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A comparative study of multiple biomarkers levels in complicated versus noncomplicated type 2 diabetic patients

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Abstract

The prevalence of diabetes mellitus is growing globally and the management of diabetes is a critical issue for public health. This study aimed to analyze the concentration of different biomarkers in patients with type 2 diabetes mellitus (T2DM) without complication, T2DM patients with complication (T2DM+C), and compared to healthy controls (HC). For this aim, there were 164 participants: 59 T2DM, 60 T2DM+C, and 45 HC. Venous blood was collected and the levels of Hemoglobin A1C (HbA1C), fasting blood glucose, Interleukin-31 (IL-31), IL-35, glutamic acid decarboxylase antibody (GADA), developmental locus-1 (Del-1), fibroblast growth factor-9 (FGF-9) and FGF-18) and lipid profile (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride) were analyzed. Results showed that IL-31 was significantly higher in T2DM compared to HC ($p<0.0001$), and compared to T2DM+C ($p<0.0001$). IL-31 was significantly lower in T2DM+C than HC ($p=0.009$). The level of serum GADA was significantly elevated in T2DM compared to HC ($p=0.0009$), and T2DM+C ($p=0.03$). There was a significant correlation between (IL-31, IL-35, GADA, Del-1, FGF-9 and FGF-18). The duration of having diabetes was significantly longer in T2DM+C compared to T2DM ($p<0.0001$). However, there was no significant difference in the level of HBA1C% between T2DM+C and T2DM patients ($p=0.98$). In conclusion, there were significant differences in biomarker concentrations between all three groups. This indicates that the monitoring of multiple biomarkers may be of value in the controlling of T2DM in the future.

Keywords: DEL-1; Diabetes; FGF-9; FGF-18; GADA; IL-31; IL-35; T2DM.

1. Introduction

Type 2 diabetes accounts for 90–95% of the estimated 347 million cases of diabetes diagnosed globally. The prevalence of type 2 diabetes mellitus (T2DM) is progressively growing with the improvement of people's living conditions, the aging of the population, and changes in people's lifestyles [1]. Diabetes-related persistent high blood sugar levels, known as chronic hyperglycemia, can lead to both small blood microvascular and macrovascular problems, ultimately leading to mortality. Notwithstanding these established repercussions, a significant number of individuals are unable to effectively control their blood glucose levels. Approximately 50% of patients with T2DM successfully reach one of the glycemic, blood pressure, or lipid targets, whereas less than 20% manage to fulfill all three simultaneously. Hyperglycemia and impaired glycemic control can be attributed to medication side effects, pharmacological interactions, other medical conditions, and hospitalizations [2,3].

The development of T2DM is marked by a gradual decrease in the effectiveness of insulin (insulin resistance) in tissues, and the reduced ability of pancreatic β cells to produce enough insulin to counteract the insulin resistance. This results in high blood sugar levels. Previous studies have indicated a complex interaction between oxidative stress and inflammation. The onset of T2DM is facilitated by intricate interactions between environmental factors and genetic variables, specifically numerous susceptibility genes [4].

Chronic diabetic patients have persistent glutamic acid decarboxylase antibodies (GADA), which are unaffected by the patient's age at disease onset. Therefore, identifying GADA is the most reliable indicator of adult-onset autoimmune diabetes. However, due to its high cost and restricted availability, the GADA test has yet to be widely utilized in primary care fields [5]. There is enormous variability in autoimmunity and β-cell dysfunction in T2DM, with GADA positivity and high C-peptide related to early insulin initiation and GADA positivity and low C-peptide increasing the risk of severe hypoglycemia. To improve the accuracy of categorization and treatment in T2DM, several tests are required [6].

Cytokines are critical immune mediators produced in an ordered process during the infectious and inflamma-

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tory response, which has a particular function in cell-to-cell contact. Several cytokines such as interleukins, chemokines, colony-stimulating factors, interferons, tumor necrosis factors, and transforming growth factors are included in the cytokine superfamily, all of which exhibit various immunological and biological functions [7].

