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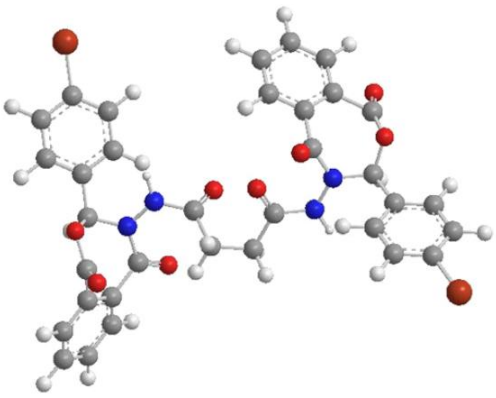

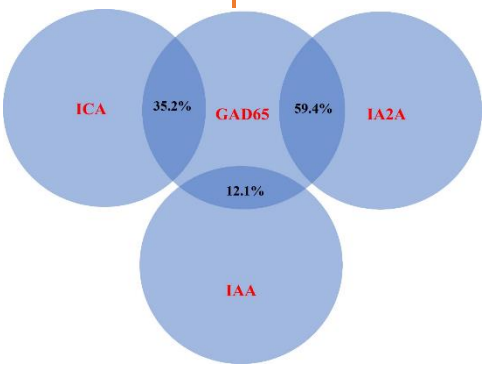

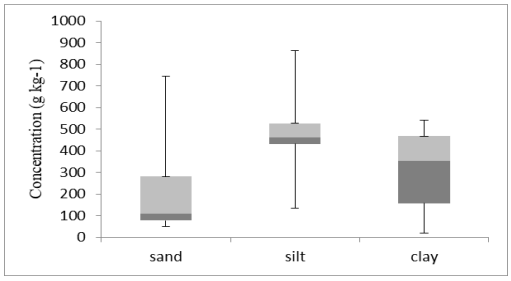

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Role of Cytokines (IL-17 & IL-33), FGF-18, and WNT-5 in the Pathogenesis of Patients with Established Type II Diabetes

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Abstract

Background: Biomarker investigation is important to detect the presence/severity of hyperglycemia, implying diabetes/pre-diabetes, or over time, as a risk factor for diabetic retinopathy, nephropathy, and other vascular complications related to diabetes. **Objectives:** To determine the role and level of certain cytokines, FGF-18, and WNT5A in the serum of diabetic patients. **Patients and Methods:** A case-control comparative study was conducted on 50 healthy individuals and 100 diabetic patients. Then; a validated questionnaire was used to collect the participants' data. Next, participants' serum level of IL-17, IL-33, FGF-18, and WNT5A was determined using the ELISA technique and the blood level of glycated hemoglobin (HbA1C). **Results:** A highly significant difference in the levels of HbA1C and FGF-18 and a significant difference in levels of IL-17 and IL-33, with no significant difference in WNT-5A level, were seen between the studied groups. There was no significant difference between the biomarkers level and each gender, age, BMI, and duration of DM in the patients ($p>0.05$). A positive correlation was observed between the number of symptoms in the patients and levels of IL-17 ($p=0.003$) and IL-33 ($p=0.018$). Also, a positive correlation between IL-17 and IL33 ($Rho=0.874$, $p<0.001$), IL-17 and FGF-18 ($p=0.023$), as well as between IL-33 and FGF-18 ($p=0.041$) were seen. **Conclusion:** It is realized that IL-17, IL33, and FGF-18 expression are directly related to DM, Additionally, a positive correlation between most biomarkers was seen. The detection of the cytokines' levels and their relations with diabetic will affect the diagnosis, knowledge about the disease immunology, and thereafter effects on the treatments of patients with diabetic depending on our findings.

