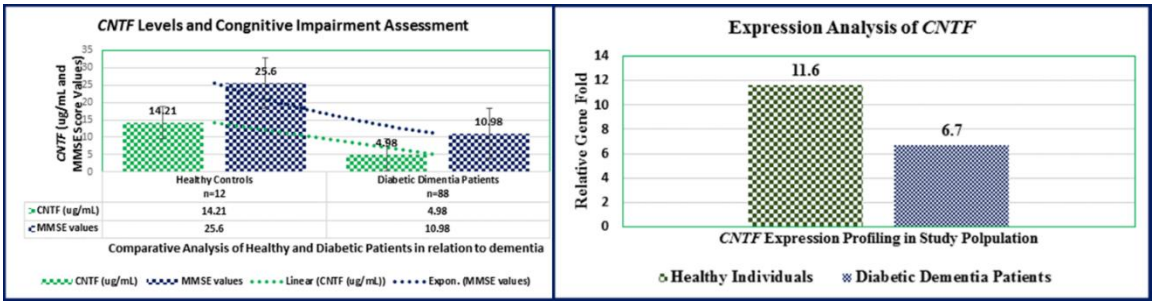

Figure 2 CNTF Assessment for Diabetic Dementia and MMSE Analysis for Cognitive Impairment, Fold change in healthy controls and diabetic dementia cases.

The results derived from these figures are crucial as they quantitatively outline the differences in CNTF serum levels and gene expression between healthy individuals and those suffering from diabetic dementia. These findings align with the demographic summary and biochemical parameters detailed earlier in Tables 2 and 3, where significant disparities in fasting glucose, HbA1c levels, CNTF concentrations, and MMSE scores were documented. The numerical values reinforce the potential link between lower CNTF levels and the severity of cognitive decline in diabetic dementia, highlighting the gene's potential role as a biomarker for the disease's progression and as a target for therapeutic intervention.

Journal of Health and Rehabilitation Research 2791-156X JHRR

DISCUSSION

Type II Diabetes Mellitus (Type II DM), a condition that affects multiple organ systems, has been found to precipitate numerous microvascular and macrovascular complications, significantly affecting neurovascular coupling and, consequently, cognitive functions, with dementia being a common outcome (12). Hyperglycemia and insulin resistance, both states associated with Type II DM, have been implicated in the increased risk of cognitive impairments. The focus of our investigation, the Ciliary Neurotrophic Factor (CNTF) gene, has been implicated in exacerbating these conditions, potentially accelerating the decline in mental capacity. This study's findings have echoed previous research, indicating that a dysfunctional expression of CNTF is associated with the progression of hyperglycemia and cognitive decline (13). Historical data from diabetic patients revealed HbA1c levels above 6.8%, indicative of poor glycemic control, which could be attributed to altered expression of the CNTF gene. This dysregulation of CNTF has been linked to the abnormal accumulation of amyloid precursor protein (AβPP) and increased amyloid beta (Aβ) levels, factors contributing to Alzheimer's disease pathology (14, 15). Our observations align with studies that report reduced CNTF expression in models of Alzheimer's disease, suggesting its contribution to the disease's pathology (16).

Moreover, the study uncovered a downregulation of CNTF in patients with dementia. An MMSE score below 10, denoting severe cognitive impairment, corresponded with a significant fold decrease in CNTF gene expression, ascertained through real-time PCR, which further highlighted the gene's underexpression in diabetic dementia cases. These results are consistent with previous findings, where a decreased activity of CNTF was reported in subjects with Alzheimer's compared to those without the disease (18). Dementia, particularly Alzheimer's disease, is characterized by a decrease in Brain-Derived Neurotrophic Factor (CNTF), leading to synaptic dysfunction and impaired neuroplasticity (19). This reduction in CNTF, along with a downregulation of its precursor, proCNTF, undermines neurotrophic support, emphasizing the potential role of peripheral indicators such as serum CNTF levels and gene expression in PBMCs as diagnostic markers (20).

The relationship between decreased serum CNTF levels and cognitive decline has underscored the importance of CNTF as a peripheral biomarker for dementia. Addressing CNTF dysregulation might offer a viable approach to mitigating cognitive deterioration and improving early detection and treatment strategies for dementia-related conditions. The findings from this study suggest that CNTF could serve as a biomarker for detecting neurodegenerative diseases, including dementia, in diabetic patients. Early detection could improve patient outcomes and provide a foundation for developing targeted treatments, thereby lessening the economic burden these diseases pose.

However, this study is not without limitations. The sample size, although adequate, represents a specific population and may not be generalizable. Future research should aim to include a broader demographic to substantiate the findings. Moreover, the study's observational nature can establish correlations but not causation. Further investigation is warranted to explore the mechanistic pathways of CNTF's role in cognitive impairment and diabetes. The study's strengths lie in its thorough analysis and the correlation it draws between systemic CNTF expression and central nervous system changes. These findings could guide future research aiming to use peripheral biomarkers to reflect central pathology, potentially opening new avenues for the diagnosis and treatment of dementia in diabetic patients.