The developmental endothelial locus-1 (DEL-1) is released by tissue-resident cells, including some subsets of macrophages endothelium, and mesenchymal stromal cells. DEL-1 can interact with a variety of integrins, such as integrins β2 (αLβ2 and αMβ2) and αv (e.g., αvβ3), as well as phospholipids. DEL-1 may be a possible therapeutic target due to these interactions, which help it control critical immunological processes that impact inflammatory and autoimmune illnesses in the preclinical research [8].

Fibroblast growth factors (FGF) are a group of peptide cytokines, including 23 members with varied functions. FGFs control several biological and cellular activities, including skeletal and embryonic development, tumor proliferation, inflammation, tissue formation, blood vessel formation, tissue healing, growth, and cellular specialization [9,10]. Furthermore, FGFs play significant roles in adult tissue repair and damage response [11,12]. The FGF family, comprising FGF-9, 16, and 20, includes the fibroblast growth factor FGF-9 subfamily. Due to their high degree of sequence homology, these molecules may share similar metabolic functions [13]. The exosome-associated gene FGF-9 was first found in human glioma cells, and it has subsequently been discovered that this gene participates in the control of glia in the central nervous system. Recent studies have shown that FGF-9 is now understood to be essential for the growth of malignancies [14]. The FGF-8 subfamily has two other members, FGF-17 and FGF-18, and they have different expression patterns during embryonic development based on similarities in their amino acid sequences [15]. FGF-18 controls chondrogenesis and osteogenesis [16,17]. In addition, FGF-18 expression was upregulated in colon [18], ovarian [19], and breast cancer [20].

IL-31 and IL-6 are related as they belong to the same family, commonly grouped due to their pro-inflammatory effects and a similar signaling route that activates the gp130 receptor subunit [21]. Different kinds of cells including helper T cell 2, macrophages, eosinophils, basophils, dendritic cells, fibroblasts, and intestinal and pulmonary epithelial cells are immune and nonimmune cells that can produce IL-31 [22,23]. IL-31 modulates signaling and affects several biological processes, including the induction of pro-inflammatory cytokines, cell proliferation, and tissue remodeling. [24]. It still needs to be determined how IL-31 works physiologically. IL-31 has been linked to several inflammatory problems such as atopic dermatitis [25], inflammatory bowel disease [26], and asthma [27]. Furthermore, In mice, the cytokine Interleukin 31, generated by activated T cells, causes dermatitis and cutaneous inflammation [28]. IL-31 was detected in both blood and vitreous specimens obtained from individuals diagnosed with proliferative diabetic retinopathy, indicating that IL-31 might have a significant role in the development of diabetes [29].

IL-35 is discovered by Collison [30] and Niedbala [31] recently. It belongs to the interleukin-12 family. IL-35 has anti-inflammatory and immunosuppressive capacities. It is composed of two subunits, IL-12α (also known as IL-12p35) subunit, and Epstein-Barr virus induced 3 (EBI3) subunit [30,32]. IL-35 has a significant function in the etiology and development of inflammatory and autoimmune disorders; all of these link adaptive and innate immune responses [33]. IL-35 is primarily created by T-regulatory cells, B-regulatory cells, and antigen-presenting cells. IL-35 can stimulate the development of Treg and Breg. On another side, it can inhibit the activity of macrophages and effector T lymphocytes, mainly helper T cell 1 and helper T cell 17 [34].

Increased knowledge about the role of cytokines in T2DM may lead to the development of improved tools for diagnosis and prognosis as well as new targets for treatment. The current research aimed to evaluate serum concentrations of GADA, DEL-1, FGF-9, FGF-18, IL-31and IL-35 in T2DM patients with and without complications compared with participants without diabetes.

2. Materials and Method

2.1. Study design

Cross-sectional and Comparative Study.

2.2. Study population

The study was performed in accordance with the World Medical Association's Code of Ethics (Declaration of Helsinki) for studies involving human beings. The research protocol (No. 75, Date 18/5/2021) was authorized by the Ethical Committee of the Sulaimani University, College of Medicine. Prior to their participation in the study, each subject provided written informed permission.