Introduction

Diabetes mellitus (DM) is a complex disease affecting almost every tissue and organ system, with metabolic ramifications extending far beyond impaired glucose metabolism [1]. DM has become a global burden due to its high prevalence, growth rate, and long-lasting related complications [2].
The International Diabetes Federation (IDF) reported that 463 million people aged 20-79 years are living with diabetes, which might increase to 700 million by 2045. In addition, diabetes is among the top ten leading causes of mortality in the world [3, 4].
Knowledge of risk predictors of other acute/chronic diseases in type 2 diabetes mellitus (T2DM) is crucial given the frequent coexistence of conditions and the fact that T2DM doubles the risk of these diseases.

Consequently, investigations of blood biomarkers are very potential for these patients to protect them from the threat of other conditions, including heart failure, as it is increasingly recognized as an important endpoint trials in T2DM. Thus, the diagnostic and prognostic performance of established biomarkers may be modified in T2DM patients [5].

Cytokines act as pleiotropic polypeptides regulating inflammatory and immune responses through practical actions on vital cells. They provide essential signals in the pathophysiology of various diseases, including DM. The role of adaptive immune cytokines in the pathogenesis of type-1 Diabetes Mellitus (T1DM) is well known [6]. Even though reports on the serum levels of T cell cytokines, including interleukin (IL)-17 and IL33 in T2DM, are scarce. However, studies revealed an elevation in IL-17 and a reduction in IL-33 levels in T2DM patients, with a marked relationship between them and the onset/progression of diabetic nephropathy (DN) [7]. Fibroblastic growth factor (FGF) plays a vital role in many physiological processes, including inflammation, angiogenesis, and skeletal development, especially FGF-18, that have a potential impact when incorporated with hydrogel and scaffolds showing implicit bone regeneration [8]. Regarding the Wingless-related integration site (WNT-5A), it was revealed that WNT receptor ROR2 is essential for WNT-5A-mediated wound healing stimulation in diabetic limbal epithelial stem cells [9]. However, the role of FGF-18 and WNT-5A in the pathogenesis of T2DM has yet to be studied. Therefore, this study was designed to find the concentrations of FGF-18, WNT-5A, IL-17, and IL-33 level in patients with T2DM.

Materials and Methods

Study design and setting

This case-control comparative study was conducted on 100 T2DM patients and 50 healthy controls from August to November 2022 at Diabetes Center and Shar Teaching Hospital in Sulaimaniyah, Iraq.

Inclusion criteria

All patients with a confirmed diagnosis had T2DM, regardless of age and gender or nationality.

Exclusion criteria

Patients with liver disease, renal failure, fever, autoimmune disease, and inflammatory disease were excluded, together with alcoholism, smokers, and pregnant/breastfeeding females.

Questionnaire

A detailed questionnaire for collecting participants' sociodemographic measures, including age, gender, body mass index (BMI), duration/symptoms of diabetes, complication, history of hospitalization, and checking of blood sugar, was used.

Collection of blood sample

About 5.0 ml venous blood was taken from each participant using the venipuncture technique, then 3.0 ml as whole blood was collected in an EDTA tube for an HbA1c test, and the remaining 2.0 ml was contained in a clot activator tube, allowed to clot at room temperature for 10 min, then centrifuged at 4500 round per minute (RPM) for 10 min. The supernatants were collected carefully, and the separated sera were stored in a freezer at -70 ^{0}C until use.

Biomarker detection

Human ELISA kits of IL-17 (Cat. No. E-EL-H0105), IL-33 (Cat. NO. E-EL-H2402), and FGF-18 (Cat. No. E-EL-H5434), using Elabscience ELISA kit from USA, and WNT5A (Code E6012Hu) by Biotechnology Laboratory (BT LAB) were used to determine the level of Biomarkers in the studied participants serum according to the manufacturers' company instructions with minor modifications based on biotin double antibody sandwich technology.

Biochemical investigation

HbA1c test

The glycated hemoglobin (Hb) in the blood was determined using Cobass c311 (Roche, Germany) in automatic analyzer according to the instructions of the manufacturer company.

Ethical considerations

The Scientific and Ethics Committees approved the study protocol at the College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq, with approval number (No.199 on September28,2021). All parameters were done according to the Declaration of Helsinki. Participants' written consent was obtained before starting the study.

