The Relationship between urinary tract infection and some cytokines in children with type I diabetes mellitus

Noor Abd-ulamer Oda*, Kawkab A. Alsaadi , Haider Hashim Mohammed Ali

Department of Biology, College of Science, University of Kerbala,56001, Kerbala, Iraq

*Corresponding Author :

Email : noor.a.oda@s.uokerbala.edu.iq

https://orcid.org/0000-0002-5430-6940

Scopus Author ID: 57202513162

ABSTRACT

Diabetes patients are suffuring from urinary tract infections, mainly related to an impaired immune response , inadequate glycemic control and diabetic microangiopathy. Irregular secretion of cytokines leads to bidirectional effect on the development of the diabetic disease and UTIs. This study aimed to assess the role of diabetes in the development of some UTI bacterial infections and studying the effect of infections and Type 1 diabetes mellitus (T1DM) in Interleukin-35 and Macrophage Migration Inhibitory Factor (MIF) concentration. total of 90 male children, whose ages ranged between 5-15 years participated in this study ,60 patients with T1DM half of them have UTIs and 30 healthy controls. Cultivation urine on agar plates method used to identify UTIs patients with substantial bacteriuria. All bacterial isolates were diagnosed by VITEK-2. The levels of IL-35 and MIF concentration were measured using ELISA assay.The major findings of the study were :1-The serum IL-35 and MIF levels were a significant increased for both patients groups of T1DM compared to control. 2-Upon bacterial stimulation, happened higher increased of IL-35 and MIF levels compared to D1TM without UTIs. 3- The most frequent bacteria isolated were Escherichia coli 35% , Pseudomonas stutzeria 19% and Kocuria rosea 19% caused of UTI in T1DM patient.These results suggested that Type 1 diabetes mellitus and Urinary tract infections affects cytokines secretion, suggesting that cytokines -directed therapies might offer new treatment opportunities for inflammatory and autoimmune diseases in the future.

Keywords *Type1 Diabetes mellitus , Urinary tract infections , IL-35 , Migration Inhibitory Factor*

يصاب مرضى السكري بالتهابات المسالك البولية بشكل متكرر ، ويعود ذلك بشكل أساسي لضعف الاستجابة المناعية وعدم التحكم في نسبة السكر في الدم واعتلال الشعيرات الدموية المصاحبة لمرضى السكري . يؤدي إفراز السيتوكينات غير المنتظم إلى تأثير ثنائي الاتجاه على تطور مرض السكري والتهاب المسالك البولية .هدفت هذه الدراسة إلى تقييم دور مرض السكري في تطور بعض الالتهابات البكتيرية في المسالك البولية ودراسة تأثير العدوى ومرض السكري من النوع الأول على عامل تثبيط هجرة البلاعم والإنترلوكين- 35 . 90 طفل من الذكور شارك في هذه الدراسة تراوحت أعمارهم بين (5-15) عامًا ، 60 طفل منهم يعانون من مرض السكري النوع الأول نصفهم مصابون بعدوى المسالك البولية و 30 طفل اصحاء استخدمو كمجموعة قياسية . استخدمت طريقة زراعة البول على أطباق أكار لتحديد المصابين بعدوى المسالك البولية. تم تشخيص جميع العزلات البكتيرية بواسطة جهاز الفايتك 2 . تم قياس مستوى تركيز إنترلوكين - 35 و تركيز عامل تثبيط هجرة البلاعم باستخدام جهاز بالمقايسة الممتز المناعي المرتبط بالأنزيم (الإليزا) . النتائج الرئيسية للدراسة هي : 1-زيادة مستويات تركيز إنترلوكين-35 و تركيز عامل تثبيط هجرة البلاعم في الدم بشكل معنوي لمجموعتي مرضى السكري من النوع الأول مقارنة بمجموعة الأطفال الأصحاء. 2-ادت الأصابة البكتيرية الى زيادة في مستويات إنترلوكين - 35 و تركيز عامل تثبيط هجرة البلاعم مقارنة بـالأطفال المصابين بداء السكري وغير مصابين بألتهابات المسالك البولية . 3- أكثر أنواع البكتيريا المعزولة التي تسبب التهاب المسالك البولية في مرضى السكري من النوع الأول هي الإشريكية القولونية بنسبة 35 ٪ و الزائفة شتوتزرية بنسبة 19 ٪ و الكوكوريا الوردية بنسبة 19 % . تشير هذه النتائج إلى أن داء السكري من النوع الأول والتهابات المسالك البولية تؤثر على إفراز السيتوكينات ، مما يشير إلى أن تنظيم السيتوكينات الموجة قد يقدم طرق علاجية جديدة للأمراض الالتهابية وأمراض المناعة الذاتية في المستقبل.

