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Differential Serum IL-37 Levels and Monomorphic CTLA-4 rs5742909 Polymorphism in Type 1 and Type 2 Diabetes Mellitus: An Iraqi Cohort Study

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ABSTRACT

***Background**: Diabetes mellitus (DM) is linked to intricate inflammatory and autoimmune mechanisms. Interleukin-37 (IL-37) functions as a significant anti-inflammatory cytokine, while CTLA-4 serves as an essential immune regulator. **Objective**: To investigate serum IL-37 levels and CTLA-4 rs5742909 gene polymorphisms in patients with type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) compared to* apparently healthy *controls. Methods: In this case-control study, 120 participants were enrolled, comprising 30 T1DM patients, 30 T2DM patients, and 60* apparently healthy *controls. Serum IL-37 levels were quantified by ELISA. Genotyping of the CTLA-4 rs5742909 polymorphism was performed using polymerase chain reaction. **Results**: Serum IL-37 levels were decreased in T2DM group (49.50 ± 5.51 ng/L) compared with those of T1DM (56.43 ± 11.62 ng/L) and control groups (60.09±12.14 ng/ L) (P=0.001). The ROC curve for the diagnosis of T2DM was 0.81. Using genetic analysis, it was determined that the polymorphism of rs5742909 was completely absent in all participants and the frequency of the homozygous CC genotype was 100%. **Conclusion**: Lower serum IL-37, a strong candidate biomarker for T2DM was observed in our Iraqi subjects and monomorphic CTLA-4 rs5742909 variant might not be an indication of susceptibility to T1DM or T2DM stressing the demand of immune-related genetic studies specific for different populations.*

Keywords: CTLA-4 Antigen; Genetic Variation; Inflammation; Autoimmunity.

Article Information

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INTRODUCTION

Diabetes Mellitus (DM) is one of today's most difficult health problems in the world, and has become an epidemic with millions of patients and a growing number of people afflicted(1,2). It is a multifactorial metabolic disease characterized by sustained hyperglycemia due to insulin secretion, action, or both (3). Both types of disease, type 1 Diabetes Mellitus (T1DM) and type 2 Diabetes Mellitus (T2DM), have a different pathophysiology, however they meet in the same metabolic disruption. T1DM is an autoimmune disease that progresses via T cell-mediated destruction of pancreatic β-cells, resulting in absolute insulin deficiency(4).

Instead, T2DM (> 90% of cases) is the result of both peripheral insulin resistance and relative insulin deficiency(5). Although traditionally considered to have different etiologies, the immune system and inflammatory responses are emerging as central players in both types of diabetes(6,7). Although an autoimmune attack is central to defining T1DM, recent research suggests that chronic low-grade inflammation plays a significant role in the development of insulin resistance and the ensuing β-cell dysfunction in T2DM (8,9).

This increasing understanding of the immunological basis of DM has prompted the study of the defined molecular triggers that drive this phenomenon. Among the cytokines involved in immune homeostasis, Interleukin-37 (IL-37) appears to be a highly potent anti-inflammatory factor(10). IL-37 belongs to the IL-1 family and acts as a central inhibitor of innate and acquired immunity(11,12). It mediates its action by nuclear translocation of the protein to repress proinflammatory gene expression and, when bound to one of its extracellular receptors (4-hydroxytamoxifen), suppresses inflammatory signaling pathways (11,12). Owing to its potent immunosuppressive activities, IL-37 has been linked to several inflammatory and immune-mediated diseases(13,14). Its holistic capacity to influence the inflammatory state of T1DM and T2DM makes an intriguing area for research, with some studies hinting at its ability to attenuate insulin resistance and GC-induced inflammation, positioning this molecule in the endocrine milieu of these diseases as a potential biomarker or even a target(15,16).