Statistical analysis

All recorded questionnaire was coded and given an identifying number (ID). The data was set into a Microsoft Excel Spreadsheet; after data sorting, the data was transported into Statistical Package for Social Science (SPSS) (version 26) software and GraphPad Prism (8). Descriptive statistics (number and percentage) were calculated for each variable, and the analytical statistic was done to find the association between the variables. The relation between variables was calculated using Person's Chi-square ($\chi 2$). The quantitative variables were presented as Mean±SD. Mann-Whitney test was used to determine the significant differences between the studied groups. $P \leq 0.05$ is considered a significant difference. Also, the Spearman correlation test (Rho) was used to determine the correlation between the biomarkers and a Rho<0.05 was considered a significant correlation.

Results

Participant's characteristics

The mean age of individuals in the healthy group was 38.22±10.91 years, and most of them (54%) were females. The mean age of patients was 57.80±10.53 years, and most (68%) were females. There was no significant difference in gender (p=0.093), with a highly considerable age difference (p<0.001) between the studied groups. Regarding the body mass index (BMI), most of the healthy individuals (46%) were overweight with a mean of 26.93±4.45 kg/m^2, while most patients (45%) were obese with a mean BMI of 30.36±6.29 kg/m^2 (p=0.022). Most patients (84%) and healthy individuals (90%) lived in Sulaimaniyah City (p=0.413) (Table 1).

The mean duration of diabetic patients was 10.01 ± 7.55 years; 40% had the disease for > 10 years, 36% for 5-10 years and 24% for <5 years. Regarding the DM symptoms in the patients, 78% had more than one symptom, 5% associated with increasing thirst, 4% had frequent urination during the night, 2% had dry mouth, and 11% had other symptoms. Most had microvascular complications (54%), 8% had macrovascular, and 38% had both difficulties. Also, most patients (63%) were admitted to the hospital more than once due to the disease; 23% had a one-time history of hospitalization, while 63% had no history of hospitalization (Table 1).

Table 1. Distribution of studied participant's characteristics.

Variable		**Healthy (*n*=50)**	**Patient (*n*=100)**	**p-value**
		Number, %		
Gender	Female	27 (54)	68 (68)	0.093
	Male	23 (46)	32 (32.0)	
Age (Years)	< 40	24 (48)	3.0 (3.0)	<0.00 **
	40-60	26 (52)	43 (43)	
	> 60	0.0 (0.0)	54 (54)	
BMI (kg/m^2)	Normal weight	16 (32)	24 (24)	0.022*
	Overweight	23 (46)	31 (31)	
	Obese	11 (22)	45 (45)	
Disease's duration (Years)	< 5	0.0 (0.0)	24 (24)	NA
	5-10	0.0 (0.0)	36 (36)	
	>10	0.0 (0.0)	40 (40)	
Symptoms	Increase thirst	0.0 (0.0)	5.0 (5.0)	NA
	Frequent urination at night	0.0 (0.0)	4.0 (4.0)	
	Dry mouth	0.0 (0.0)	2.0 (2.0)	
	More than one symptom	0.0 (0.0)	78 (78)	
	Others	0.0 (0.0)	11 (11)	

Complication	Microvascular	0.0 (0.0)	54 (54)	NA
	Macrovascular	0.0 (0.0)	8.0 (8.0)	
	Both	0.0 (0.0)	38 (38)	
History of hospitalization	None	0.0 (0.0)	63 (63)	NA
	One time	0.0 (0.0)	23 (23)	
	> One time	0.0 (0.0)	14 (14)	

BMI= Body mass index; NA= not applicable; *: Significant difference; **: Highly significant difference

Most patients (41%) were not checked their blood glucose continuously, 21% checked, and 38% checked once in a while. Most patients were checked in the morning (38%), followed by the afternoon (20%), and then by evening (1%). Moreover, most (94%) patients were taking medication for DM; 65.96% were taking pills, 1.06% had injections, 32.98% were taking both, and 6% did not use any drug for their disease (Table 2).

Table 2. Characteristics of the patients.