1. INTRODUCTION

Diabetes patients have urinary tract infections (UTIs) more frequently than the normal population. UTIs is an infections of any part of the urinary system, including kidney infection , urethritis and the cystitis [1]. The clinical manifestations of UTIs vary with age,levels of the activity of the immune system , stage of infection, and type of bacteria causing the infection [2].

T1DM is an autoimmune condition that is defined by the immune system's destruction of pancreatic β-cells, which results in a lifelong reliance on exogenous insulin and places a significant burden on individuals and healthcare resources [3]. Additionally adding to the burden of this condition, T1DM is linked to an increased risk of comorbidities like bacterial infections, cardiovascular disease, retinopathy, and diabetic kidney disease[4].

Cytokines are low molecular weight extracellular proteins that mediate immunological responses. They function in incredibly intricate pathways that control T1DM and the inflammatory response to bacterial infection, and they are necessary to carry out the lesion site response [5]. When it comes to the development of T1DM, cytokines are essential for coordinating the intricate multicellular interactions between immune cells and pancreatic beta-cells [6]. It is believed that cytokines like IL-35, which can promote anti-inflammatory responsibilities (regulatory functions), can improve immunological tolerance and guard against -cell damage [7]. Contrarily, cytokines like MIF that encourage the differentiation and operation of immune cells that because diabetes are hypothesized to cause the beginning and development of T1DM [8]. However, because the pro-inflammatory or anti-inflammatory functions of cytokines, which are in charge of β-cell degeneration, are context dependent, targeting these dysregulated cytokine networks does not always produce consistent effects [9].

Patients with diabetes are more likely to get infections that affect their mucous membranes and soft tissues [10]. Additionally, diabetic rats' lungs have been shown to have lower levels of tumor necrosis factor (TNF), interleukin (IL)-1 and intercellular adhesion molecule (ICAM)-1, as well as less ICAM-1 expression, which results in fewer leukocyte-endothelial interactions [11].

Diabetes and bacterial infections are linked in both directions. Chronic infections like periodontitis are associated with higher proinflammatory cytokines, which can exacerbate insulin resistance and compromise glycemic management, whereas diabetes increases susceptibility to bacterial infections and their consequences. [12]. There is growing evidence that diabetes development may be significantly influenced by anomalies in the composition of the microbiome[13]. For effective prevention and quick treatment, it is crucial to understand the intricate links between diabetes and the accompanying bacterial infections. [6]. Therefore, this study was aimed to investigate IL-35 and MIF concentration in T1DM and the effect of urinary tract infections on it, compared with healthy individuales.

2. MATERIALS AND METHODS

2.1 Ethics Statement

The clinic's ethical committee approved the current study of Imam Hussein Hospital No. 240 - in 14 /2/2022 in Karbala, Iraq. All participants in this study willingly participated, and parents of children were contacted prior to enrollment to get their informed written consent for sample collection and research.

2.2 Patients and clinical parameters tests

The practical side of the study was performed at the Al-Husain Teaching Hospital / Karbala, All samples were collected from February 2021 till July, 2021.A total of 90 male children, whose ages ranged between 5-15 years participated in this study ,were diagnosed by podiatrist, 60 patients with T1DM half of them have UTIs and 30 healthy controls (Figure 1). The criteria based on WHO

recommendations were used to diagnose T1DM [14]. The diagnosis of T1DM was based on a typical history of hyperglycaemia according to HbA1C, at least one classic anti-glutamic acid decarboxylase(anti-GAD) were investigated by ELISA, Elabscience – China[15], and patients exhibited impaired connecting peptide (C-peptide) secretion. 30 healthy controls had no history of autoimmune diseases, or malignant diseases. HbA1C and C-peptide were investigated by cobas-e-411 analyzer(Cobas HbA1C and C-peptide kit, Roche–Switzerland)[16]. All patients were confirmed to be free of cancer, allergies, or chronic infection diseases, and they did not take any immunosuppressive medications. Venous blood samples (6 mL) were collected from each person into two types of tubes: 3 ml in EDTA tube for Flow cytometry and HbA1c, the other one is gel tube was allowed to clot at 37°C for 15-20 minutes and then centrifuged at 3000xg for approximately 12-15 minutes in deep freezer (-80°C) [17]. The collected serum from patients and control were used for the measurements of anti-GAD antibodies and C-peptide secretion tests, Interleukin-35 and Macrophage Migration Inhibitory Factor.