The study was started from May 2021 to February 2023. Patients with T2DM have participated in this study. Diagnosis of diabetes was performed by a specialist physician (Internal physician and endocrinologist) based on the diagnostic criteria for T2DM, and they were admitted to the Diabetes and Endocrine Center, Shar Hospital, and private healthcare sectors in Sulaymaniyah City. The study approached 277 subjects, with ages ranging from 30-80 years. Among them, 164 individuals were selected and the other 103 were excluded based on exclusion criteria and missing data. The exclusion criteria were systemic steroid use, other autoimmune diseases, smoking, alcoholism, and pregnancy.

The individuals were grouped as 59 T2DM patients without complications (female 30, male 29), 60 T2DM patients with complications (female 32, male 28), and 45 subjects representing healthy controls (HC) (female 22, male 23). The group with complications had one of the following: diabetic foot ulcer (DFU), diabetic retinopathy or diabetic peripheral neuropathy (DPN). The HC were individuals without diabetes and other diseases including hypertension.

2.3. Sampling

Venipuncture was performed to collect blood from patients and controls, and collected in two different test tubes; one with gel and clot activator to obtain serum and one with EDTA for whole blood. The serum tubes were left in the rack in a vertical position without shaking for 10 to 15 minutes to permit the blood to clot, then centrifuged (6 minutes, 5000 rpm) to separate serum from clotted parts. Serum samples were divided into two parts, the first part was used to perform several tests including fasting blood

glucose (FBG), hemoglobin A1C (HbA1C), triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol, while the second part was divided into three aliquots and stored at -70°C until testes by Enzyme-Linked Immunosorbent Assay (ELISA) for different biomarkers (GADA, DEL-1, FGF-9, FGF-18, IL-31, and IL-35).

2.4. Analytical methods

COBAS C 111 (Roche) was used to determine HbA1C, while COBAS C 311 (Roche) was used for FBG, triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol.

2.5. Qualitative and quantitative analysis of cytokines and FGFs

A special ELISA kit was used for each type of cytokines, fibroblast growth factors, and other biomarkers. Anti-GAD autoantibody ELISA (Cat No. E7259Hu), Human Developmental Endothelial Locus-1 ELISA (Cat No. E4505Hu), IL-31 ELISA (Cat No. E3254Hu), IL-35 ELISA (Cat No. E0042Hu), FGF-9 ELISA (Cat No. E5289Hu) and FGF-18 ELISA (Cat No. E5287Hu). Serum samples were thawed completely, and based on manufacturer instructions ELISA kits were left at room temperature for 30 minutes. The standard solution was diluted for each kit to prepare standards from one to five. All standards were tested alongside serum samples based on the assay procedure step by step. The optical density of each ELISA plate was read by a microplate reader (Biotek ELX800 Microplate Reader). A standard curve was made using known concentrations of standards with optical density, and the standard curve was used to determine the concentration of each analyte in each kit by GraphPad Prism 9.0 software.

2.6. Statistical Analysis

The results of T2DM and T2DM+C were compared to a control group consisting of individuals of the same age and sex who were in good health. The statistical analysis was performed using GraphPad Prism 9.0 software. The results were presented as the mean value plus or minus the standard deviation (SD) and were examined using a Mann-Whitney-U test for two groups, or a one-way analysis of variance (ANOVA). The study employed ANOVA to compare numerical variables across the three groups. The Spearman correlation analysis was employed to figure out the linear association between two variables and detect correlations among biomarkers, parameters, BMI, age, symptom score, and duration of diabetes. A significance level of 0.05 or lower was employed as the threshold for determining statistical significance.

3. Results

3.1. Patients' characteristics

The characteristics of the individuals who took part are clearly outlined in (Table 1).

Table 1 and Figure 1A demonstrate that the mean age of T2DM+C patients was significantly higher than that of T2DM patients and the HC group. Furthermore, the T2DM+C patients exhibited a considerably longer duration of diabetes compared to the T2DM patients, as seen by the data presented in Table 1 and Figure 1B. In addition, the HbA1C level was significantly increased in both T2DM and T2DM+C patients compared to HC. However, there was no notable difference in the HbA1C level between T2DM+C patients and T2DM patients (Table 1 and Figure 1C).