CONCLUSION

This study substantiates the potential of the CNTF gene as a biomarker for the early detection of dementia in diabetic patients, underscoring its significance in improving patient outcomes and guiding targeted therapeutic strategies. The implications for human healthcare are considerable; early diagnosis could prolong the quality of life for affected individuals and alleviate the economic impact of neurodegenerative diseases on healthcare systems. These findings advocate for the integration of genetic screening in routine diabetes care, potentially facilitating early intervention and personalized medicine approaches in the management of dementia.

REFERENCES

1. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Phys Ther. 2008;88(11):1322-35.
2. Ehtewish H, Arredouani A, El-Agnaf O. Diagnostic, Prognostic, and Mechanistic Biomarkers of Diabetes Mellitus-Associated Cognitive Decline. Int J Mol Sci. 2022;23(11):6144.
3. Flores-Dorantes MT, Díaz-López YE, Gutiérrez-Aguilar R. Environment and Gene Association With Obesity and Their Impact on Neurodegenerative and Neurodevelopmental Diseases. Front Neurosci. 2020;14:863.
4. Taylor R. Insulin resistance and type 2 diabetes. Diabetes. 2012;61(4):778-9.

5. Rezende LF, Santos GJ, Santos-Silva JC, Carneiro EM, Boschero AC. Ciliary neurotrophic factor (CNTF) protects non-obese Swiss mice against type 2 diabetes by increasing beta cell mass and reducing insulin clearance. Diabetologia. 2012;55(5):1495-504.
6. Lemper M, De Groef S, Stangé G, Baeyens L, Heimberg H. A combination of cytokines EGF and CNTF protects the functional beta cell mass in mice with short-term hyperglycaemia. Diabetologia. 2016;59(9):1948-58.
7. Silva S, Nitsch D, Fatumo S. Genome-wide association studies on coronary artery disease: A systematic review and implications for populations of different ancestries. PLoS One. 2023;18(11):e0294341.
8. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. Immunity. 2022;55(1):31-55.
9. Kohlgruber A, Lynch L. Adipose tissue inflammation in the pathogenesis of type 2 diabetes. Curr Diab Rep. 2015;15(11):92.
10. Massaro AR, Soranzo C, Carnevale A. Cerebrospinal-fluid ciliary neurotrophic factor in neurological patients. Eur Neurol. 1997;37(4):243-6.
11. Kazim SF, Iqbal K. Neurotrophic factor small-molecule mimetics mediated neuroregeneration and synaptic repair: emerging therapeutic modality for Alzheimer's disease. Mol Neurodegener. 2016;11(1):50.
12. Spinelli M, Fusco S, Grassi C. Brain Insulin Resistance and Hippocampal Plasticity: Mechanisms and Biomarkers of Cognitive Decline. Front Neurosci. 2019;13:788.
13. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights. 2016;11:95-104.
14. Rajmohan R, Reddy PH. Amyloid-Beta and Phosphorylated Tau Accumulations Cause Abnormalities at Synapses of Alzheimer's disease Neurons. J Alzheimers Dis. 2017;57(4):975-99.
15. Jackson RJ, Rudinskiy N, Herrmann AG, Croft S, Kim JM, Petrova V, Ramos-Rodriguez JJ, Pitstick R, Wegmann S, Garcia-Alloza M, Carlson GA, Hyman BT, Spires-Jones TL. Human tau increases amyloid β plaque size but not amyloid β-mediated synapse loss in a novel mouse model of Alzheimer's disease. Eur J Neurosci. 2016;44(12):3056-66.
16. Jiao F, Jiang D, Li Y, Mei J, Wang Q, Li X. Amyloidogenesis and Neurotrophic Dysfunction in Alzheimer's Disease: Do They have a Common Regulating Pathway? Cells. 2022;11(20):3201.
17. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016;8(6):595-608.
18. Guo Q, Li H, Cole AL, Hur JY, Li Y, Zheng H. Modeling Alzheimer's disease in mouse without mutant protein overexpression: cooperative and independent effects of Aβ and tau. PLoS One. 2013;8(11):e80706.
19. Benussi L, Binetti G, Ghidoni R. Loss of Neuroprotective Factors in Neurodegenerative Dementias: The End or the Starting Point? Front Neurosci. 2017;11:672.
20. Arosio B, D'Addario C, Gussago C, Casati M, Tedone E, Ferri E, Nicolini P, Rossi PD, Maccarrone M, Mari D. Peripheral blood mononuclear cells as a laboratory to study dementia in the elderly. BioMed Res Int. 2014;2014:169203.