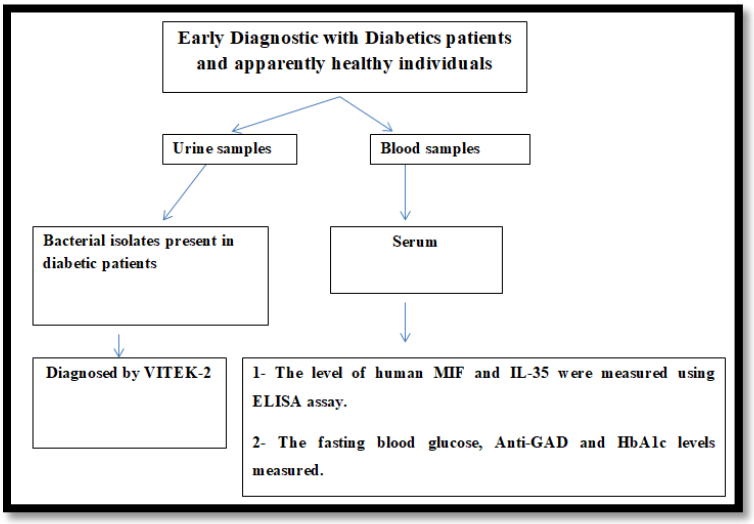

Figure 1: The experiment design scheme.

2.3 Measurement of Interleukin-35 and Macrophage Migration Inhibitory Factor

The level of human IL-35 and MIF were measured using ELISA assay. More specifically, the sandwich ELISA for the quantitative determination of human immunological by used Elabscience kits – China [18].

2.4 Isolation and Identification of Bacteria

Ten milliliters urine samples were collected from all patients and controls. Patients with UTI were separated according to result of inoculated the sample on MacConkey agar and blood agar base aerobically at 37°C for 24 hour. To identify urine samples with substantial bacteriuria, bacterial colonies that developed on the plates following the incubation time were counted. Plates with growth of ≥100 colonies (105 cfu/ml) were considered positive bacteriuria and involved in patient group, while ≤ 100 colonies were excluded[19] . All bacterial isolates present in diabetic patients with urinary tract infections were diagnosed by VITEK-2 instrument (bioMérieux, France) [20].

2.5 Statistical Analysis

The statistical analysis of this study was carried out using the software statistical package for social sciences (SPSS) version 22, where data were expressed as the Mean ± standard Error of the mean, independent-sample T-test with their 95% confidence interval (CI), were used to find the association between the categorical variables, $P \leq 0.05$ was considered statistically significant.The significance value were indicated as * between the groups.The level of probability was indicated as *$P \leq 0.05$, **$P \leq 0.01$ *** $P \leq 0.001$ and **** $P \leq 0.0001$.

3. RESULTS

3.1 Demographic and Clinical Characteristics

Ninety participants were enrolled in the current study, The T1DM males patients included 30 with UTIs and 30 without UTIs . The fasting blood glucose, Anti-GAD and HbA1c levels of the both groups of diabetic patients were significantly higher , the mean ± SE amount were approx 274.97 ± 18.76 mg/dl , 295.72 ± 132.72 U/ml and 10.33 ± 0.42 % , respectively than that of healthy controls. than that of healthy controls . Of note, the C-peptide both groups of T1-DM was lower than that of healthy controls average concentration estimated 0.176 ± 0.07 ng/ml.

3.2 Interleukin 35 (IL-35) concentration

As shown in (Figure 2), serum IL-35 levels were higher for both patients groups of T1DM compared to control . Additionally, the outcomes revealed a substantial rise in IL-35 levels for T1DM without bacterial infection the mean was (7.091 ±1.85) and with bacterial infection the mean was (10.42±1.56) ng/ml) on level ($P \leq 0.0001$ and $P \leq 0.001$) respectively compared to control (5.514 ±0.20) ng/ml . When compared to diabetic patients without bacterial infections, IL-35 levels in those patients were significantly higher on level $p \leq 0.05$.