Variable		**Number, %**
How often check blood sugar	Yes	21 (21)
	No	41 (41)
	Once in a while	38 (38)
What time of day check blood glucose	Not	41 (41)
	Morning	38 (38)
	Afternoon	20 (20)
	Evening	1.0 (1.0)
Take medication for diabetes	Yes	94 (94)
	No	6.0 (6.0)
Medication dosage form	Pill	62 (65.96)
	Injection	1.0 (1.06)
	Both	31 (32.98)

There was a highly significant difference in the HbA1C level between healthy individuals (4.89±0.42) and the patients (9.84±1.87) ($p<0.001$) (Table 3).

Table 3. The mean level of HbA1C in healthy individuals and patients.

	Healthy (*n* =50)	**Patient (*n*=100)**	**p-value**
	Mean ± SD		
HbA1C	4.89 ± 0.42	9.84 ± 1.87	<0.001 **

HbA1C: Glycated hemoglobin; **: Highly significant difference

The highest mean serum concentration of IL-17, IL33, and FGF-18 was seen in patients (8.84±7.38, 10.87±6.11, and 27.24±22.17, respectively) than in healthy individuals (4.97±4.70, 4.14±3.58, and 6.30±4.82, respectively) with p-value of p=0.047, p=0.014 and$p<0.001$) respectively. There was no significant difference in WNT-5A level between both studied groups (p=0.129) (Figure 1).