3.2. Comparing the levels of cytokines, fibroblast growth factors, Del-1, and GADA between HC and T2DM

As shown in (Figure 2A) a higher level of serum GADA was found in T2DM compared to HC. Likewise, a significantly higher concentration of GADA was found in T2DM than in T2DM+C. As shown in (Figure 2 B, C, D, and F) there were no significant differences in the concentrations of DEL-1, FGF-9, FGF-18, and IL-35 between the three groups. The concentration of IL-31 in blood was significantly elevated in T2DM patients compared to HC (Figure 2 E). The group with T2DM+C had significantly lower concentrations of IL-31 than both those with T2DM and the HC group (Figure 2 E). In addition, the levels of all

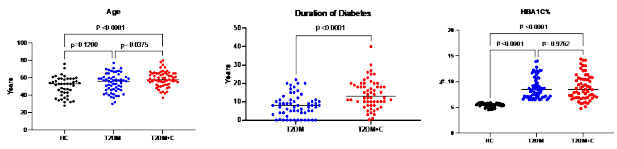

Fig. 1. The difference in (A) HBA1C%, (B) Age among HC, T2DM, and T2DM+C. (C) the difference in duration of having diabetes between T2DM and T2DM+C.

Table 1. Demographic and clinical characteristics of diabetic patients and controls.

Parameters	T2DM (N=59)	T2DM+C (N=60)	Controls (N=45)	p-value
M/F	29/30	28/32	23/22	
Age (yr) (Mean±SD)	54.3± 10.8	58.9±8.59	50.0±11.1	0.0001
Duration (yr) (Mean±SD)	7.9±6.1	14.1±7.6	-	<0.0001
HbA1c Mean (%)	8.8	8.9	5.4	<0.0001
Family history (Yes)	34	39	-	
Symptom scores	4.5	5.3	-	0.23
Hypertension (Yes)	18	20	-	
Pills for diabetes (only)	34	30	-	
Pills and insulin for diabetes	11	17	-	
Insulin injection (only)	2	8	-	
No treatment for diabetes	12	1	-	
Pills for other diseases	Yes (38)	Yes (26)	-	

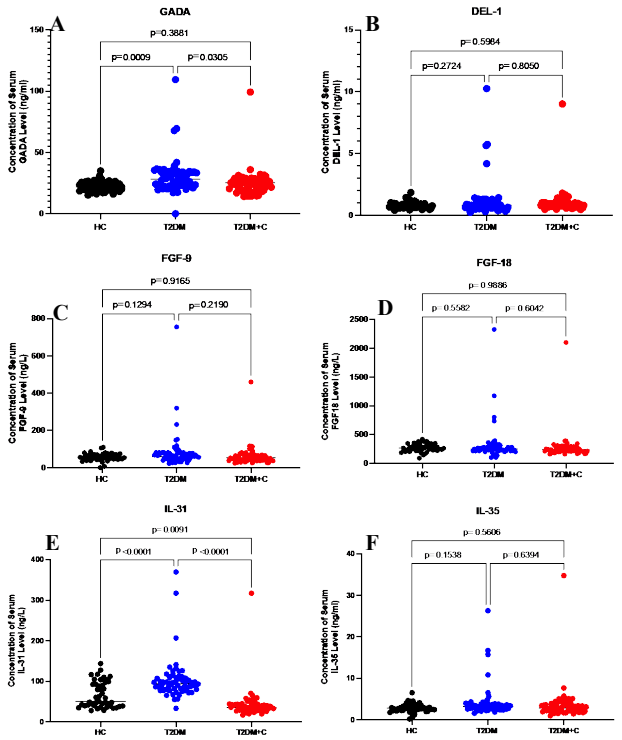

Fig. 2. The difference in the concentration of serum (A) GADA, (B) DEL-1, (C) FGF-9, (D) FGF-18, (E) IL-31, and (F) IL-35) levels among HC, T2DM, and T2DM+C.

biomarkers were compared among different diabetic patients with complications (DR, DN, and DF), and the differences were not statistically significant (data not shown).