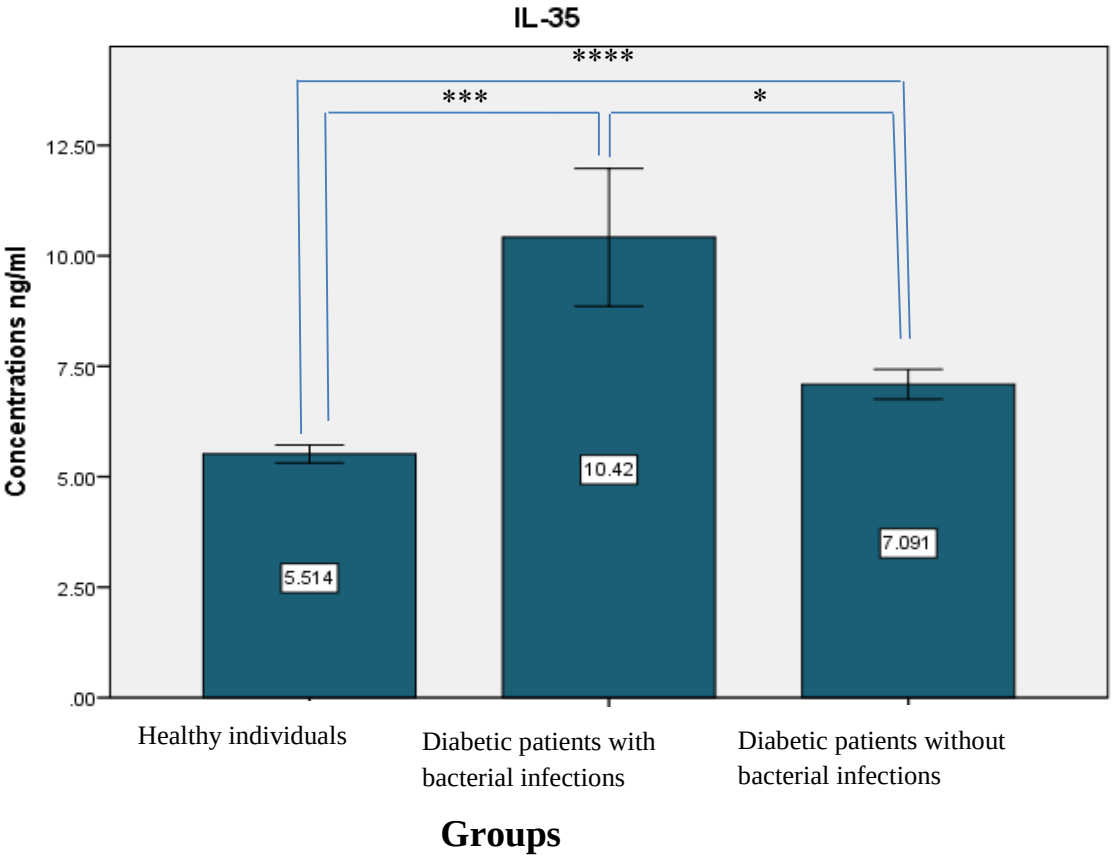

Figure 2: The concentration of IL-35 in healthy individuals,diabetic patients with bacterial infections and diabetic patients without bacterial infections. The significance value were indicated as * between the groups.The level of probability was indicated as *$P \leq 0.05$, **$P \leq 0.01$ *** $P \leq 0.001$ and **** $P \leq 0.0001$.

3.3. Macrophage Migration Inhibitory Factor (MIF) concentration

Data results show in (Figure 3), serum MIF levels were higher for both patients groups of T1DM compared to control . Additionally, the outcomes revealed a substantial rise in MIF levels for T1DM with bacterial infection the mean was (29.68 ±7.18) ng/ml and T1DM without bacterial infection the mean was (9.047 ±1.47) ng/ml on level ($P \leq 0.001$) compared with healthy individuals (4.685 ±0.52) ng/ml . MIF levels were significantly elevated for diabetic patients with bacterial infections compared with patients without bacterial infection on levels ($P \leq 0.001$).