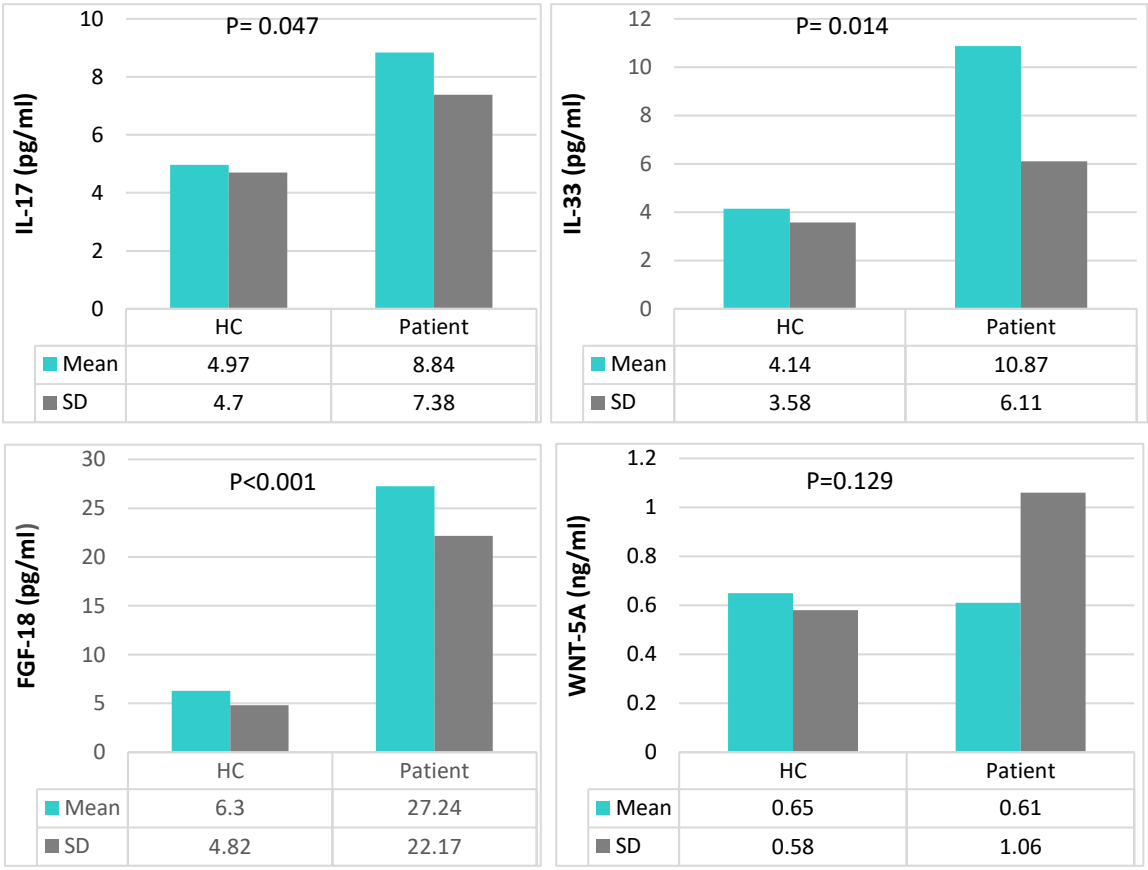

Figure 1. Comparisons of the serum concentration of IL-17 (upper left), IL-33 (upper right), (FGF-18 (lower left) and WNT-5A (lower right) between the studied groups. HC: Healthy control; IL: Interleukin; FGF: Fibroblastic growth factor; WNT-5A: Wingless-related integration site

There was no significant difference between the biomarkers level and each gender, age, BMI, and duration of DM in the patients (p>0.05) (Table 4).

Table 4. Serum level of the biomarkers in diabetic patients.

Variable		**Biomarker Level (Mean ± SD)**			
		IL-17	**IL-33**	**FGF-18**	**WNT-5A**
Gender	Female	10.01 ± 15.14	11.25 ± 18.20	30.45 ± 40.42	0.59 ± 1.14
	Male	6.67 ± 8.44	10.04 ± 19.71	20.44 ± 24.00	0.68 ± 0.86
	p-value	0.776	0.174	0.474	0.263
Age (Years)	< 40	12.53 ± 11.95	19.00 ± 16.45	5.07 ± 4.39	0.70 ± 1.21
	40-60	8.44 ± 14.06	11.21 ± 20.36	28.27 ± 27.99	0.48 ± 0.78
	> 60	9.13 ± 13.15	10.14 ± 17.42	27.66 ± 42.34	0.71 ± 1.24
	p-value	0.740	0.563	0.245	0.504
Duration of Diabetes (Years)	< 5	12.16 ± 15.65	14.41 ± 20.15	24.34 ± 23.61	0.60 ± 1.52
	5-10	8.32 ± 13.85	9.04 ± 16.90	20.57 ± 23.22	0.68 ± 0.89
	>10	7.56 ± 11.46	10.39 ± 19.28	35.00 ± 48.99	0.56 ± 0.87
	p-value	0.356	0.551	0.558	0.593
BMI	Normal Weight	7.18 ± 11.38	7.45 ± 14.98	17.27 ± 26.38	0.60 ± 0.78
	Overweight	10.34 ± 15.66	13.22 ± 20.78	35.66 ± 53.70	0.74 ± 0.89
	Obese	8.91 ± 12.92	11.07 ± 18.88	26.76 ± 22.56	0.54 ± 1.28
	p-value	0.401	0.105	0.091	0.115

IL: Interleukin; FGF: Fibroblastic growth factor; WNT-5A: Wingless-related integration site

A positive correlation was observed between the number of symptoms in the patients and serum concentration of IL-17 (p=0.003, Rho=0.301) and IL-33 (p=0.018, Rho=0.238). Otherwise, no correlation was noted (Table 5).

Table 5. Correlation between the number of symptoms in the patients and the biomarkers' level.

Number of symptoms	Correlation	Biomarker			
		IL-17	IL-33	FGF-18	WNT-5A
	Spearman's Rho	0.301	0.238	0.001	0.05
	p-value	0.003**	0.018*	0.923	0.625

IL: Interleukin; FGF: Fibroblastic growth factor; WNT-5A: Wingless-related integration site
*: Significant correlation at level 0.05, **: Significant correlation at level 0.01

In respect of the correlation between the biomarkers, there was a positive correlation between IL-17 and IL33 (Rho=0.874, p<0.001), IL-17 and FGF-18 (Rho=0.228, p=0.023), as well as between IL-33 and FGF-18 (Rho=0.204, p=0.041)(Figure 2).

