

Original Article

# Pathophysiology Crossroads: Examining the Role of Adiponectin (ADIPOQ) in the Intersection of Type II Diabetes and Dementia

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**Conflict of Interest: None.**

Shakir ZA., et al. (2024). 4(1): DOI: <https://doi.org/10.61919/jhrr.v4i1.582>

## ABSTRACT

**Background:** Type II Diabetes Mellitus (Type II DM) is intricately linked with an increased risk of cognitive impairment and dementia. The ADIPOQ gene encodes adiponectin, a hormone that plays a critical role in glucose regulation and fatty acid oxidation and may be implicated in the pathophysiology of both diabetes and dementia. Understanding the expression of ADIPOQ in diabetic patients with dementia could offer insights into the mechanisms at play and aid in the development of potential therapeutic targets.

**Objective:** This study aims to analyze the expression of the ADIPOQ gene in diabetic patients diagnosed with dementia and to correlate these findings with cognitive impairment as assessed by Mini-Mental State Examination (MMSE) scores.

**Methods:** We collected 5ml EDTA blood samples from 88 diabetic dementia patients and 12 healthy controls after obtaining written informed consent. The study adhered to the Declaration of Helsinki guidelines. Serum adiponectin levels were measured via Chemiluminescent immunoassay (CLIA), and genomic DNA was isolated for methylation-specific PCR and expression analysis. ADIPOQ gene expression was quantified using real-time PCR, and data were analyzed using SPSS version 25, employing descriptive and inferential statistics.

**Results:** The diabetic dementia patient group demonstrated significantly lower adiponectin levels ( $5.62 \pm 1.08 \mu\text{g/mL}$ ) and MMSE scores ( $10.68 \pm 4.56$ ) compared to healthy controls (adiponectin:  $15.68 \pm 4.64 \mu\text{g/mL}$ , MMSE:  $25.9 \pm 3.12$ ). Real-time PCR results indicated a relative fold decrease of 4.9 in ADIPOQ gene expression in the dementia cohort.

**Conclusion:** The ADIPOQ gene expression is inversely associated with cognitive function in diabetic patients, highlighting its potential as a biomarker for early detection and targeted therapeutic interventions in dementia associated with diabetes.

**Keywords:** ADIPOQ, Type II Diabetes Mellitus, Dementia, Cognitive Impairment, MMSE, Gene Expression, Biomarker, Neurodegenerative Diseases.

## INTRODUCTION

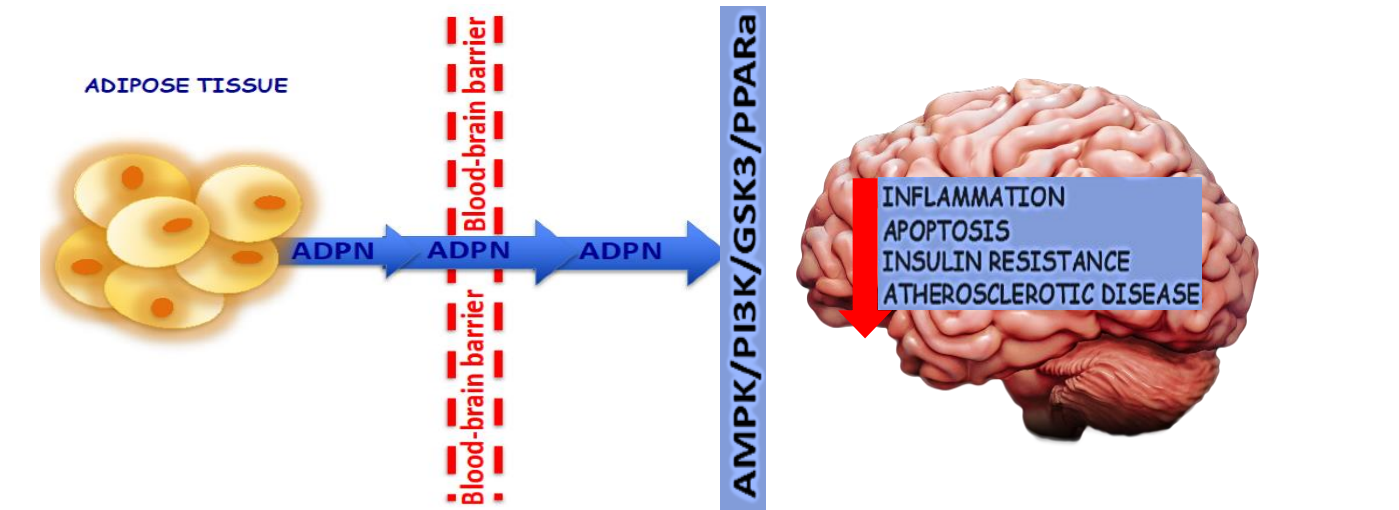
Diabetes Mellitus, particularly Type II Diabetes Mellitus (Type II DM), stands as the sixth leading cause of death globally, manifesting as a multifaceted metabolic disorder characterized by a spectrum of microvascular and macrovascular complications (1). Type II DM is predominantly associated with a range of cognitive impairments, extending from mild cognitive decrements to the severe cognitive dysfunction observed in dementia. Dementia itself encompasses a group of neurological disorders, with Alzheimer's disease (AD) being the most prevalent, accounting for approximately 70% of all dementia cases (2). The etiology of dementia and, consequently, AD, involves a complex interplay of genetic, lifestyle, and environmental factors, including the presence of genetic variants such as the ApoE  $\epsilon$ 4 allele, APP, PSEN1/PSEN2, alongside modifiable risk factors such as depression, smoking, hypertension, and notably, diabetes mellitus (3).