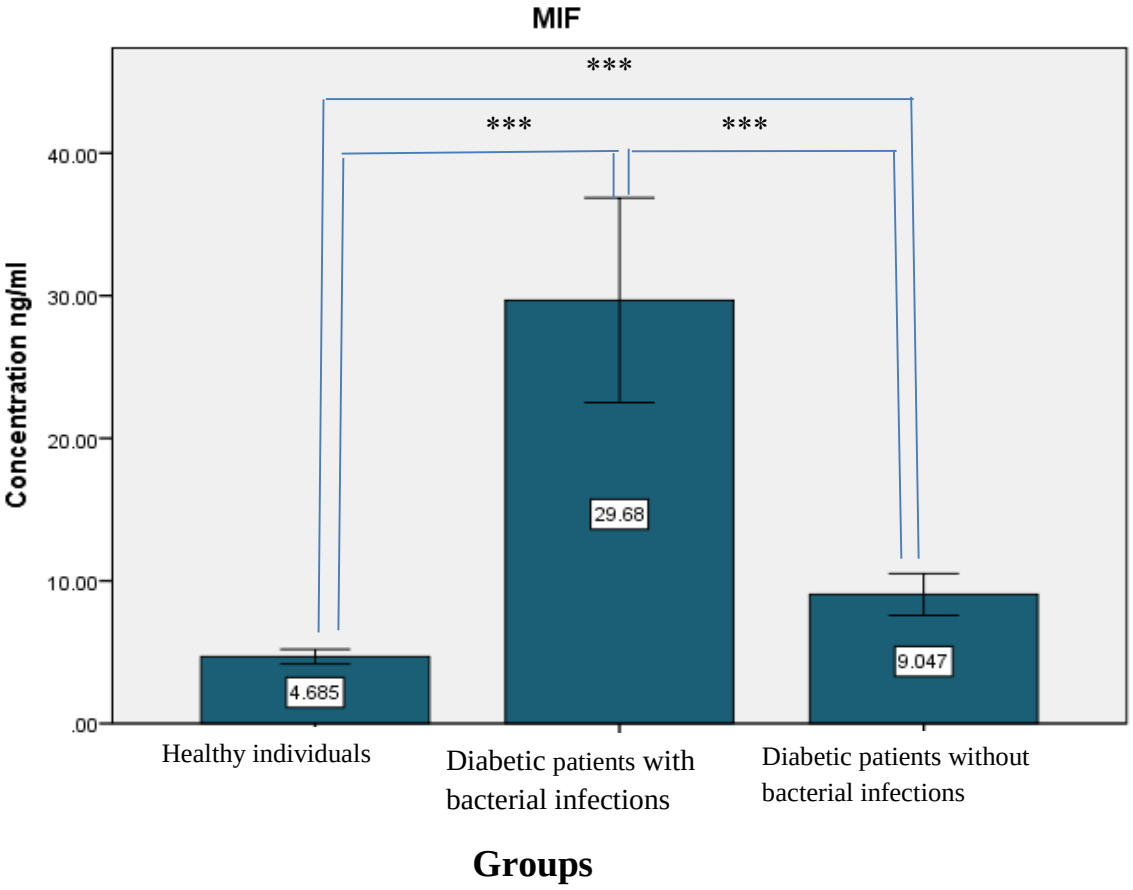

Figure 3: The concentration of MIF in healthy individuals, diabetic patients with bacterial infections and diabetic patients without bacterial infections. The significance value were indicated as * between the groups.The level of probability was indicated as *$P \leq 0.05$, **$P \leq 0.01$ *** $P \leq 0.001$ and **** $P \leq 0.0001$.

3.4. The bacterial etiology of urinary colonization in diabetes

Data results showed in (Table 1) ,The bacterial pathogens that causing UTI belongs to gram negative 54% and gram-positive bacteria 46%. Among Gram negative pathogens, *E. coli* li is the principal etiology of UTI in diabetic individuals If estimated at 35% followed by *P. stutzeria* while among Gram positives, *K. rosea* is the major uropathogen estimated at 19% followed by *S. thoraltensis* , *S. gallolyticus* , *S. haemolyticus* , *K. kristinae*.

Table 1. Bacterial etiology of UTI in diabetic individuals

No.	Types of bacteria	Number of strains	%

4.