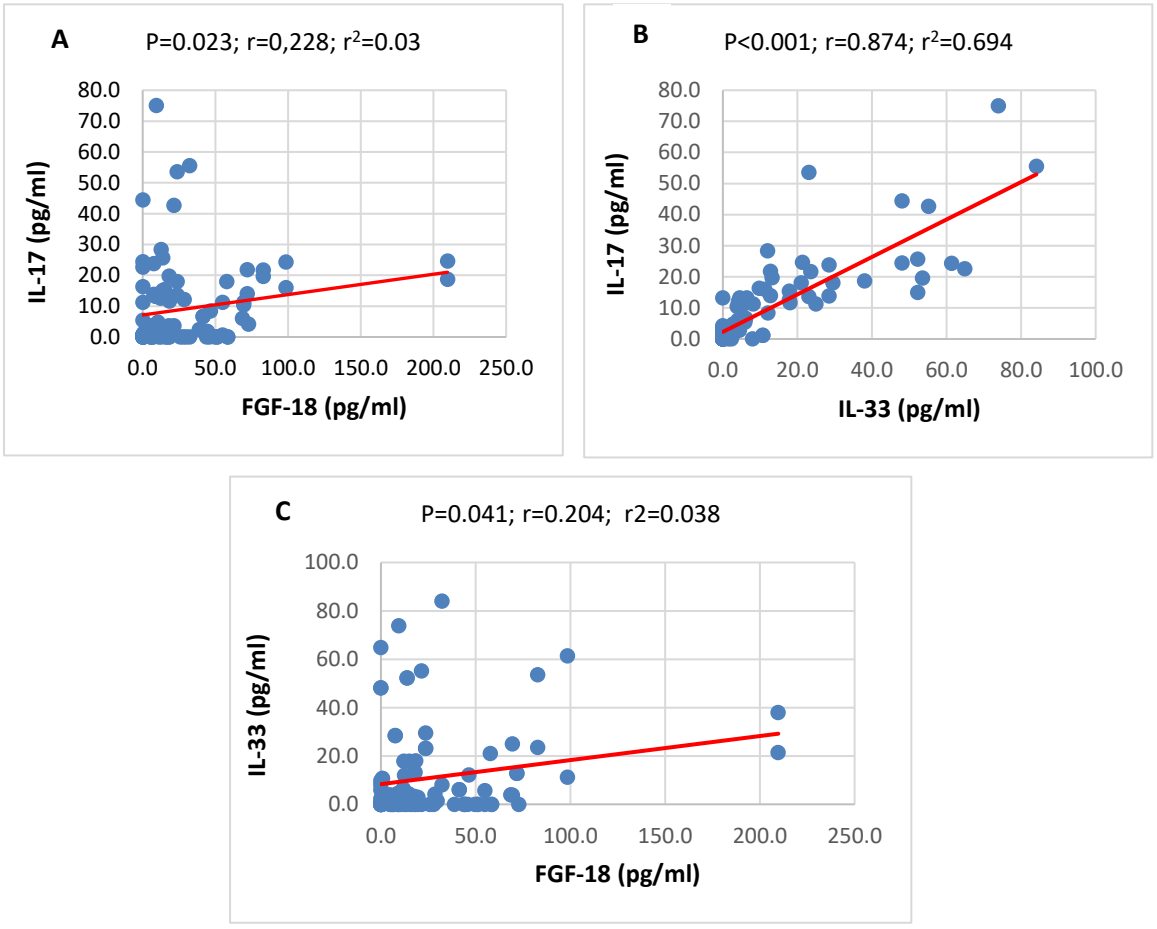

Figure 2. Correlation between IL-17 and FGF-18 (A); IL-17 and IL-33 (B); IL-33 and FGF-18 (C) in the studied patients. IL: Interleukin; FGF: Fibroblastic growth factor; WNT-5A: Wingless-related integration site.