Within this intricate network of risk factors and pathophysiological mechanisms, the protein encoded by the ADIPOQ gene, adiponectin, emerges as a significant player. Adiponectin is renowned for its insulin-sensitizing properties, which play a pivotal role

in mitigating insulin resistance, a critical factor in the pathogenesis of Type II DM (4). Beyond its metabolic functions, adiponectin exerts anti-inflammatory effects, a property of paramount importance given the established link between chronic inflammation and the development of both diabetes and dementia. By attenuating inflammation, adiponectin not only contributes to reducing the risk of these conditions but also supports improved cognitive function, potentially lowering the risk of dementia in individuals, irrespective of their diabetic status (5). However, reduced levels of adiponectin have been correlated with increased generation of Reactive Oxygen Species (ROS), elevated glucose levels, and the accumulation of Amyloid  $\beta$  and Tau proteins, factors that exacerbate the risk of obesity-linked cardiovascular diseases and, by extension, cognitive impairments (6,7). Genome-wide association studies (GWAS) have shed light on several candidate genes implicated in Alzheimer's dementia, among which the ADIPOQ gene has been identified as a novel potential gene of interest (8).

Neurophysiology of Adiponectin (9)	Neuropathology of Adiponectin (10)
<ul style="list-style-type: none"><li>• Insulin-sensitizing, anti-inflammatory, angiogenic, and vasodilatory Properties</li><li>• Can cross the brain barrier and be detected in the cerebrospinal fluid.</li><li>• Controls important brain functions such as energy homeostasis, hippocampal neurogenesis and Synaptic Activity</li><li>• Controls neurogenesis and synaptic plasticity</li><li>• AdipoR1 is expressed primarily in the hippocampus.</li><li>• Significant effect on cognitive functions</li></ul>	<ul style="list-style-type: none"><li>• Reduction in the levels of adiponectin or reduction in the adiponectin signalling activity can promote the progression of Alzheimer's disease and cause cognitive impairment.</li><li>• Decreased plasma levels of adiponectin are a risk factor for women with Alzheimer's disease.</li><li>• May have a neuroprotective effect on Alzheimer's disease</li></ul>

The neurophysiological attributes of adiponectin underscore its significance in brain health. Despite not crossing the blood-brain barrier, adiponectin influences the secretion of pro-inflammatory cytokines such as interleukin-6 from brain endothelial cells, indicative of its peripheral actions (11). The presence of adiponectin receptors, AdipoR1 and AdipoR2, in critical brain regions such as the hypothalamus, hippocampus, and cortex, further elucidates its central role. AdipoR1 is implicated in regulating insulin sensitivity via the AMP-activated protein kinase (AMPK) pathway, whereas AdipoR2 is involved in enhancing neural plasticity through the peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ) pathway, which collectively contribute to adiponectin's neuroprotective effects by reducing inflammatory markers like C-reactive protein (CRP), interleukin 6 (IL6), and Tumor Necrosis Factor alpha (TNFa) (12,13).



