Original Article

Tumor Necrosis Factor-alpha (TNF-α) Expression and Role of Cytokines in Type 2 Diabetes Mellitus

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ABSTRACT

Objective: The basic aim of this study is to find the TNF-α expression and role of different cytokines in type 2 diabetes mellitus patients.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the CMH Rawalakot from June 2025 to August 2025.

Methods: Patients diagnosed with diabetes and having age >18 years were included in the study. Patients with Type 1 diabetes mellitus and individuals with malignancies or autoimmune diseases were excluded. Plasma levels of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β, were measured using enzyme-linked immunosorbent assay (ELISA) kits.

Results: Data were collected from 550 participants in this study. Mean age of participants was 58.6 ± 10.2 year and mean BMI was 29.4 ± 5.6 kg/m^2. Mean duration of DM in participants was 8.5 ± 5.3 years. Patients in the uncontrolled diabetes group had higher fasting plasma glucose levels (168.9 ± 38.2 mg/dL vs. 142.6 ± 28.9 mg/dL, $p < 0.001$) and higher BMI (31.5 ± 6.2 kg/m² vs. 28.1 ± 4.9 kg/m², $p < 0.001$). TNF-α gene expression was moderately correlated with HbA1c levels ($r = 0.42$, $p < 0.001$), indicating that higher TNF-α expression is associated with poorer glycemic control. There was also a positive correlation with BMI ($r = 0.35$, $p < 0.001$), suggesting a link between increased TNF-α expression and higher body mass index.

Conclusion: Elevated TNF-α gene expression and pro-inflammatory cytokine levels are prominent features of Type 2 Diabetes Mellitus (T2DM), correlating with glycemic control and metabolic dysfunction.

Key Words: TNF-α, T2DM, Glycemic control, Pro-inflammatory, BMI, Correlation

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by insulin resistance, impaired insulin secretion, and chronic inflammation. One of the critical molecular players implicated in the pathogenesis of T2DM is Tumor Necrosis Factor-alpha (TNF-α), a pro-inflammatory cytokine[1]. The following are the factors that have been associated with T2DM development; Genetic and environmental factors are the main causes of T2DM and the condition is also associated with obesity and being overweight[2].

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Obesity prompts adipose tissue hypertrophy and shifts in stromovascular cell population to solve pro-inflammatory state to foster the interactions between adaptive cells and adipose tissue macrophage to shift their activation status[3]. It is also proposed that the inflammatory and immune-mediated T2DM due to cytokines like IL-6, TNF-a, IL-1, IL-10, and TGF-ß are another factor causing DM. Also, genetic variation in specific genes of pro-inflammatory and anti-inflammatory cytokines such as TNF-α and IL-10 is being reported as one of the most common risk factor for diabetic population[4].

Interleukin 1β (IL-1β), Interleukin 6 (IL-6), and Tumor Necrosis Factor alpha (TNF-α), are frequently associated with the development of Type-2 DM[5]. In particular, it has been described that the rise in IL-1β is capable of enhancing the killing of pancreatic β-cells (Banerjee and Saxena 2012 Additionally, TNF-α and IL-6 can induce insulin resistance in peripheral tissues by altering the expression of insulin signaling pathway components. IL-10 has been described as having potent and pleiotropic anti-inflammatory effects, including the down regulation of proinflammatory cytokines and APCs, stimulation of tissue repair mechanisms, and regulation of excessive inflammation[6]. IL-10 is known to have multiple effects in the immune system, and

thus, is an important modulator of the balance that is required to allow the body to protect itself from infection[7]. This prevents the expression of several inflammatory cytokines while having a positive effect on the proliferation of B-lymphocytes and the prevention of cell growth and apoptosis[8]. Within this context, IL-10 provides broad and manifold anti-inflammatory actions, including the suppression of pro-inflammatory cytokines and antigen presenting cells, the support of tissue repair mechanisms and it is crucial in the regulation of inflammation. However, the mechanism of IL-10 in preventing β-cell damage and in the development of T1DM remains a point of debate[9]. In the context of T2DM, pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and TNF-α are upregulated, leading to a chronic low-grade inflammatory state. This inflammation is primarily driven by adipose tissue macrophages, which increase in number and secrete higher levels of cytokines in obese individuals, a common condition associated with T2DM[10].

METHODS

This study was a cross-sectional study conducted at CMH Rawalakot from June to August 2025, included total 550 participants suffering from T2DM. Patients diagnosed with diabetes and having age >18 years were included in the study. patients with Type 1 diabetes mellitus, pregnant or lactating women, and individuals with malignancies or autoimmune diseases. These criteria ensured a homogeneous study population representative of the typical T2DM patient profile. Blood samples were collected and approximately 10 mL of venous blood was drawn into EDTA tubes. These samples were used for both plasma and peripheral blood mononuclear cells (PBMC) isolation.

