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EVALUATION OF INFLAMMATORY BIOMARKERS (CRP, IL-6, TNF-A) AND NF-KB EXPRESSION IN INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT
Type 2 diabetes mellitus (T2DM) has emerged as a complex disorder encompasses both metabolic impairments and sustained inflammatory processes. This study measured the concentrations of inflammatory proteins in the bloodstream, such as CRP, IL-6, and TNF-α, along transcriptional activity of NF-κB, in 40 patients diagnosed with T2DM and 20 healthy matched controls. Blood samples were processed for both biochemical and molecular assessments using immunoturbidimetry, ELISA, and qRT-PCR. NF-κB expression and inflammatory marker levels were found to be considerably greater in diabetes individuals. underscoring the central role of inflammation in the disease's progression. These findings support the inclusion of inflammatory profiling as part of T2DM management and may inform future anti-inflammatory therapeutic strategies.

KEYWORDS: Type 2 Diabetes Mellitus, Inflammatory markers, TNF-alpha, IL-6, NF-κB

1. INTRODUCTION

The chronic and persistent ailment known as type 2 diabetes mellitus, or T2DM, is characterized by persistently high blood glucose levels, insulin resistance and gradual β-cell decline. Although traditionally viewed as a metabolic disorder, an increasing body of evidence has revealed a strong immunological dimension that plays a central role in its onset and progression [3,10].

One of the majors signaling mechanisms implicated in the inflammatory aspect of T2DM is the nuclear factor kappa B (NF-κB) pathway. The NF-κB signaling cascade controls transcription of several inflammation-related cytokines including IL-6 and TNF-α and tumor necrosis factor-alpha (TNF-α), which are known to disrupt insulin signaling and contribute to tissue inflammation [1].
C-reactive protein (CRP), another well-established inflammatory biomarker, is synthesized by hepatic cells following stimulation with IL-6, is also linked to elevated cardiovascular disease risk in diabetic individuals [11]. The combined elevation of CRP, IL-6, and TNF-α not only reflects systemic inflammation but also offers potential value for risk stratification and disease monitoring[7] .
by detecting blood levels of these inflammatory indicators and evaluating NF-κB expression in diabetic patients relative to healthy controls, the existing study attempts to explore the association between these markers and type 2 diabetes. This approach seeks to provide further insight into the inflammatory processes that accompany metabolic dysfunction in diabetes to uncover molecular factors that can guide therapeutic interventions.

2. MATERIALS AND METHODS

study design included 40 participants in a cross-sectional format, including 20 individuals previously diagnosed with type 2 diabetes mellitus and 20 healthy controls matched by age and sex. prior to sample collection, all subjects gave their informed permission, and the institutional review board gave its ethical approval.
Venous blood samples were collected using standard aseptic techniques. after centrifugation, the serum was stored at -20°C until it became required for immunological and biochemical testing. the concentrations of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) were quantifiedA high-sensitivity immunoturbidimetric technique was employed to measure CRP, and ELISA kits were utilized in accordance with manufacturer guidelines to detect TNF-α and IL-6 [13].
Each sample was processed in duplicate, and values were reported as mean ± standard deviation. Peripheral blood mononuclear cells (PBMCs) were purified from ethylenediamine tetra acetic acid (EDTA) blood using a density gradient method. RNA samples were extracted using TRIzol reagent, and complementary DNA (cDNA) synthesis

was achieved via reverse transcription [2]. Using GAPDH as the internal reference gene, real-time PCR (qRT-PCR) of NF-κB was used for evaluating target gene expression [8].
Gene expression differences were determined by applying the comparative Ct method (2^−ΔΔCt), the data was statistically analyzed using SPSS version 25. The groups were analyzed using independent sample t-tests, with a p-value threshold of less than 0.05 considered statistically significant.

3. RESULTS AND DISCUSSION

The findings indicated a marked increase in serum concentrations of inflammatory markers—CRP, IL-6, and TNF-α—with significant differences ($p < 0.05$) observed between individuals with type 2 diabetes mellitus (T2DM) and the control group [4,9]
The elevated CRP values in diabetic patients point to a chronic systemic inflammatory state that may contribute to metabolic irregularities.
Likewise, IL-6 and TNF-α levels were notably increased in the diabetic population, reflecting a sustained imbalance in immune system function These pro-inflammatory cytokines have been connected to both insulin resistance and β-cell dysfunction, and they are known to disrupt insulin signaling [5]. The current findings support previous evidence that inflammation interferes with glucose metabolism.
Peripheral blood mononuclear cells (PBMCs) from diabetics showed a substantial increase of NF κB gene expression on the genetic level ($p < 0.01$), highlighting the pathway's role in inflammation in type 2 diabetes. As a master regulator, NF-κB promotes a pro-inflammatory loop that may worsen metabolic degradation through raising the construction of TNF-α and IL-6 [14,15].
The concurrent increase in cytokine levels and their upstream regulator reinforces the notion that T2DM involves not only metabolic dysfunction but also immunological activation. These findings highlight the therapeutic potential of targeting inflammatory mediators—especially NF-κB—as a strategy in diabetes care [6,12].

4. CONCLUSION

This study validates an important rise in inflammatory biomarkers, including CRP, IL-6, and TNF-α, among individuals suffering from type 2 diabetes mellitus compared to healthy persons. These findings strengthen existing evidence that links low-grade chronic inflammation to the progression of T2DM beyond its metabolic characteristics.
In particular, the upregulation of NF-κB expression highlights its critical function in orchestrating inflammatory signaling, notably by regulating cytokine gene activity. This suggests that NF-κB holds promise both as a diagnostic marker and as a potential point of therapeutic intervention in managing diabetes.
Incorporating both serum cytokine measurements and molecular-level gene expression profiling into clinical assessments could improve the precision of diagnosis and guide the design of personalized anti-inflammatory treatment strategies aimed at minimizing inflammation-related complications in diabetic populations.