3.3. Comparing (BMI, Cholesterol, Triglyceride, and LDL) among T2DM, T2DM+C, and HC

Regarding BMI, cholesterol, triglyceride, and LDL, no significant differences were seen between the three groups (T2DM, T2DM+C and HC) (Data not shown). However, significantly increased concentration of HDL was seen in the HC individuals than in the group of T2DM patients (Figure 3).

3.4. Comparing the levels of GADA, DEL-1, FGF-9, FGF-18, IL-31 and IL-35 in males and females

There was no statistically significant sex difference in the concentration of GADA, Del-1, FGF-9, IL-31, and IL-35 in any of the three groups (Data not shown). However, only the serum level of FGF-18 was significantly higher in females than males in the group of T2DM patients with complications (Data not shown).

3.5. Correlation between cytokines, FGFs, GADA, and DEL-1 in T2DM

As shown in Table 2 in the T2DM patients, there was a significant, positive correlation between GADA and FGF-18, IL-31, and IL-35 in the group with T2DM. A significant association was also found between DEL-1 and FGF-9, FGF-18, and IL-35 (Table 2). FGF-9 was statistically significantly correlated with FGF-18, IL-31 and IL-35 (Table 2). A statistically significant correlation was seen between FGF-18 IL-31 and IL-35 (Table 2). Likewise, there was a significant positive association between IL-31 and IL-35 (Table 2).

3.6. Correlation between cytokines, FGFs, GADA, and DEL-1 in T2DM+C

As shown in Table 3, in the group of T2DM+C patients, there was no significant correlation between GADA and any of the other biomarkers. A significant, positive correlation between DEL-1 and FGF-9, FGF-18, IL-31 and IL-35 (Table 3). FGF-9 was significantly correlated with FGF-18, IL-31, and IL-35 while FGF-18 correlated significantly with IL-31 and IL-35 (Table 3). A strong positive association was seen between IL-31 and IL-35 in the T2DM+C patient group (Table 3).

3.7. Correlation between cytokines and other T2DM-related variables

There was no significant correlation between cyto-

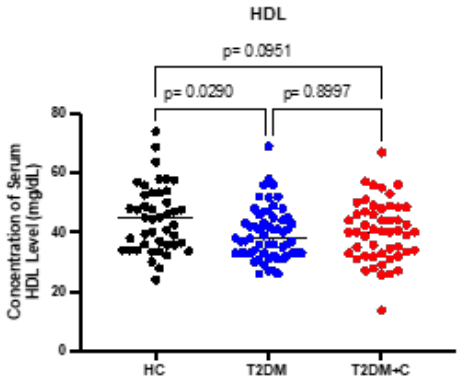

Fig. 3. The difference in the concentration of serum HDL levels among HC, T2DM, and T2DM+C.

Table 2. Correlation between different biomarkers in patients with T2DM (Spearman correlation (rho) and p-value.

Biomarkers		DEL-1 (ng/ml)	FGF-9 (ng/L)	FGF-18 (ng/L)	IL-31 (ng/L)	IL-35 (ng/ml)
GADA (ng/ml)	rho	0.21	0.14	0.52	0.35	0.51
	p-value	0.1211	0.3109	<0.0001	0.0068	<0.0001
DEL-1	rho		0.54	0.31	0.22	0.32
	p-value		<0.0001	0.0165	0.0991	0.0130
FGF-9	rho			0.37	0.50	0.45
	p-value			0.0040	0.0001	0.0004
FGF-18	rho				0.49	0.43
	p-value				0.0001	0.0007
IL-31	rho					0.38
	p-value					0.0036

Table 3. Correlation between different biomarkers in patients with T2DM+C Spearman correlations coefficient (rho) and p-value.