RNA Extraction and TNF-α Gene Expression Analysis: PBMCs collected from the subjects were then subjected to the Total RNA extraction using TRIzol reagent as per the described protocols to obtain the highest yield and quality of RNA. The extracted RNA was used to synthesize cDNA through the reverse transcription process using a commercial reverse transcription kit, thereby preparing the sample for an analysis of its quantities. They wanted to ascertain the expression levels of the TNF-α gene thus quantitative Real-Time PCR (qRT-PCR) was used on tissues samples and mouse ears, utilizing specific primers for TNF-α gene while GAPDH was used as the reference gene.

Cytokine Measurement: Enzyme-linked immunosorbent assay (ELISA) kits were used to measure plasma concentrations of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β. ELISA provided sensitive and specific quantification of these cytokines, here, the test was done as directed by the manufacturer to enable quantifications that could be easily tabulated.

Clinical and Biochemical Assessments: Participants underwent a comprehensive clinical evaluation, which included measuring body mass index (BMI) and blood pressure. Fasting plasma glucose (FPG) and HbA1c levels were assessed to evaluate glycemic control. Lipid profiles, including total cholesterol, LDL-C, HDL-C, and triglycerides, were measured to assess cardiovascular risk factors. These clinical and biochemical assessments provided a detailed characterization of the study population, allowing for a thorough analysis of the relationships between metabolic parameters and inflammatory markers.

Statistical Analysis: Data analysis was performed using SPSS v29 software. Comparative analyses of TNF-α expression and cytokine levels between different subgroups versus uncontrolled diabetes, were conducted using t-tests.

RESULTS

Data were collected from 550 participants in this study. Mean age of participants was 58.6 ± 10.2 year and mean BMI was 29.4 ± 5.6 kg/m^2. Mean duration of DM in participants was 8.5 ± 5.3 years. Patients in the uncontrolled diabetes group had higher fasting plasma glucose levels (168.9 ± 38.2 mg/dL vs. 142.6 ± 28.9 mg/dL, $p < 0.001$) and higher BMI (31.5 ± 6.2 kg/m² vs. 28.1 ± 4.9 kg/m², $p < 0.001$). Additionally, the uncontrolled group exhibited elevated total cholesterol (200.5 ± 35.7 mg/dL vs. 185.2 ± 26.3 mg/dL, $p = 0.012$), LDL cholesterol (125.8 ± 30.6 mg/dL vs. 110.6 ± 20.5 mg/dL, $p = 0.008$), and triglycerides (198.3 ± 55.6 mg/dL vs. 165.4 ± 48.9 mg/dL, $p < 0.001$), along with lower HDL cholesterol levels (40.4 ± 6.5 mg/dL vs. 45.7 ± 7.2 mg/dL, $p < 0.001$).

Table No.1: Comparison of clinical parameters between controlled and uncontrolled DM

Clinical Parameter	Controlled Diabetes (HbA1c < 7%)	Uncontrolled Diabetes (HbA1c ≥ 7%)	p-value
Fasting Plasma Glucose (mg/dL)	142.6 ± 28.9	168.9 ± 38.2	<0.001
BMI (kg/m²)	28.1 ± 4.9	31.5 ± 6.2	<0.001
Total Cholesterol (mg/dL)	185.2 ± 26.3	200.5 ± 35.7	0.012
LDL Cholesterol (mg/dL)	110.6 ± 20.5	125.8 ± 30.6	0.008
HDL Cholesterol (mg/dL)	45.7 ± 7.2	40.4 ± 6.5	<0.001
Triglycerides (mg/dL)	165.4 ± 48.9	198.3 ± 55.6	<0.001

In T2DM patients, the relative expression of the TNF-α gene was markedly higher (2.5 ± 1.3) compared to the control group (1.0 ± 0.5). Plasma TNF-α levels were also significantly elevated in T2DM patients (15.4 ± 5.2 pg/mL) versus controls (7.8 ± 2.1 pg/mL). Additionally, pro-inflammatory cytokines IL-6 and IL-1β were substantially higher in the T2DM cohort, with IL-6 levels at 12.6 ± 4.8 pg/mL compared to 4.2 ± 1.5 pg/mL in controls, and IL-1β levels at 8.3 ± 3.2 pg/mL versus 2.9 ± 1.1 pg/mL in the control group.