Discussion

Currently, there is a limited number of studies on the performance of biomarkers for patients with T2DM in Iraq, including the Kurdistan region. More studies have investigated the role of biomarkers for the

prognostication of general risk, but without referring mainly to the position of biomarkers in the pathogenesis of the disease [10]. Thus, this study is designed to determine the expression of some biomarkers, including IL-17, IL-33, FGF-18, and WNT-5, in patients with T2DM and their role in the pathogenesis of the disease.

In the current study, the sociodemographic characteristic of diabetic patients showed that most of respondents were females (68%), aged >60 years old (54%), obese (45%), had the disease for >10 years (40%), presented with more than one symptom (thirsty, frequent urination at night, dry mouth, etc.) (78%), had microvascular complication (54%), and non-hospitalized due to the T2DM (63%). In this regard, Sangar et al. 2023 in Sulaimaniyah, Iraq, realized that most diabetic patients were males (51.6), aged >60 years (66.2%), non-insulin-dependent (74.2%), had diabetes for 10-15 years (35.5%) with normal BMI (18.5-24.5 Kg/m^2) and independent premorbid activity (87.1%) [11]. Similarly, another study reported that most diabetic patients were males (72.3%), with an average age of 66.1 years, had the disease for >18 years, and 83.5% had macrovascular complications [12]. However, another study reported that most diabetic patients were females (60.7%) and had an average age of 63.92±1.07 years with an average disease duration of 10.59±15.6 years [13]. These variations might be related to the family history of the disease, educational level, economic status, severity of the disease, lifestyle (physical activity, diet, stress), sample size, environmental condition, and personnel factors (type/dose of used medications for the disease and disease follow-up). T2DM develops at various ages, but its incidence rate, prevalence rate, and mortality tend to increase sharply with age. Therefore, age at diagnosis and duration of the disease are considered to be very important indexes for determining treatment and prognosis [14].

Furthermore, in this study, most patients were from Sulaimaniyah City (90%), followed by Halabja (6%), and then Erbil (4%). These variations might be related to the sample collections being done inside Sulaimaniyah Hospitals, and most patients were Sulaimaniyah residents. Additionally, most patients (41%) were not checking their blood sugar continuously, 38% were checking their sugar in the morning, and 94% were taking medication for T2DM; in the form of a pill (65.96%) with a mean HbA1c of 9.84±1.87%. Generally, these factors are affected by the patient's care about the disease's seriousness, correct medical/clinical guidance, family support and remaindering, educational level, availability of the tests, and proper following up of the patients. In this respect, a study in Korea on 651 T2DM patients revealed that their BMI was 24.7±4.2 kg/m^2 (overweight), their age at diagnosis was 45.3±11.6 years, the duration of diabetes was 8.4±7.9 years, and HbA1c was 9.6 ± 3.8% [14] which is similar to the value of this study.

Moreover, some emerging proinflammatory biomarkers (cytokines), including IL-17 and IL-33, were investigated in this study. The results showed the highest serum mean of serum IL-17 and IL33 in patients to be 8.84±7.38 and 10.87±6.1 pg/mL, respectively, rather than in healthy individuals (control group) that were 4.97±4.70 and 4.14±3.58 pg/mL, respectively, with significant difference between both studied groups ($p<0.05$). In this respect, a study in Croatia on 190 T2DM patients found the value of IL-17 to be 3.291 pg/mL [14], which is not aligned with our outcomes. Regarding the IL-33 in diabetic patients, a recent Cohort study in Sweden on 40 subjects (20 control and 20 diabetics) matched for age, gender, and BMI indicated higher IL-33 gene and protein expression in patients than in control. Also, they reported that IL-33 mRNA expression was positively correlated with biomarkers of HbA1c, insulin resistance, and BMI [15].

On the other hand, another study conducted on 91 diabetic patients in Kuwait determined that adipose tissue IL-33 was associated with glycated haemoglobin (HbA1c) and mediators of inflammation and immune regulation in patients with varying degrees of glycemia [16]. Thus, the set of inflammatory biomarkers is needed to indicate the capacity of patients in the clusters for inflammatory cell recruitment from the circulation to the tissues and, subsequently, for the progression of end-organ damage and vascular complications. The hypothalamus–pituitary–thyroid hormonal axis and the cytokine IL-33 may have a suppressive, inflammation-regulatory role. Thus, these results may help physicians with their clinical reasoning by reducing the complexity of diabetic patients [17].