In parallel with the study of inflammatory cytokines, genetic factors that predispose individuals to immune dysregulation are of significant interest. CTLA-4 is a critical negative regulator of T-cell activation and a key checkpoint for maintaining immune tolerance(17). By competing with the co-stimulatory molecule CD28 for binding to its ligands (CD80 and CD86), CTLA-4 effectively raises the threshold for T-cell activation, thereby preventing excessive immune responses(18). Genetic polymorphisms within CTLA-4 have been robustly associated with an increased risk of numerous autoimmune diseases, including rheumatoid arthritis, and T1DM (19,20). The single nucleotide polymorphism (SNP) rs5742909, located in the promoter region of the gene, is of particular interest because it may influence gene expression and, consequently, the efficacy of immune regulation (21). However, its association with diabetes remains an area requiring further exploration, with some studies showing conflicting results in different populations(22).

Although inflammation and immune regulation are well-recognized factors in diabetes, there is a paucity of literature focusing on the simultaneous study of these closely tied pathways in T1DM and T2DM, within a clearly defined cohort. To the best of our knowledge, previous studies have only investigated IL-37 levels or CTLA-4 polymorphisms among patients with diabetes separately, and there are few reports on the combined effects. The association between the levels of circulating IL-37, T1DM, and T2DM has not been fully clarified, as some studies have provided conflicting results (16,23), which is likely due to population differences. Other reasons as to why some studies have found a significant association of the CTLA-4 rs5742909 polymorphism and others have not, may reflect gene-gene interactions with other susceptible SNPs in non-autoimmune genes or SNP environmental trigger (viral infection, eat antigen) which was already shown by Ebrahim showed that HLA-A*0201+ patients with T1DM are frequently characterized by expression of TLR3 on CD8+cellshowever, it can be assumed but without evidence that no strong relationships between this particular SNP and T1DM has been reported in Iraqi children (24). The limited scope of this study has prevented a comprehensive investigation into the shared and divergent immunopathological processes underlying these diseases. Thus, a study evaluating anti-inflammatory cytokines such as IL-37 and an immuneregulatory gene polymorphism such as CTLA-4 rs5742909 in T1DM and T2DM patients is important to identify possible associations between these and provide comprehensive information regarding the immunogenetic background of DM.

METHODOLOGY

Study Design and Participants

This research was conducted between November 27, 2024 and March 21, 2025 in the general diabetes center at Al-Diwaniyah Teaching Hospital with cooperation from AL-Hussein Children's Hospitals and maternity hospitals for children and gynecology. One hundred and twenty individuals between 18-65 years were recruited into three groups according to disease status, T1DM group (N = 30), T2DM patients group (N = 30), and control participants otherwise apparently healthy N = 60), matched with the diabetic subjects in age, sex. The diagnosis of T1DM was supported by the clinical course, young age at presentation, and complete reliance on insulin therapy. T2DM was defined on the basis of medical history, treatment with oral hypoglycemic agents, no insulin (users of metformin alone were kept in the non-medicamentous user group), and corroboratory laboratory tests of fasting blood glucose and HbA1C. All groups met the exclusion criteria for autoimmune or chronic inflammatory diseases and use of immunosuppressive drugs. Demographic and clinical details were recorded for all patients.

Sample Collection and Preparation

After an 8 hour fast, 5mL of venous blood was obtained from each subject then put in gel tubes without anticoagulants (Afco, Jordan). The samples were clotted at room temperature for 15 min. Serum was obtained by centrifugation at 5000 rpm for 3 min (OHAUS, Germany). Aliquots of the resultant serum were prepared in sterile Eppendorf tubes (Eppendorf, Germany) and preserved at −20°C for analysis. For the genetic studies, 2 mL of venous blood was collected in EDTA-containing tubes to avoid clotting.

Genomic DNA was isolated from peripheral blood leukocytes using the AddBio DNA Extraction Kit (AddBio, South Korea), according to the manufacturer's protocol. The cells were lysed with proteinase K and the DNA was bound to a silica column. Two washes with wash buffer I and one with wash buffer II were performed followed by elution. Recovered DNA was quantified and purity was checked with a spectrophotometer (Thermo Fisher Scientific, USA) at A260/A280 over 1.8 - 2 to demonstrate high level of purity for DNA extraction. The purified DNA was stored at -20°C until used.