Biomarkers		DEL-1 (ng/ml)	FGF-9 (ng/L)	FGF-18 (ng/L)	IL-31 (ng/L)	IL-35 (ng/ml)
GADA (ng/ml)	rho	-0.06	0.07	-0.09	0.05	0.13
	p-value	0.6500	0.5747	0.4802	0.7182	0.3381
DEL-1	rho		0.52	0.51	0.62	0.53
	p-value		<0.0001	<0.0001	<0.0001	<0.0001
FGF-9	rho			0.43	0.64	0.78
	p-value			0.0006	<0.0001	<0.0001
FGF-18	rho				0.44	0.39
	p-value				0.0006	0.0021
IL-31	rho					0.71
	p-value					<0.0001

kines and FBG, HbA1C, Cholesterol, Triglyceride, HDL, and LDL in any of the two T2DM groups. Likewise, no significant correlation was found between cytokines and symptom score or duration of diabetes. Nonetheless, in the group with T2DM, there is a significant correlation between IL-35 and BMI (rho=0.26, p=0.048). Similarly, In the group of T2DM+C patients, the correlation between age and FGF-9 was significant (rho=0.26, p=0.035,) (CI [0.01 - 0.50]).

4. Discussion

The main findings of this research were that there were differences in biomarker levels among all three groups studied. For many of the biomarkers, the highest levels were found in the group with T2DM without complication while somewhat surprisingly the group with T2DM with complication often had levels that were even below the HC group.

In the present study, a significantly higher level of serum GADA was found in T2DM compared to HC. This is compatible several earlier findings [35,36]. A significantly higher concentration of GADA was found in T2DM compared to T2DM+C. Moreover, in both T1DM and T2DM, GADA can have pathological effects such as neurological syndromes [37], and the destruction of insulin-producing pancreatic cells in T1DM [6]. GADA positivity is an indicator of the course of diabetes, and it can also be detected in non-diabetic patients with autoimmune diseases [38].

In the current study, a higher level of DEL-1 was found in the serum of T2DM and T2DM+C compared to HC, while the differences were not significant. So far, this is the first study in which the serum DEL-1 was estimated in T2DM, and T2DM+C compared to HC. Recent studies determined that DEL-1 participates in the host inflammatory response at various stages from neutrophil production, their recruitment and their eventual clearance by macrophages during the resolution of inflammation [39,40]. Del-1 is participated in the control of inflammation and angiogenesis, and it may be related to diabetes through its role in the regulation of endothelial cell function and its association with T2DM.

Higher level of FGF-9 and FGF-18 was found in the serum of T2DM and T2DM+C compared to HC, while the differences were not significant. According to previous findings, FGF-9 may serve as a novel immuno-associated and prognostic biomarker for ovarian cancer [41,42]. Furthermore, a recent study has concluded that FGF-9, FGF-16, and FGF-20, may one day be used to treat cardioprotection and revascularization [13]. The level of FGF-18 has been studied in other diseases and was upregulated in colon [18], ovarian [19], and breast cancer [20]. FGF has shown potential therapeutic benefits in diabetes by regulating metabolic processes [43], improving insulin resistance, and promoting adipose tissue homeostasis.

In the present investigation, a significantly higher concentration of serum IL-31 was detected in T2DM compared to HC while the difference was not significant. Until now, this is the first study in which the blood level of IL-31 was studied in T2DM and T2DM+C. IL-31 has been studied in other diseases, and a higher blood concentration of IL-31 was reported in the serum of individuals with cutaneous lesions and people with atopic dermatitis compared to HC [44,45]. However, surprisingly, we found a significantly lower level of serum IL-31 in T2DM+C compared to T2DM and HC. In accordance with the current study, the levels of other inflammatory cytokines were higher in HC compared to T2DM+C, [46]. The precise mechanism of IL-31's involvement in diabetes remains incompletely clarified, therefore more research is needed to understand its potential involvement in T2DM. Similar to other pro-inflammatory cytokines, we imagine that IL-31 might participate in the initiation of inflammation during diabetes.

In the current study, a higher concentration of IL-35 was found in the serum of T2DM and T2DM+C compared to HC. Studies about IL-35 in T2DM and T1DM are limited. A recent study found that the difference in the serum concentration of IL-35 between HC and T2DM was not significant [47]. In line with our research, patients with T1DM exhibited elevated levels of IL-35 in their serum [48]. IL-35 might affect the progression of T1DM and T2DM. It was hypothesized that IL-35 would reduce inflammatory cytokine production [49]. It has been shown that IL-35 manages the suppression of Tregs, blocks Th17 cell differentiation, and thus tends to reduce the continuous immune damage of beta-cells [50].