FGF regulates several human processes essential for normal development. Even though FGF has been implicated in the pathological development of diabetic nephropathy, the underlying mechanisms are not well understood [18]. FGF-18 regulates the endocrine system and hormone release (insulin). Thus, in the present study, the FGF-18 level was investigated, and its value was 27.24±22.17 pg/mL in diabetic patients, which was significantly several times greater than its value in healthy control group ($p<0.001$). However, no study on this topic in the literature compares our results.

Moreover, WNT-5A signalling plays an essential role in obesity/diabetes-induced metabolic dysfunction and inflammation, but its explicit molecular mechanisms and biological function in diabetic nephropathy remain unknown [18]. Thus, its value is significant for diabetic patients [19]. In the current study, the WNT-5A value in diabetic patients was recorded to be 0.61±1.06 pg/mL, which was almost close to that found in the control group (0.65 ± 0.58 pg/mL) ($p\geq0.05$). In this regard, a study in China found that CD146 directly binds to WNT-5A-induced noncanonical signalling, contributing to renal tubular inflammation in diabetic nephropathy. Also, they concluded that the concentration of serum/urine CD146 could be a potential biomarker to predict renal outcomes in diabetic patients [18]. Another study in China on 25 T2DM patients also stated that WNT5A expression and inflammatory factors were elevated in diabetic patients [19].

Regarding the studies on serum biomarkers in Iraq, in 2022, a study was conducted in Baghdad on 115 male diabetic patients with various complications. The level of irisin was increased highly significantly ($p<0.001$) in diabetic nephropathy patients, while the IL-8 level was decreased highly significantly ($p<0.001$) in patients with diabetic cardiovascular disease [20].

In the current study, no significant difference between the level of the biomarker with each gender, age, and BMI of the patients, as well as the duration of diabetes ($p>0.05$), was found. However, a positive relationship between the number of symptoms in patients and serum concentration of IL-17 and IL-33 ($p=0.018$, $Rho=0.238$) was detected, but not other biomarkers (FGF-18 and WNT-5A). Finally, regarding the correlations between the studied biomarkers, there was a positive correlation between IL-17 and IL33, IL-17 and FGF-18, and IL-33 and FGF-18. To the best of our knowledge, no study in the literature correlated between biomarkers and diabetic patients' sociodemographic data, nor correlations between biomarkers level in diabetic patients. This is the first research that found these correlations with potential outcomes.

Hence, the identification of potential biomarkers for the accurate prediction of other diseases, including heart failure and cardiovascular events in patients with T2DM, may enhance patient management and, at the same time, inform clinical trials and indicate novel pathogenetic and therapeutic targets that may further improve clinical practice among diabetic populations in the community.

For future studies, we recommend in silico analyses be established to investigate the pathways for *in vivo*/*in vitro* mechanisms for identifying proteins with potential therapeutic significance. Additionally, targeting of therapeutic strategy, targeting drug delivery systems, and more biomarker identification in diabetic patients (especially diabetic nephropathy) using proteomic tools such as mass spectrometric analysis and microarray analysis are strongly recommended.

Conclusions

This study concluded a direct correlation between the serum levels of IL-17, IL33, FGF-18, and T2DM, but not for WNT-5A. The biomarker's serum concentration does not affect each patient's age, BMI, and disease duration. Ultimately, a positive correlation between most biomarkers was seen, especially IL-17 and IL33, IL-17 and FGF-18, and IL-33 and FGF-18. Moreover, a positive correlation was observed between the number of symptoms in the patients and IL-17 and IL-33 levels, but symptoms were not correlated to FGF-18 and WNT-5A levels.

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Conflict of interest

The authors confirm that they are not affiliated with or involved in any organization or entity with financial interests.

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