Laboratory Analyses

Serum IL-37 levels were measured using a Human IL-37 ELISA Kit, employing the sandwich enzyme-linked immunosorbent assay (ELISA) methodology. All reagents were equilibrated to room temperature prior to use. A standard curve was established through serial dilutions of the 480 ng/L standard, resulting in concentrations of 240, 120, 60, 30, and 15 ng/L. The assay was conducted following the manufacturer's, measured at 450 nm utilizing a BioBase microplate reader (BioBase, South Korea).

The rs5742909 (C>T) (SNP in the CTLA-4 gene was genotyped using a T-ARMS PCR method. Primers for this assay were specifically designed for this study. The forward outer primer had the sequence 5′-TTTTTTTCTCTTAACCAAATGCTAAATG G-3′ and the reverse outer primer had the sequence 5′-ATATGTATTACACATGTGCACACACAGA AG-3,' which together amplified a 500 bp control fragment. The forward inner primer, specific for the C allele, had the sequence 5′-CAAGTCTCCACTTAGTTATCCAGATCAT C-3′ and produced a 223 bp fragment. The reverse inner primer, specific for the T allele, had the sequence 5′-TTGAAACTGAAGCTTCATGTTCACATTG TA-3′ and produced a 334 bp fragment.

PCR was conducted in 20 µL, using 10 µL of 2x Master Mix (AddBio, Korea), 1 µL each of four primers, 1 µL of DNA template (50-100 ng/µL), and the remaining nuclease-free water. The amplification was done using a BIO-RAD T100 Thermal Cycler (USA). The thermal profile consisted of initial denaturation at 95°C for 5 min, 39 cycles of denatation (95°C for 35 seconds), annealing (65°C for 35 secs), and extension (72°C for 35 secs), and a final extension at 72°C for 5 min. PCR products were electrophoresed on a 1.5% agarose gel stained with ethidium bromide and viewed under UV light using a gel documentation system (Syngene, Taiwan). Based on band patterns, the CC genotype had a 223 bp band, the TT genotype a 334 bp band, and the CT genotype both bands, plus the 500 bp control band in all successful reactions.

Statistical Analysis

Statistical analyses were conducted in GraphPad Prism (version 8.4.3). Patient characteristics were summarized using descriptive statistics. P<0.05 was considered as statistically significance.

RESULTS

Table 1 Demographic and baseline clinical characteristics of the 120 subjects received a classification into T1DM, T2DM, and apparently healthy control (HC) cohort. The mean age (35.26±11.51 years for T1DM and T2DM; 35.58±11.51 for HC: P = 0,86) was matched to the gender distribution (P = 0,61) and to the average BMI (22,9±5,03 kg/m² for T1DM, 21,6 ±5,03 kg/m²for T2DM and23,49±4,43 kg/m²: P=0.69).. Unsurprisingly, markers of glycemic HC were much higher in the diabetic group than in apparently healthy participants. The average (± SD) HbA1c values were 10.8 ± 1.9% in T1DM, 9.6 ± 3.7% in T2DM, and significantly greater than that in HCs (4.6 ±0.8%, both P < 0.001). Similarly, average fasting blood sugar (FBS) concentrations were significantly higher in T1DM (272 ± 92.6 mg/dL) and T2DM subjects (382 ± 88.7 mg/dL) than in HCs (91 ±11.5 mg/dL), P < 0.001).

Table 1: Demographics and Clinical Features of Study Participants.