1	***Escherichia coli***	19	35
2	***Pseudomonas stutzeria***	10	19
3	***Kocuria rosea***	10	19
4	***Staphylococcus haemolyticus***	2	4
5	***Streptococcus gallolyticus***	4	7
6	***Kocuria kristinae***	1	2
7	***Streptococcus thoraltensis***	8	15
		∑= **54**	

DISCUSSION

Lower immunity and recurring bacterial infections are common in T1DM.The diagnosis is made on the basis of the confirmatory screening test, if a patient meets the diabetes criterion of the HbA1c results ≥ 6.5% and FPG ≤125 mg/ dL, that person should be considered to have diabetes. After confirming that the children had diabetes, screening tests were conducted to prove that they had type 1 diabetes such as detect autoantibodies produced by B-cell activation to GAD and the concentration of C-peptide [21]. The children were chosen to investigated altered frequency of regulatory B- cells in the target groups in the current study, because the disease is in its early stages and provides more accurate information about the changes that occur in the immune system [22].

A protein produced inside the body that suppresses the immune system and has anti-inflammatory properties is known as IL-35 [23]. Our results revealed that diabetes patients' serum IL-35 levels were significantly higher than those of controls. These outcomes are in line with a study carried out by Espes *et al.*, 2017, and Zhang *et al.*, 2023 demonstrated that the elevated levels of anti- inflammatory cytokines in T1DM patients may be due to the production from a compensatory mechanism to the rise of pro-inflammatory cytokines [24,25]. For this reason, the cytokines linked to the pathogenesis of T1DM are essential for the onset of the disease as they can act alone or in a cascade to control inflammation both positively (pro-inflammatory cytokines) and negatively (anti-inflammatory cytokines) [26].

In comparison to controls, T1DM patients had higher serum levels of MIF. This finding is consistent with Ismail, *et al.*, 2016 who noted that this cytokine contributes to the destruction of pancreatic beta cells by the immune system, favoring the inflammatory and autoimmune response that is typical of type 1 diabetes [27]. Furthermore, our results are in accordance with results obtained by do Nascimento de Oliveira, *et al*., 2018 who found that newly diagnosed T1DM patients had high levels of inflammatory markers, indicating that systemic inflammation may have contributed to the disease's onset. The elevated peripheral MIF levels in children, however, point to a powerful function throughout the early years of the disease that may help to explain the cytokine storm connected to the first stage of T1DM. Strategies for primary prevention that focus on inflammatory-mediated comorbidity may shield these patients from secondary problems in the future [28].

During insulitis, high levels of MIF are secreted by effector T-cells to trigger the β-cell destruction process . Additionally, MIF has been identified as a key player in the progression of T1DM complications like diabetic foot disease and has been shown to support inflammatory cytokines and palmitic acid-induced pancreatic islet apoptosis [29].

MIF has been described as a critical mediator of LPS-induced shock, suggesting its detrimental role in septic shock by Gram-negative bacteria. Recent findings demonstrate that MIF modulates Toll-like receptor 4 (TLR4). The TLR4 senses LPS, the abundant cell-wall component of Gram-negative bacteria [8]. MIF was suggested as a potential target for the therapy of T1DM following the successful neutralization of MIF by pharmacological and antibody-mediated mechanisms [30].

A bidirectional relationship exists between infectious diseases and T1DM: on the one hand, poor glycemic control raises the risk of infections, and on the other, infectious diseases can occasionally be the trigger for metabolic decompensation leading to diabetic ketoacidosis in both newly diagnosed and long-term patients [31,32]. High blood sugar levels alter tissues, the skin, and blood flow, which all raise the risk of infections by reducing the immune system's activity. [33].

The most common bacteria isolated in the current study were *E. coli* 35% , *P. stutzeria* 19% and *K. rosea*19% . This was shown by a previous study conducted by Akash et al. (2020) who reported that *E.coli* and *Klebsiella* spp. were commonly associated with UTIs for T1DM [34].

E coli is the most frequent cause of UTI in both men and women with DM, however multiple series show that this organism only accounts for a small part of both nosocomial and community-acquired UTI in diabetic patients when compared to age-matched nondiabetic individuals [35]. Although the exact causes of UTIs in diabetics being higher than in non-diabetics are unknown, some studies have suggested that immunodeficiency, a modified urothelium (which results in a higher bacterial adhesion), and chronic neurologic bladder dysfunction may be to blame. Urine glucose excretion has also been linked to UTIs [36].

CONCLUSIONS

These results suggested that Type 1 diabetes mellitus and Urinary tract infections affects cytokines secretion, suggesting that cytokines -directed therapies might offer new treatment opportunities for inflammatory and autoimmune diseases in the future.

ACKNOWLEDGMENTS

Authors would like to express gratitude to Parents of Children and all the people participating in this study.

Conflicts of interest

No potential conflict of interest.