**Figure 1.** Adiponectin (ADPN), a molecular neuroprotective target for dementia.

Given these multifaceted roles, adiponectin, particularly the ADIPOQ gene, emerges as a molecular nexus at the intersection of Type II diabetes and dementia. This gene's involvement not only links to the prevalence of diabetes but also to cognitive impairments in diabetic individuals, underlined by events such as hyperglycemia or insulin resistance. Our study is dedicated to exploring the ADIPOQ gene's role in the confluence of Type II diabetes and dementia, investigating its expression in diabetic patients suffering

from dementia and its correlation with neural disturbances as assessed by their Mini-Mental State Examination (MMSE) scores. Through this examination, we aim to unravel the complex interplay between metabolic dysfunctions and cognitive decline, shedding light on potential therapeutic targets for mitigating the impact of these conditions on patient health.

## MATERIAL AND METHODS

The study was meticulously designed to investigate the role of the ADIPOQ gene in patients suffering from Type II Diabetes Mellitus (Type II DM) with concurrent dementia, in comparison to a control group comprised of healthy individuals. The research protocol adhered strictly to the ethical standards of the Declaration of Helsinki and received approval from the institutional review boards of Jinnah Hospital Lahore and Shaikh Zayed Hospital Lahore. A total of 100 participants were enrolled in the study, including 88 patients diagnosed with diabetes and dementia and 12 healthy controls, following the acquisition of written informed consent. The sample collection involved the procurement of 5ml of EDTA blood from each participant, adhering to predefined inclusion and exclusion criteria. The inclusion criteria targeted individuals over the age of 40, of any gender, diagnosed with Type II DM and dementia for the patient group, and healthy controls. Exclusion criteria were set to omit individuals lacking a clinical history and diagnostic records, those refusing to provide informed consent, and cases of secondary diabetes.

Following collection, the blood samples were transported under standard protocols to designated diagnostic laboratories, with which memoranda of understanding (MOU) were in place, ensuring the samples' integrity until further processing.

The quantitative determination of serum adiponectin levels was performed using a Chemiluminescent immunoassay (CLIA) based on an ELISA kit (IHUADPNKTC # IH0515), following the manufacturer's instructions. Concurrently, genomic DNA was extracted from peripheral blood samples utilizing the QIAgen blood kit (QIAamp#56604), adhering strictly to the provided protocol. The integrity and quality of the isolated DNA were assessed through UV spectrophotometry, fluorometry, and gel electrophoresis, ensuring optimal absorbance ratios (260/280 and 260/230), visualization of intact bands, and confirmation of amplifiability via PCR, which are critical for reliable molecular analyses.

Further, the study delved into the epigenetic regulation of the ADIPOQ gene by conducting bisulfite DNA modification and Methylation Specific PCR. This process utilized the ZYM bisulphite conversion kit (ZYM, D#5024), treating 1.8-2µg of DNA with a specific mixture to prepare it for subsequent methylation profiling at a pre-determined CpG site located at the -74 nt sequence of the E-box. Amplification of bisulfite-treated DNA was executed on a Mastercycler instrument (Eppendorf) with defined PCR conditions to ensure the accuracy of methylation status assessment.

Primer design was an integral part of the methodology, with primers for the ADIPOQ gene being meticulously crafted to match the consensus CDS sequence from the NCBI database. The specificity and universality of these primers were validated through primer-BLAST or BLASTn, ensuring the precision of the PCR amplifications. The primers underwent optimization for their melting temperatures ( $T_m$ ) and amplicon properties using a gradient PCR thermocycler (Bio-Rad T100-Thermocycler, USA), facilitating optimal amplification conditions.

For data analysis, the study employed SPSS version 25, analyzing the gathered data through a combination of descriptive statistics and inferential analyses. Demographic data were illustrated via bar charts, while the frequencies of relative morbid conditions and expression analyses were also presented in a similar format. The statistical significance of differences among the groups was evaluated using one-way ANOVA, with a p-value threshold of  $>0.05$  considered indicative of significant variance.