Characteristic	T1DM Patients (n=30)	T2DM Patients (n=30)	Apparently healthy Controls (n=60)	P-value
Age (years, Mean ± SD)	35.26 ± 11.51	35.26 ± 11.51	35.58 ± 11.51	0.86
Gender (Female/Male, n (%))	16 (53.3%) / 14 (46.7%)	13 (43.3%) / 17 (56.7%)	30 (50.0%) / 30 (50.0%)	0.61
BMI (kg/m², Mean ± SD)	22.9 ± 5.03	24.6 ± 5.03	23.49 ± 4.43	0.69
- Normal Weight,n (%)	17 (66.7%)	17 (56.7%)	32 (53.3%)	0.21
- Overweight, n (%)	6 (20.0%)	8 (26.7%)	25 (41.7%)	
- Obese, n (%)	4 (13.3%)	5 (16.3%)	3 (5.0%)	
HbA1c (%, Mean ± SD)	10.8 ± 1.9	9.6 ± 3.7	4.6 ± 0.8	<0.001
FBS (mg/dL, Mean ± SD)	272 ± 92.6	382 ± 88.7	91 ± 11.5	<0.001

IL-37 serum levels There was a statistically significant difference in the level of IL-37 between the study groups (P = 0.001) (Table 2). The mean IL-37 concentration of T2DM group (49.5 ± 5.51 ng/L) is significantly decreased than those of apparently healthy HC group (60.09 ± 12.14 ng/L) and T1DM group (56.43 ± 11.62 ng/L). No significant was found between patients with T1DM and HC.

The ROC curve was used to assess the diagnostic value of IL-37 (Figure 1). IL-37 showed good diagnostic value for differentiating T2DM cases from apparently healthy HCs in an AUC value of 0.81 (95% CI: 0.68–0.89; P =.000). A cutoff value of 51.29 ng/L resulted in a sensitivity of 80.0% and a specificity of 78.3%. For T1DM, IL-37 had a poor diagnostic value (AUC = 0.59 [95%CI: 0.45–0.74],

P = 0.226). In addition, irrespective of disease duration (<5 years vs. ≥5 years), there were no significant differences in IL-37 levels in either the T1DM (P = 0.133) or T2DM (P = 0.332) subgroups.

Table 2: Comprehensive Analysis of Interleukin-37 in Study Groups

Analysis Type	T1DM Patients	T2DM Patients	Healthy Controls	P-value
IL-37 Level (Mean ± SD)	56.43 ± 11.62^{A}	49.50 ± 5.51^{B}	60.09 ± 12.14^{A}	0.001**
Diagnostic Accuracy (ROC Analysis)				
- Cutoff Value	> 56.93	> 51.29		
- AUC (95% CI)	0.59 (0.45- 0.74)	0.81 (0.68- 0.89)		
- Sensitivity (%)	60.0%	80.0%		
- Specificity (%)	65.0%	78.3%		
- PPV (%)	46.2%	64.9%		
- NPV (%)	76.5%	88.7%		
P value	0.226	<0.001		
IL-37 Level by Disease Duration (Mean ± SD)		**< 5 years**	**≥ 5 years**	
T1DM patients		59.86 ± 12.71	52.33 ± 9.71	0.133
T2DM patients		50.91 ± 4.43	48.52 ± 6.11	0.332
Means denoted by distinct superscript letters (A, B) are statistically different. **: statistically significant at P < 0.01.				

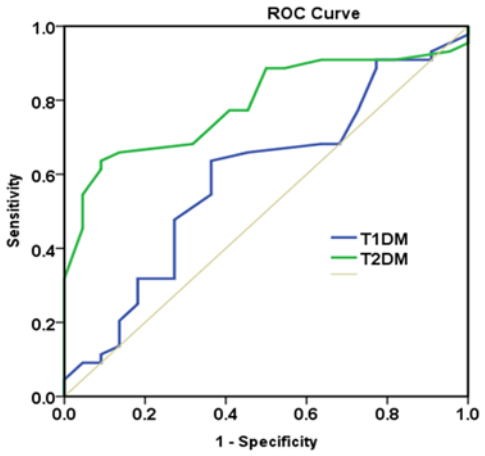

Figure 1: The blue curve is the receiver operating characteristic curve for IL-37 levels in differentiating T1DM patients from apparently healthy HC participants. Receiver operating characteristic curve (green curve) for IL-37 levels to differentiate T2DM patients from apparently healthy HC participants**.**