REFERENCES

1. Tullus, K., & Shaikh, N. (2020). Urinary tract infections in children. *The Lancet*, *395*(10237), 1659-1668.

2. Chaurasia, R., & Jain, A. (2019). Self Assessment & Review of Microbiology & Immunology. JP Medical Ltd.

3. Herold, K. C., Vignali, D. A., Cooke, A., & Bluestone, J. A. (2013). Type 1 diabetes: translating mechanistic observations into effective clinical outcomes. *Nature Reviews Immunology*, *13*(4), 243-256.

4. Lv, Q., Li, Z., Sui, A., Yang, X., Han, Y., & Yao, R. (2022). The role and mechanisms of gut microbiota in diabetic nephropathy, diabetic retinopathy and cardiovascular diseases. *Frontiers in Microbiology*, *13*.

5. Gouda, W., Mageed, L., Abd El Dayem, S. M., Ashour, E., & Afify, M. (2018). Evaluation of pro-inflammatory and anti-inflammatory cytokines in type 1 diabetes mellitus. *Bulletin of the National Research Centre*, *42*(1), 1-6.

6. Lu, J., Liu, J., Li, L., Lan, Y., & Liang, Y. (2020). Cytokines in type 1 diabetes: mechanisms of action and immunotherapeutic targets. *Clinical & translational immunology*, *9*(3), e1122.

7. Francisco, L. M., Sage, P. T., & Sharpe, A. H. (2010). The PD-1 pathway in tolerance and autoimmunity. *Immunological reviews*, *236*(1), 219-242.

8. Kim, B. S., Tilstam, P. V., Arnke, K., Leng, L., Ruhl, T., Piecychna, M., ... & Bucala, R. (2020). Differential regulation of macrophage activation by the MIF cytokine superfamily members MIF and MIF-2 in adipose tissue during endotoxemia. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*, *34*(3), 4219.

9. Chen, Z., Bozec, A., Ramming, A., & Schett, G. (2019). Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nature Reviews Rheumatology*, *15*(1), 9-17.

10. Martínez de Marañón Peris, A. (2021). Study of mitochondrial dynamics and function and autophagy and their relationship with cardiovascular complications in type 2 diabetes.

11. Peleg, A. Y., Weerarathna, T., McCarthy, J. S., & Davis, T. M. (2007). Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. Diabetes/metabolism research and reviews, 23(1), 3-13.

12. Mirza, B. A., Syed, A., Izhar, F., & Ali Khan, A. (2010). Bidirectional relationship between diabetes and periodontal disease: review of evidence. *J Pak Med Assoc*, *60*(9), 766-8.

13. Pascale, A., Marchesi, N., Govoni, S., Coppola, A., & Gazzaruso, C. (2019). The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases. *Current opinion in pharmacology*, *49*, 1-5.

14. American Diabetes Association Professional Practice Committee, & American Diabetes Association Professional Practice Committee:. (2022). 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2022. Diabetes Care, 45(Supplement_1), S17-S38.

15. Sasano, M., Kimura, S., Maeda, I., & Hidaka, Y. (2017). Analytical performance evaluation of the Elecsys® Cyclosporine and Elecsys® Tacrolimus assays on the cobas e411 analyzer. *Practical laboratory medicine*, *8*, 10-17.

16. Dahman, L. S. B., Sumaily, K. M., Sabi, E. M., Hassan, M. A., Thalab, A. M. B., Sayad, A. S., ... & Alhadhrmi, F. M. (2022). A comparative study for measuring serum ferritin levels with three different laboratory methods: enzyme-linked immunosorbent assay versus Cobas e411 and Cobas Integra 400 methods. *Diagnostics*, *12*(2), 320.

17- Akram, N. N., Abdullah, W. H., & Ibrahim, B. A. (2022). Factors contribute to elevated blood pressure values in children with type 1 diabetes mellitus: A review. *Medical Journal of Babylon*, *19*(2), 126.

18. Xie, S., Fan, R., Tang, Q., Cai, X., Zhang, H., Wang, F., ... & Jiang, W. (2022). Identification of robust biomarkers for early predicting efficacy of subcutaneous immunotherapy in children with house dust mite-induced allergic rhinitis by multiple cytokine profiling. *Frontiers in Immunology*, *12*, 5866.