Table No.2: TNF- α expression and cytokine levels

Parameter	T2DM Patients (n=550)	Control Group (n=100)
TNF-α Gene Expression (Relative to Control)	2.5 ± 1.3	1.0 ± 0.5
TNF-α (pg/mL)	15.4 ± 5.2	7.8 ± 2.1
IL-6 (pg/mL)	12.6 ± 4.8	4.2 ± 1.5
IL-1β (pg/mL)	8.3 ± 3.2	2.9 ± 1.1

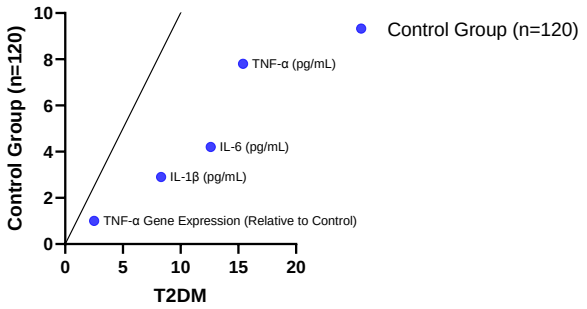

Figure No.1: Control Groups

TNF-α gene expression was moderately correlated with HbA1c levels ($r = 0.42$, $p < 0.001$), indicating that higher TNF-α expression is associated with poorer glycemic control. There was also a positive correlation with BMI ($r = 0.35$, $p < 0.001$), suggesting a link between increased TNF-α expression and higher body mass index. Additionally, TNF-α expression correlated with fasting plasma glucose levels ($r = 0.39$, $p < 0.001$).

Table No.3: Correlation between TNF- α and clinical parameters in DM

Clinical Parameter	Correlation (r)	p-value
HbA1c (%)	0.42	<0.001
BMI (kg/m²)	0.35	<0.001
Fasting Plasma Glucose (mg/dL)	0.39	<0.001

TNF-α gene expression was strongly correlated with IL-6 levels ($r = 0.58$, $p < 0.001$), indicating a robust association between these two inflammatory markers. Additionally, there was a moderate positive correlation with IL-1β levels ($r = 0.47$, $p < 0.001$).

Table No. 4: Correlation between TNF- α and cytokine levels

Cytokine	Correlation with TNF-α Expression (r)	p-value
IL-6 (pg/mL)	0.58	<0.001
IL-1β (pg/mL)	0.47	<0.001

Table No. 5: Comparative analysis of controlled vs uncontrolled DM

Parameter	Controlled Diabetes (HbA1c < 7%)	Uncontrolled Diabetes (HbA1c ≥ 7%)
TNF-α Gene Expression (Relative to Control)	1.7 ± 0.8	3.1 ± 1.4
TNF-α (pg/mL)	12.3 ± 3.6	17.2 ± 5.8

DISCUSSION

The result of this research confirms increased TNF-α gene expression and increased pro-inflammatory cytokines concentrations in T2DM patients such as TNF-alpha, IL-6, and IL-beta. These findings agree with studies performed earlier where inflammation was identified as playing a critical step in the development of T2DM[11]. The COI observation and HI and LO investigation revealed that the TNF-α gene participates in the development of chronic low-grade inflammation in T2DM by increasing its expression and causing insulin resistance and metabolic disorder[12]. The Concierge Strategy of TNF-α was related to the severity and progression of T2DM by revealing the positive relationships of TNF-α expression with such clinical determinants. TNF-α was directly associated with HbA1c, more heightened inflammation exacerbating glycemic control[13]. Moreover, TNF-α levels also showed a significant positive correlation with BMI and fasting plasma glucose supporting inflammation relation with metabolic deficits in T2DM. Baseline comparison of both control and uncontrolled diabetes stated higher level of TNF- α gene expression and plasma concentration in uncontrolled diabetes[14]. This brings to light that spontaneous secretion of TNF-α might play a role in the compromised glycemic management of T2DM patients. Further, the compliance between TNF-α expression and the HbA1c score highlighted TNF-α's application for informing disease progression and therapeutic outcomes in T2DM[15]. This clearly points towards the fact that there appears to be a link between TNF-α and insulin resistance, which means that targeting the cytokine and other inflammatory cytokines has the potential to provide beneficial results in the treatment of T2DM patients[16]. Inflammation is a modifiable factor, and strategies that seek to modulate inflammation including anti-TNF-α therapies or diet and exercise interventions to reverse adipose tissue inflammation could be

valuable approaches to enhance glycaemia and diminish the threat of diabetes complications[17,18]. However, several limitations of this study are, despite the fact that this study has highlighted the role of TNF-α and cytokines in T2DM, there is still a possibility that the findings of this study could be biased. Also, the present study only investigated the role of one cytokine pathway, therefore, more experimental studies should be done in the context of understanding the cross talk between TNF-α and other inflammatory markers in T2DM aetiopathogenesis.

CONCLUSION

It is concluded that elevated TNF-α gene expression and pro-inflammatory cytokine levels are prominent features of Type 2 Diabetes Mellitus (T2DM), correlating with glycemic control and metabolic dysfunction. These findings highlight the critical role of inflammation in T2DM pathogenesis and highlight TNF-α as a potential biomarker for disease severity and therapeutic target. Targeting inflammatory pathways, including TNF-α, may offer promising avenues for improving treatment outcomes and reducing the burden of T2DM-related complications.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Adnan Jehangir, Farhana Ayub
Drafting or Revising Critically:	Adnan Jehangir, Ayesha Almas
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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