Genetic analysis of the CTLA-4 polymorphism at the locus rs5742909 was conducted in all 120 participants. The results presented in Table 3 show the complete lack of polymorphisms in the study cohort. All individuals in the T1DM, T2DM, and apparently healthy HC groups were found to be homozygous for the C allele, exhibiting a 100% frequency of the CC genotype. Consequently, the T allele and the corresponding heterozygous (C/T) and homozygous (TT) genotypes were absent in all participants. Agarose gel electrophoresis analysis confirmed these findings, consistently showing only the 223 bp band corresponding to the C allele alongside the 500 bp HC band in all samples (Figure 2). Due to the monomorphic nature of the rs5742909 locus in this population, no further statistical analysis of its association with either T1DM or T2DM could be performed.

Table 3: Genotypic and Allelic Frequencies of CTLA-4 (rs5742909) Polymorphism.

CTLA-4 (rs5742909)	T1DM (n=30)	T2DM (n=30)	Control (n=60)	T1DM vs C (P-value, OR)	T2DM vs C (P-value, OR)

Genotype Frequency					
- TT	0	0	0		
- C/T	0	0	0		
- CC	30 (100%)	30 (100%)	60 (100%)	Reference	Reference
Allele Frequency					
- T	0	0	0		
- C	60 (100%)	60 (100%)	120 (100%)		

OR: Odds Ratio; CI: Confidence Interval.

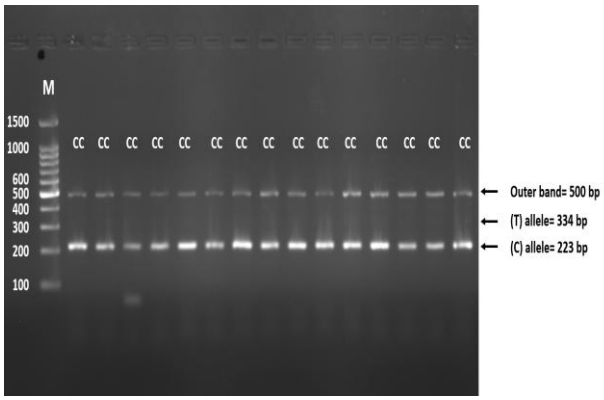

Figure 2: agarose gel electrophoresis displaying the ARMS-PCR product analysis of the CTLA-4 (rs5742909) gene polymorphism in patient blood samples.

DISCUSSION

The most remarkable observation concerning IL-37 was the decreased serum levels in T2DM patients compared with both HC and T1DM individuals. This is supported by the hypothesis that low levels of this pivotal anti-inflammatory cytokine underlie the chronic low-grade inflammation state typical of T2DM (9). This finding is consistent with several reports. For example, Zhang et al. documented significantly reduced IL-37 expression in T2DM, which, when overexpressed, may inhibit high glucose-induced inflammation and podocyte death (25). Another research on gestational diabetes has shown a reduction in IL-37 levels that may be associated with metabolic dysregulation (23). (23). The preventive impact of IL-37 has been confirmed, as it restored insulin sensitivity and mitigated obesity-induced inflammation.(15,16).

However, the evidence regarding IL-37 in type 2 diabetes mellitus is conflicting. Conversely, several studies have shown increased IL-37 levels in T2DM populations. Research conducted by Alfadul et al. in a Saudi Arabian cohort revealed markedly elevated circulating IL-37 levels in individuals with T2DM compared to apparently healthy HCs (26). A recent research conducted by AL-Sahi et al. in an Iraqi community similarly revealed elevated levels of IL-37 in diabetic individuals (27). Other reports corroborate the findings of upregulated IL-37 levels in T2DM(28,29). This discrepancy may stem from several factors, including differences in study populations and ethnicities, variations in disease duration or the presence of complications, and different laboratory methodologies (ELISA vs. Luminex). It is also plausible that IL-37 levels change dynamically throughout the course of the disease. An initial decrease might contribute to the onset of inflammation, followed by compensatory upregulation in an attempt to counteract the persistent pro-inflammatory state, a phenomenon that our cross-sectional study could not capture. Our ROC analysis, which indicated good diagnostic accuracy for T2DM, supports the clinical relevance of our findings in this specific population, despite the broader controversy.

