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Evaluation of Serum Toll-Like Receptor 9 (TLR9) Levels Among Iraqi Patients with Type 2 Diabetes Mellitus

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Abstract: This study aimed to evaluate serum levels of Toll-like receptor 9 (TLR9) in Iraqi patients with type 2 diabetes mellitus (T2DM) and to compare them with healthy controls. A total of 45 T2DM patients and 35 healthy individuals were enrolled. Serum TLR9 levels were measured using the enzyme-linked immunosorbent assay (ELISA) technique.

The mean TLR9 concentration was significantly lower in T2DM patients (173.75 ± 8.52 ng/mL) compared to healthy controls (270.79 ± 20.61 ng/mL) ($P < 0.00001$). When analyzed by gender, male diabetic patients had higher levels than females (189.09 ± 11.45 vs. 159.09 ± 12.03 ng/mL, $P = 0.0391$). However, both sexes in the diabetic group had significantly lower levels compared to the healthy group. No significant difference was observed between males and females in the control group.

These findings suggest that reduced serum TLR9 levels are associated with T2DM and may reflect an immune-inflammatory imbalance that contributes to disease pathogenesis.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic, multifactorial metabolic disease characterized by persistent hyperglycemia caused by insulin resistance and/or reduced insulin secretion. It has become one of the most prevalent global health challenges, currently affecting more than 500 million people worldwide (Saeedi et al., 2019).

Emerging evidence suggests that chronic low-grade inflammation plays a pivotal role in T2DM progression. Toll-like receptors (TLRs), especially TLR9, are essential components of the innate immune system. TLR9 recognizes unmethylated CpG DNA motifs found in bacterial and viral genomes, activating downstream signaling pathways that lead to the production of pro-inflammatory cytokines (Hemmi et al., 2000).

Given the role of inflammation in the pathogenesis of T2DM, this study focuses on evaluating serum levels of TLR9 in diabetic patients and healthy individuals to assess its potential relevance as a biomarker.

Materials and Methods

This case-control study was conducted at the Department of Biology, College of Education for Pure Sciences, University of Wasit, from July 1 to October 30, 2024.

Participants

- **T2DM group:** 45 patients (24 males, 21 females) aged 40–78 years (mean ± SD = 57.38 ± 7.67).
- **Control group:** 35 healthy individuals (15 males, 20 females), age-matched with the T2DM group.

Participants were recruited from Al-Zahraa General Hospital and private clinics in Wasit Province, Iraq. All patients were diagnosed according to international diabetes diagnostic criteria.

Sample Collection and Processing

Five milliliters of venous blood were collected in plain tubes. After clotting, samples were centrifuged at 3000 rpm for 15 minutes. The separated sera were stored at −20°C in Eppendorf tubes until analysis.

TLR9 Measurement

Serum TLR9 levels were quantified using a human-specific ELISA kit (Bioassay Technology Laboratory), following the manufacturer's instructions.

Results

Serum TLR9 Levels in T2DM Patients vs. Healthy Controls

The mean serum TLR9 level was significantly lower in T2DM patients compared to healthy controls:

Group	Mean ± SE (ng/mL)	P-value	Significance
Control	270.79 ± 20.61		
T2DM Patients	173.75 ± 8.52	< 0.00001	Highly Significant

Serum TLR9 Levels by Gender

When stratified by gender, male T2DM patients had higher TLR9 levels than female patients. However, both sexes had significantly lower levels than the corresponding control group.

Group	Male (ng/mL)	Female (ng/mL)	P-value	Significance
Control	270.91 ± 20.18	270.72 ± 32.05	0.43	Not Significant
T2DM Patients	189.09 ± 11.45	159.09 ± 12.03	0.0391	Significant

Discussion

The current study demonstrates a marked reduction in serum TLR9 levels among patients with T2DM compared to healthy individuals. This decrease was observed regardless of gender, although male patients had slightly higher TLR9 levels than female patients.

The role of TLR9 in detecting microbial DNA and initiating innate immune responses is well-established. Reduced expression or secretion of TLR9 in T2DM patients may reflect a suppressed immune-inflammatory state or dysregulation of immune signaling pathways. Chronic hyperglycemia and oxidative stress in T2DM are known to impair immune responses, which may contribute to this downregulation.

In previous studies that have investigated the association of other biomarkers among patients with type2 DM from Wasit province, (Yousif and Ghali,2021), revealed that IL-10 is a major contributor to the onset of type 2 diabetes mellitus and there may be a correlation between low levels of interleukin-10 and type two diabetes .(Al-Sarray and Ahmed ,2021) found that may be a correlation between high levels of TNF-α and type 2 diabetes mellitus.(Shamkhi and Ahmed ,2021), displayed that levels of SIRT1 may be not associated with type2 diabetes mellitus. Furthermore, the cell free mitochondrial DNA increases significantly in patients with type2 diabetes mellitus (Hussein and Ghali,2022). COX-1 is a major contributor to the onset of type 2 diabetes and there may be an association between low levels of cyclooxygenase-1and type 2 diabetes (Jebil and Ghali,2021). (Mahmood and Ghali,2022), found also that there may be a correlation between high levels of OPG and T2DM.

These findings align with previous studies that link TLR dysfunction with metabolic disorders. Since TLR9 plays a role in inflammation and immune regulation, its serum concentration may serve as a biomarker for disease progression and immune imbalance in T2DM.

Conclusion

Based on the results of this study, the following conclusions can be drawn:

1. Serum TLR9 levels are significantly reduced in patients with type 2 diabetes mellitus compared to healthy individuals.
2. Both male and female T2DM patients show lower TLR9 levels than controls, though male patients exhibit slightly higher levels than females.
3. The consistent decrease in TLR9 levels suggests a disease-related downregulation of innate immune activity.
4. TLR9 may be considered a potential immunological marker for T2DM-related inflammation and metabolic dysregulation.

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