19. Ness, D., & Olsburgh, J. (2020). UTI in kidney transplant. *World Journal of Urology*, *38*(1), 81-88.

20. Paul, A.G.(2019). Lab Manual and Workbook in Microbiology 12th Edition .

21.Khudhur KH, Al-Ani MH. Hematological parameters in children with type-1 diabetes. Med J Babylon 2019;16:184-7

22. Chiang, J. L., Maahs, D. M., Garvey, K. C., Hood, K. K., Laffel, L. M., Weinzimer, S. A., ... & Schatz, D. (2018). Type 1 diabetes in children and adolescents: a position statement by the American Diabetes Association. *Diabetes care*, *41*(9), 2026.

23. Li, M., Eckl, J., Geiger, C., Schendel, D. J., & Pohla, H. (2017). A novel and effective method to generate human porcine-specific regulatory T cells with high expression of IL-10, TGF-β1 and IL-35. *Scientific Reports*, *7*(1), 1-13.

24.Espes, D., Singh, K., Sandler, S., & Carlsson, P. O. (2017). Increased interleukin-35 levels in patients with type 1 diabetes with remaining C-peptide. *Diabetes Care*, *40*(8), 1090-1095.

25.Zhang, S. M., Liang, J., Xia, J. P., Li, L., Zheng, L., Wang, Y. L., ... & Lu, Y. (2023). Interleukin 35: protective role and mechanism in type 1 diabetes. *Central European Journal of Immunology*, *48*(1).

26. Chatzigeorgiou, A., Harokopos, V., Mylona-Karagianni, C., Tsouvalas, E., Aidinis, V., & Kamper, E. (2010). The pattern of inflammatory/anti-inflammatory cytokines and chemokines in type 1 diabetic patients over time. *Annals of medicine*, *42*(6), 426-438.

27. Ismail, N. A., Abd El Baky, A. N., Ragab, S., Hamed, M., Hashish, M. A., & Shehata, A. (2016). Monocyte chemoattractant protein 1 and macrophage migration inhibitory factor in children with type 1 diabetes. *Journal of Pediatric Endocrinology and Metabolism*, *29*(6), 641-645.

28. do Nascimento de Oliveira, V., Lima-Neto, A. B. M., Van Tilburg, M. F., de Oliveira Monteiro-Moreira, A. C., Duarte Pinto Lobo, M., Rondina, D., ... & Guedes, M. I. F. (2018). Proteomic analysis to identify candidate biomarkers associated with type 1 diabetes. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 289-301.

29. Sánchez-Zamora, Y. I., & Rodriguez-Sosa, M. (2014). The role of MIF in type 1 and type 2 diabetes mellitus. *Journal of diabetes research*, *2014*.

30. Wołoszyn-Durkiewicz, A., & Myśliwiec, M. (2019). The prognostic value of inflammatory and vascular endothelial dysfunction biomarkers in microvascular and macrovascular complications in type 1 diabetes. *Pediatric Endocrinology Diabetes and Metabolism*, *25*(1), 28-35.

31. Piccolo, G., De Rose, E. L., Bassi, M., Napoli, F., Minuto, N., Maghnie, M., ... & d'Annunzio, G. (2022). Infectious diseases associated with pediatric type 1 diabetes mellitus: A narrative review. *Frontiers in Endocrinology*, *13*.

32. Nada, S. Z., & Abdulhasan, F. A. (2022). Prediction of Chronic Kidney Disease in Type 2 Diabetes Mellitus patients. *HIV Nursing*, *22*(2), 1209-1213.

33. Sellami, M., Gasmi, M., Denham, J., Hayes, L. D., Stratton, D., Padulo, J., & Bragazzi, N. (2018). Effects of acute and chronic exercise on immunological parameters in the elderly aged: can physical activity counteract the effects of aging?. *Frontiers in immunology*, *9*, 2187.

34. Akash, M. S. H., Rehman, K., Fiayyaz, F., Sabir, S., & Khurshid, M. (2020). Diabetes-associated infections: development of antimicrobial resistance and possible treatment strategies. *Archives of microbiology*, *202*, 953-965.

35. Stapleton, A. (2002). Urinary tract infections in patients with diabetes. *The American journal of medicine*, *113*(1), 80-84.

36. Lee, Y. C., Chen, T. H., Hsiao, M. C., Hung, P. H., Tung, S. H., & Hsiao, C. Y. (2020). Glycated hemoglobin< 6.5% is associated with uroseptic shock in diabetic patients with urinary tract infection. *Frontiers in Medicine*, *7*, 515506.