There was a positive correlation between GADAs and other biomarkers in T2DM. Studying these biomarkers is new, therefore finding related work is limited. Similar to this finding, recent research observed a positive correlation between GADAs and (IL-1β, IL-3, and IFN-γ) in T1DM and T2DM patients [35]. In agreement with this study, a significant correlation was observed between GADA and (IL-6 and IL-1β) in T2DM in a previous study [36].

In T2DM and T2DM+C, positive correlations were found between DEL-1 and other biomarkers (FGF-9, FGF-18, and IL-35). In addition, in T2DM+C, there was

a positive correlation between DEL-1 and IL-3, while the difference between DEL-1 and IL-31 in T2DM was not significant. However, a previous study found a negative correlation between salivary Del-1 and IL-17 expression levels in T2DM patients with periodontitis [51]. We expect that DEL-1 seems to be involved in the control and resolution of inflammation. Previous studies showed that DEL-1 stimulates macrophage efferocytosis [39] and inflammation clearance, and it suppresses inflammation [52].

We found a positive correlation between FGF-9 and FGF-18 in both T2DM and T2DM+C. Further research may be required to discover the connection between FGF-9 and FGF-18. Moreover, in this study, we found that in T2DM and T2DM+C, there was a correlation between FGF-18 and other biomarkers (IL-31 and IL-35). In this study, we found that in both groups T2DM and T2DM+C, there was a positive correlation between FGF-9 and IL-31. In contrast to our finding, a previous study claimed that FGF-9 therapy significantly decreased the levels of circulating pro-inflammatory cytokines, M1 macrophage differentiation, and invading monocytes [53]. In the present study, a significant positive association was observed between FGF-9 and IL-35. Similar to this finding, a recent study on mice demonstrated that the release of anti-inflammatory cytokines was also boosted by FGF-9 therapy [53]. According to the situation, FGF-9 and FGF-18 may have pro-inflammatory [54,55], or anti-inflammatory effects [53,56].

Interestingly, the current study found a significant correlation between IL-31 and IL-35 in both groups of patients T2DM and T2DM+C. However, there is limited information on the correlation between IL-31 and IL-35. We assume that during diabetes, the initiation of inflammation is promoted by several pro-inflammatory cytokines containing IL-31. This inflammation might cause damage, and because of that anti-inflammatory cytokines such as Il-35 will be produced to reduce undesired effects of inflammatory reactions. Additional investigations may be required to fully understand the relationship between IL-31 and IL-35.

In the present study, the level of serum HDL was significantly lower in the blood of T2DM patients compared to the HC group. A similar result was recorded by a previous study as they found decresed levels of HDL in the blood of T2DM patients [57,58]. Recent findings suggest a connection between HDL levels and the development and prognosis of T2DM. Hyperglycemia causes a decrease in levels of HDL and impairs it's function. This occurs through changes in the proteins and lipids present in HDL particles. Consequently, decreased levels of HDL and altered HDL functionality have a negative effect on the functioning of vital organs involved in maintaining glucose balance, such as the pancreas and skeletal muscles [59].

5. Conclusion

There were significant differences in biomarker concentrations among each of the three groups. This indicates that the monitoring of multiple biomarkers may be of value in the management of T2DM in the future. Furthermore, our findings suggest that the levels of cytokines could decline as the duration of the disease advances. It has still to be discovered whether this diversity is caused by the illness process, the prescribed drugs, or if patients with lower levels of inflammatory mediators are more at risk for acquiring diabetes and its related microvascular and macrovascular problems.

Interest conflict

All authors declare that they have no conflict of interest.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

The study was carried out by the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. The Ethical Committee of the Sulaimani University, College of Medicine approved the research protocol (No. 75, Date 18/5/2021), and written informed consent was obtained from each subject before his enrolment in the study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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