Another remarkable observation in our study was the lack of the T allele for the CTLA-4 rs5742909 polymorphism. This is an

important observation because it stands in sharp contrast to many studies in other populations, which have reported that the rs5742909 polymorphism (also named -318 C/T) connected with susceptibility to T1DM and other autoimmune illnesses (30) CTLA-4 serves as a crucial negative regulator of T-cell activation, and mutations influencing its expression or function are considered significant genetic risk factors for autoimmunity(18).

Nevertheless, our findings are not unprecedented in this region. A study by Khudair et al. investigating this polymorphism in Iraqi (Baghdad) children with T1DM also identified that the CTLA-4(-318 C/T) was not a predisposing factor for their patient group(31). Despite that they did not show full monomorphism, there as well as our data indicate strongly resent that rs5742909 polymorphism is unlikely to be a major risk factor for diabetes in the Iraqi population. These findings highlight the established concept of ethnic diversity in genetic architecture of complex diseases. Although other *CTLA-4* polymorphisms such as +49A/G (rs231775) have been associated with T1DM in Iraqi and other Middle Eastern populations(22,32), our data provide compelling evidence that rs5742909 is not a useful marker in this population. This result has important implications for future risk studies in this population, suggesting that, instead of continuing further efforts to study this SNP, research should be conducted on other presumably more functional/susceptible genetic variants within the CTLA-4 gene or even other immune-related genes.

Limitations of the Study

Although this study provides important population-specific findings, we should recognize some limitations. The limitation of a cross-sectional study is the inability to infer causality; thus, it remains unknown whether the observed lower levels of IL-37 in T2DM are a cause or an effect. Prospective studies are needed to follow the kinetics of IL-37 expression throughout the development of pulmonary tuberculosis. Second, the small sample size of both the overall and patient subgroups could reduce the statistical power of the findings and make it difficult to generalize our results to the entire Iraqi population. Third, this was a single-center study and may have a selection bias. This was confirmed in a larger multicenter study from different areas and populations. Lastly, we looked at a single CTLA-4 SNP (rs5742909). For a more complete understanding of the genetic factors that increase the risk of diabetes in this group, it is necessary to investigate additional polymorphisms in immune-related loci as well as CTLA-4.

CONCLUSION

In conclusion, the present study offers novel population-based information regarding the immunogenetics of diabetes. In the present study, we found a dramatic reduction in IL-37 levels in T2DM patients, which contradicted the literature and justified its status as a potential biomarker, as well as revealing conflicting opinions in the field. We particularly revealed that the polymorphism CTLA-4 rs5742909 is monomorphic and therefore cannot act as a candidate for diabetes risk in this Iraqi population, which provides valuable information to further narrow down the genetic regions contributing to T1D in the examined cohort. Future longitudinal studies are required to clarify the dynamic variation of IL-37 in diabetes, and additional genetic studies should investigate other SNPs to characterize the inherited risk of autoimmune-related diabetes at this location.

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Declarations

Competing interests: No interests

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Author Contribution

Conceptualization: NAWH and GMAA; methodology: NAWH and GMAA; validation: NAWH and GMAA; formal analysis: GMAA; investigation: NAWH and GMAA; resources: NAWH; data curation: GMAA; writing – original draft preparation: NAWH and GMAA; writing – review and editing: all authors; visualization: GMAA; supervision: NAWH; project administration: NAWH; and funding acquisition: NAWH and GMAA. All authors have read and agreed to the published version of the manuscript.

Ethical considerations

This study complied with the ethical standards for human research described in the Declaration of Helsinki. The study protocol, including all aspects of participant recruitment, data collection, and sample analysis, the College of Biotechnology at the University of AL-Qadisiyah's Institutional Review Board and Ethics Committee on January [2171 January 24, 2024].

Statement of Permission and Conflict of Interests

The authors declare no conflicts of interest related to this study.