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Conflicts of Interest: The authors have no relevant financial or non-financial interests to disclose

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Table 1: Preparation Scheme for Blank, Standard, and Test Samples in Serum Glucose Determination via Colorimetric Method

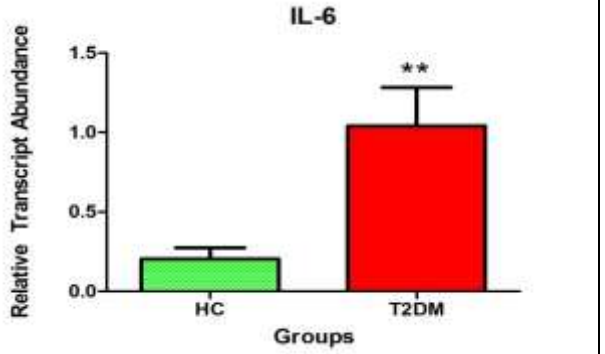
Sequence of addition	Blank (ml)	Standard (ml)	Test (ml)
Glucose reagent	1.0	1.0	1.0
Distilled water	0.01	-	-
Glucose standard	-	0.01	-
Sample	-	-	0.01

Table 2: Enrolment of healthy and diabetic subjects in the study

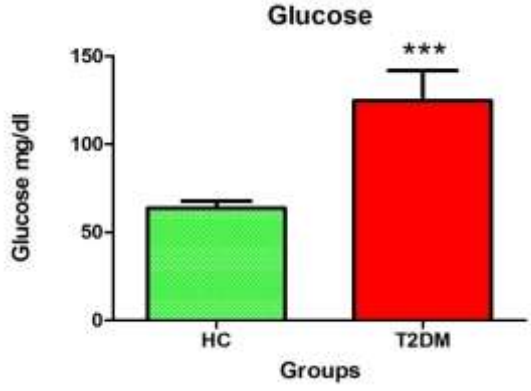
	Diabetic Subjects (n=20)		**Healthy Subjects (n=20)**	
Age	**Male (n=10)**	**Female (n=10)**	**Male (n=10)**	**Female (n=10)**
< 50 years	6	5	5	4
> 50 years	4	5	5	6

Table 3. Primer sequences for selected genes used in qRT-PCR.

SrNo.	Primer name	Sense	Sequence (5'----3')
Housekeeping gene			
1.	Glyceraldehyde-3-phosphate dehydrogenase	Forward	AGTTCAACGGCACAGTCAAG
		Reverse	TACTCAGCACCAGCATCACC
Transcription factor			
1.	Nuclear Factor-κβ (NFkβ)	Forward	AAAAACGAGCCTAGAGATTG
		Reverse	ACATCCTCTTCCTTGTCTTC
Inflammatory markers			
1.	Tumor Necrosis Factors-α (TNF-α)	Forward	CTCACACTCAGATCATCTTC
		Reverse	GAGAACCTGGGAGTAGATAAG
2.	Interleukin- 6 (IL-6)	Forward	CAGAGTCATTCAGAGCAATAC
		Reverse	CTTTCAAGATGAGTTGGATGG



(**c**)

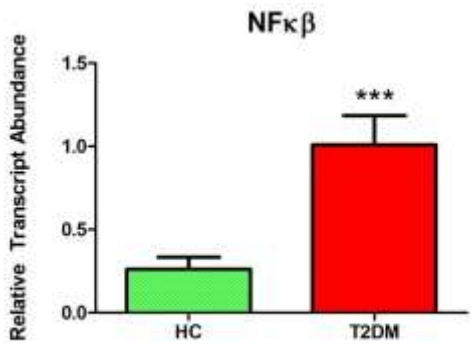


(**d**)

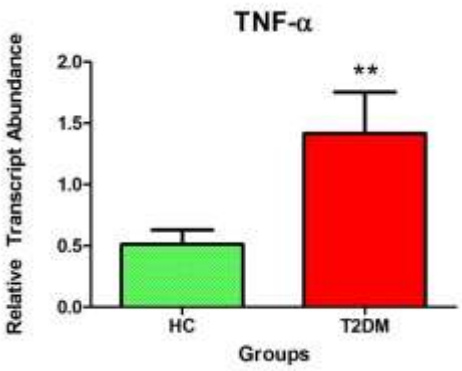
Figure 2.

Table 4: Primers for the selected genes

(a) The values represent as mean ± SE (n=20 for each group and reactions were performed in duplicate).
**P≤0.01 as compared to healthy individuals (unpaired t-test).
HC: Healthy control, T2DM: Type 2 diabetes mellitus
(b) The values represent as mean ± SE (n=20 for each group and biochemical estimations were performed in triplicates).
***P≤0.001 as compared to healthy individuals (unpaired t-test)
HC: Healthy control, T2DM: Type 2 diabetes mellitus***P≤0.001 as compared to healthy individuals (unpaired t-test)
HC: Healthy control, T2DM:
Type 2 diabetes mellitus



(a)



(b)

Figure 2.
(a) The values represent as mean ± SE (n=20 for each group and reactions were performed in duplicate)
***P≤0.001 as compared to healthy individuals (unpaired t-test). HC: Healthy control, T2DM: Type 2 diabetes mellitus

(b) The values represent as mean ± SE (n=20 for each group and reactions were performed in duplicate).
**P≤0.01 as compared to healthy individuals (unpaired t-test). HC: Healthy control, T2DM: Type 2 diabetes mellitus