This comprehensive approach, encompassing ethical considerations, meticulous sample collection and processing, advanced molecular techniques, and rigorous data analysis, ensured the robustness and reliability of the study's findings regarding the ADIPOQ gene's role in the intersecting pathologies of Type II DM and dementia.

**Table 1. Sequences of Designed Primers**

ADIPOQ Primers Pair	ADIPOQ sequence (5/ to 3/), -74 nt sequence
Froward	TGCCCCATCTTCTGTTGCTG
Reverse	AACTCGATGAGGGCCAGAGG

## RESULTS

In the demographic breakdown of the study population, which comprised 88 confirmed cases of diabetic dementia patients, a higher prevalence was observed in females, accounting for 59% of cases with an average age of 59.1 years (Table 1). The standard deviation for this group indicated less variability in age, at  $\pm 4.6$  years. The male participants represented 41% of the cases, with a slightly lower average age of 55.4 years, but a broader age range, as denoted by a higher standard deviation of  $\pm 8.5$  years (Table 1).

Biochemical assessments were conducted to compare healthy controls with diabetic dementia patients, involving a cohort of 100 participants (Table 2). Fasting glucose levels presented a stark contrast between the two groups; the healthy controls had an average fasting glucose level of 5.01 mmol/L with a standard deviation of  $\pm 0.56$ , while the diabetic dementia patients had a significantly elevated average of 9.01 mmol/L, and a standard deviation of  $\pm 0.86$ , a difference that was statistically significant with a p-value of 0.008. Hemoglobin A1c (HbA1c) levels further emphasized the disparity, standing at 4.31% ( $\pm 0.67$ ) for the controls, compared to 10.59% ( $\pm 1.62$ ) for the patient group, again with a significant p-value of 0.049.

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

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

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)	5.01 $\pm$ 0.56	9.01 $\pm$ 0.86	0.008*
HbA1c Levels (%)	4.31 $\pm$ 0.67	10.59 $\pm$ 1.62	0.049*
Adiponectin ( $\mu$ g/mL)	13.37 $\pm$ 4.64	5.37 $\pm$ 1.08	0.016*
MMSE Scores	24.1 $\pm$ 3.12	12.26 $\pm$ 4.56	0.007*

Adiponectin levels were also markedly different between the two cohorts. The healthy controls' mean adiponectin concentration was recorded at 13.37  $\mu$ g/mL ( $\pm 4.64$ ), more than double the 5.37  $\mu$ g/mL ( $\pm 1.08$ ) observed in the diabetic dementia group, with the difference being statistically significant (p=0.016). The impact of these biochemical differences on cognitive function was evident in the Mini-Mental State Examination (MMSE) scores, where the control group had an average score of 24.1 ( $\pm 3.12$ ), indicative of normal cognitive function, contrasting with the diabetic dementia patients' average of 12.26 ( $\pm 4.56$ ), which not only underscores cognitive impairment but also aligns with a statistically significant p-value of 0.007 (Table 2).