REFERENCES

1. Pardhan S. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. 2023;
2. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Al Kaabi J. Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. J Epidemiol Glob Health. 2020 Mar;10(1):107–11.
3. Abel ED, Gloyn AL, Evans-Molina C, Joseph JJ, Misra S, Pajvani UB, et al. Diabetes mellitus—Progress and opportunities in the evolving epidemic. Cell. 2024;187(15):3789–820.
4. Aamodt KI, Powers AC. The pathophysiology, presentation and classification of Type 1 diabetes. Diabetes Obes Metab. 2025 Aug;27 Suppl 6(Suppl 6):15–27.
5. Iheagwam F, Iheagwam O. Diabetes Mellitus: The Pathophysiology as a Canvas for Management Elucidation and Strategies. Med Nov Technol Devices. 2025 Mar 1;25:100351.
6. Wadai G. The impact of active and passive smoking on IL-6 serum levels for Iraqi healthy people. Bionatura. 2023 Aug 15;8:1–7.
7. Alexander M, Cho E, Gliozheni E, Salem Y, Cheung J, Ichii H. Pathology of Diabetes-Induced Immune Dysfunction. Int J Mol Sci. 2024 Jun;25(13).
8. Zhao L, Hu H, Zhang L, Liu Z, Huang Y, Liu Q, et al. Inflammation in diabetes complications: molecular mechanisms and therapeutic interventions. MedComm. 2024 Apr;5(4):e516.
9. Berbudi A, Rahmadika N, Tjahjadi AI, Ruslami R. Type 2 Diabetes and its Impact on the Immune System. Curr Diabetes Rev. 2020;16(5):442–9.
10. Su Z, Tao X. Current Understanding of IL-37 in Human Health and Disease. Front Immunol. 2021;12:696605.
11. Ye L, Jiang B, Deng J, Du J, Xiong W, Guan Y, et al. IL-37 Alleviates Rheumatoid Arthritis by Suppressing IL-17 and IL-17-Triggering Cytokine Production and Limiting Th17 Cell Proliferation. J Immunol. 2015 Jun;194(11):5110–9.
12. Boraschi D, Lucchesi D, Hainzl S, Leitner M, Maier E, Mangelberger D, et