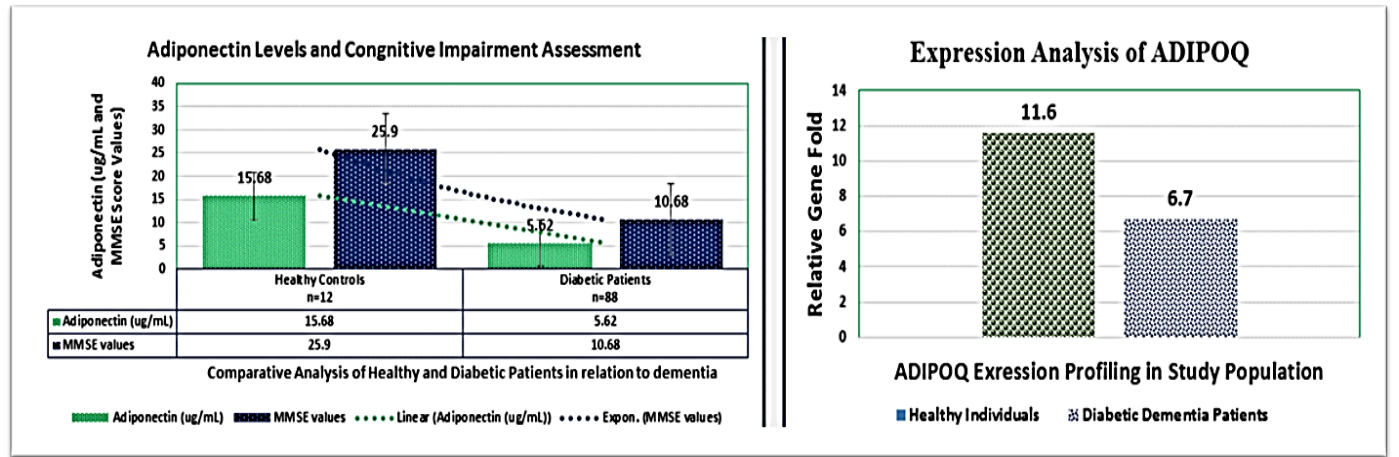


Figure 1 ADIPOQ and Adiponectin Levels

DISCUSSION

In the exploration of the intersection between Type II Diabetes Mellitus (Type II DM) and cognitive impairment, our study centered on the ADIPOQ gene due to its proposed influence on metabolic and cognitive pathways. Type II DM, a condition known to precipitate a range of systemic complications, is also implicated in neurovascular alterations that predispose individuals to cognitive deficits, prominently dementia (14). The hyperglycemic state and insulin resistance characteristic of diabetes are believed to exacerbate these cognitive challenges. The role of ADIPOQ in this context is multifaceted, as our findings suggest that its dysregulated expression may exacerbate hyperglycemia and cognitive impairment. Patients in our cohort with a recorded HbA1c level above 6.8% signified poor glycemic control, which was concomitant with altered ADIPOQ gene expression, potentially contributing to this dysregulation (15).

Our results parallel previous studies where an underexpression of ADIPOQ was correlated with increased amyloid beta metabolism, hinting at a mechanism whereby a decrease in ADIPOQ protein may foster Alzheimer's disease-like pathology (17). This was further

corroborated by research suggesting that altered ADIPOQ gene expression affects the trafficking and accumulation of A $\beta$ PP, a precursor protein to amyloid-beta, thereby impacting amyloidogenesis and the formation of amyloid plaques, a hallmark of Alzheimer's disease (16,19). Furthermore, the neuroprotective potential of ADIPOQ was highlighted, albeit the high activity of the gene could paradoxically contribute to amyloidogenesis (18).

Our study also reaffirms the role of AdipoR1 and AdipoR2 receptors in mediating the actions of adiponectin, as evidenced by siRNA studies and observations in AdipoR1/AdipoR2 double-knockout mice, which underscore the indispensability of these receptors (20). A diminished expression of ADIPOQ in our subjects with dementia, coupled with MMSE scores indicative of severe cognitive decline, further reinforces the gene's putative role in neurodegeneration. The relative fold change, determined by real-time PCR, showed a decrease in the dementia group, mirroring the findings of other studies reporting reduced ADIPOQ activity in Alzheimer's disease subjects compared to non-AD individuals (21,22).

Reflecting on the strengths of our investigation, the study underscores the potential of the ADIPOQ gene as a biomarker for early detection of neurodegenerative diseases in diabetic patients. However, the limitations include the modest sample size and the inherent complexity of interpreting gene expression data in the context of multifactorial diseases like dementia. Additionally, our study was cross-sectional, which precludes the establishment of a causal relationship between ADIPOQ gene expression and cognitive decline.

Recommendations for future research include longitudinal studies to ascertain the temporal relationship between ADIPOQ expression and cognitive changes in diabetes, and the expansion of sample size to enhance the generalizability of findings. The exploration of the therapeutic modulation of ADIPOQ expression or function could also hold promise for mitigating the trajectory of dementia in diabetic individuals.

## CONCLUSION

In conclusion, the study presented ADIPOQ as a significant biomarker for dementia in patients with diabetes, offering a potential avenue for early diagnosis and the development of targeted therapies. Such advancements could not only improve patient outcomes but also alleviate the economic burden of these chronic diseases.

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