al. IL-37: A new anti-inflammatory cytokine of the IL-1 family. Eur Cytokine Netw. 2011 Oct 29;22:127–47.
13. Mousa HM, Wadai GMA. The infiltration of T-bet in bladder tumors in sample of Iraqi patients. Biochem Cell Arch. 2018;18(1):659–63.
14. Zeng H, Zhou K, Ye Z. Biology of interleukin-37 and its role in autoimmune diseases (Review). Exp Ther Med. 2022 Aug;24(2):495.
15. Ballak DB, van Diepen JA, Moschen AR, Jansen HJ, Hijmans A, Groenhof GJ, et al. IL-37 protects against obesity-induced inflammation and insulin resistance. Nat Commun. 2014 Sep;5:4711.
16. Li T, Li H, Li W, Chen S, Feng T, Jiao W, et al. Interleukin-37 sensitize the elderly type 2 diabetic patients to insulin therapy through suppressing the gut microbiota dysbiosis. Mol Immunol. 2019 Aug;112:322–9.
17. Demerdash DM El, Saber MM, Ayad A, Gomaa K, Morad MA. Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) gene polymorphisms in a cohort of Egyptian patients with immune thrombocytopenia (ITP). Blood Res. 2024;59:8.
18. Ting WH, Chien MN, Lo FS, Wang CH, Huang CY, Lin CL, et al. Association of Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4) Gene Polymorphisms with Autoimmune Thyroid Disease in Children and Adults: Case-Control Study. PLoS One. 2016;11(4):e0154394.
19. Chen Y, Chen S, Gu Y, Feng Y, Shi Y, Fu Q, et al. CTLA-4 +49 G/A, a functional T1D risk SNP, affects CTLA-4 level in Treg subsets and IA-2A positivity, but not beta-cell function. Sci Rep. 2018 Jul;8(1):10074.
20. Sheikhian F, Golparvar MM, Ahmadi I, Anvari E, Majdolashrafi F, Ghazanfari Jajin M, et al. CTLA-4 rs5742909 but not ADAM33 rs2280091 is a predictor factor for COVID-19 mortality. J Infect Public Health. 2025 Jan;18(1):102618.
21. Fayedeh F, Khorashadizadeh S, Yousefi M, Abbasifar S, Erfanian N, Rafiee M, et al. CTLA-4 expression and polymorphisms in Schizophrenia; a systematic review of literature. Mol Biol Rep. 2024;51(1):431.
22. Ren D, He L, Pang X. Association of CLTA-4 Gene Polymorphisms with Diabetes Mellitus: A Study Based on the Han Population of Northern China. Diabetes Metab Syndr Obes. 2022;15:2705–12.
23. Zhang X, Zhu Y, Zhou Y, Fei B. Interleukin 37 (IL-37) Reduces High Glucose-Induced Inflammation, Oxidative Stress, and Apoptosis of Podocytes by Inhibiting the STAT3-Cyclophilin A (CypA) Signaling Pathway. Med Sci Monit Int Med J Exp Clin Res. 2020 Sep;26:e922979.
24. Ebrahim E, Teklu T, Tajebe F, Wondmagegn T, Akelew Y, Fiseha M. Association of cytotoxic T-lymphocyte antigen-4 gene polymorphism with type 1 diabetes mellitus: in silico analysis of biological features of CTLA-4 protein on ethiopian population. Diabetes, Metab Syndr Obes Targets Ther. 2022;2733–51.
25. Yu Z, Liu J, Zhang R, Huang X, Sun T, Wu Y, et al. IL-37 and 38 signalling in gestational diabetes. J Reprod Immunol. 2017 Nov;124:8–14.
26. Alfadul H, Sabico S, Ansari MGA, Alnaami AM, Amer OE, Hussain SD, et al. Differences and Associations of NLRP3 Inflammasome Levels with Interleukins 1α, 1β, 33 and 37 in Adults with Prediabetes and Type 2 Diabetes Mellitus. Biomedicines. 2023 Apr;11(5).

27. AL-Sahi MM, AL-Hasnawi SMJ, Ali MM. Evaluation of Immunological Levels of IL-37, IL-38, and IL-17A in Iraqi Patients with Diabetic Foot Ulcers. Karbala J Pharm Sci. 2023;14(23):116–24.
28. Alhayali FE, Yücel AA, Ashoor ZF. Comparison of Serum Interleukin-37 Level in Obese, Nonobese Type II Diabetic Patients and Healthy Controls. Mustansiriya Med J [Internet]. 2021;20(2). Available from: https://journals.lww.com/mtmj/fulltext/2021/20020/comparison_of_serum_interleukin_37_level_in_obese,.10.aspx
29. manager K journal of M, Duaa M. Qasim, Abeer T. Al-Hasnawi, Masar R. Al-Mousawi1, Akram A. Al-Abbad. Evaluation of Interleukin-17A and Interleukin- 34 in Patients with Diabetic Foot Ulcer. Karbala J Med [Internet]. 2025 Jun 30;18(1 SE-Research Articles). Available from: https://mail.journals.uokerbala.edu.iq/index.php/kj/article/view/3856
30. Douroudis K, Prans E, Uibo R. CTLA-4 promoter polymorphisms are associated with latent autoimmune diabetes in adults. Hum Immunol. 2009 Nov;70(11):921–4.
31. Khudair EA, Al-Shuwaikh AM, Abdoun DS. Investigation of CTLA-4 gene polymorphisms in a sample of Iraqi children with newly diagnosed type 1 diabetes mellitus. J Nephropharmacology. 2025;15(1):e12702–e12702.
32. Al-Isawi HR, Safi F, Muhammed HA. Association of CTLA-4 (+ 49A/G) gene Polymorphism with type 1 diabetes mellitus in Iraqi children. Cell Mol Biol. 2022;68(5):1–6.