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Elevated IL-31 and IL-31RA with dyslipidemia in T2DM patients

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Abstract--Type 2 Diabetes mellitus (T2DM) is characterized by elevated of blood glucose, and considered as a chronic metabolic disease. Uncontrolled blood glucose levels cause many dangerous complications. One of these complications are diabetic foot ulcers (DFU). About 15% of these patients suffering of DFU that affected on their lives. Approximately 8.5% Iraqi suffer from T2DM. Sixty T2DM patients (32 male and 28 female) aged 39-65 years that participated in the present study. Thirty apparently healthy subjects (12 male and 18 female) were classified as a control group. Age and BMI are not significantly different between patients and controls. This was made to exclude the influence of body mass and age on the results. There is an expected significant increase in fasting blood glucose (FBG) ($p < 0.001$) and glycated hemoglobin (HbA1c). The results showed a significant increase ($p < 0.001$) in serum triglyceride (TG), VLDLc in T2DM as comparison with the control group, while There were no significant differences among T2DM patients and control group in total cholesterol (TC), HDLc and LDLc. This result maybe was by the effect of diabetic drug on lipid profile where some drugs such as metformin that reducing cholesterol. There is a significant increase ($p = 0.032$) in serum interleukin 31 (IL-31) level in T2DM patients as compared with controls. Increase this interleukin indicate the inflammation in patients with T2DM. Also, there is a significant increase ($p < 0.001$) in serum interleukin 31 receptor alpha (IL-31RA) level in T2DM patients than controls. This increase could be resulted from the increasing of IL-31 level in T2DM. In DFU, there was a significant increase in the HbA1c and HDL. There was no significant difference in serum IL-31 level with DFU and T2DM patients, while a significant increase ($p = 0.0019$) in the serum IL-31RA with the DFU patients than T2DM. Increased levels of IL-31 have now been shown in various inflammatory skin diseases. IL-31RA was increase when skin damage or lesion. It can conclude, the patients with T2DM have a high level of

inflammatory interleukins and dyslipidemia which leads to complications. DFU have a high level in IL-31RA which indicate a lesional skin.

Keywords--diabetes foot ulcers, inflammation, lipids, interleukins.

Introduction

Type 2 Diabetes mellitus (T2DM) is characterized by elevated of blood glucose, and considered as a chronic metabolic disease (WHO 2016). The main factor that characterize T2DM is insulin resistance particularly in the liver and muscles, and also the defective insulin secreted from the pancrease (Chatterjee et al., 2017). T2DM is characterized by peripheral insulin resistance (IR) in organs such as liver, adipose tissue, and skeletal muscles, and it arises when β -cells are unable to adjust for the peripheral IR (Rachdaoui et al 2020). In another words, T2DM due to both the action of insulin is insufficient IR and reduced insulin released by pancrease (ALHILLAWI et al 2022). Un controlled blood glucose levels causes many microvascular complications (such as retinopathy, nephropathy, and neuropathy) and macrovascular complications (such as stroke) are common in people with T2DM (DeFronzo et al 2015). There are several factors that contribute to increase the blood glucose levels and lead to T2DM. Environmental variables including obesity, excessive eating, lack of exercise, stress, and age, as well as genetic factors linked to decreased insulin production and insulin resistance. In most cases, it's complicated, including a variety of genetic and environmental factors in various degrees.

(Galicia-Garcia et al 2020). The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people) (Saeedi et al., 2019). About 8.5% Iraqi suffer from T2DM (WHO 2018). Continuously high and untreated blood glucose level leads to many dangerous complications that threaten the patient's life, such as damage to the eyes, kidneys, nerves, heart and peripheral vascular system (Mouri et al 2022). Diabetic foot ulcers (DFU) are complication of diabetes and affects about 15% of diabetic patients at some point in their lives (Brocco et al 2018), which linked to diabetic neuropathy and peripheral vascular disease, DFU prevalence has risen in recent years (Bus et al 2020). patients with DFU are at an elevated risk of all-cause mortality, which is thought to be more than twice as high as those with diabetes who don't have this complication (Saluja et al 2020). The pro-inflammatory cytokine is important in wound healing because it regulates the inflammatory response. Continuously increased levels inflammation interleukins on the other hand, can prolong the inflammatory phase, which is linked to chronic, non-healing wounds that do not heal (Xu et al., 2013). The study aims of the potential effects interleukins (IL-31 and IL-31R) on IR and lipid profile in patients with T2DM and highlight on inflammatory role of IL-31 and IL-31R in T2DM.

Materials and Methods

Subjects

Sixty T2DM patients (32male and 28 female) aged 39-65 years have been included in the present study. The specimens were collected from Al-Sader medical city in Najaf governorate-Iraq for the period November 2021 till February 2022. T2DM patients were diagnosed according to the WHO criteria (WHO 2011) where they had fasting plasma glucose ≥ 7.0 mM, and HbA1c $> 6.5\%$. The assessment of patients was carried out by full medical history to explore the presence of any systemic diseases, which might have an effect on the studied parameters, kidney diseases, liver diseases and heart diseases were excluded from the study. Thirty apparently healthy subjects (12 male and 18 female) were classified as a control group. The ages were the same as the patients. Subjects were selected to be free of diabetes or other systemic or inflammatory disorders. All patients and controls have negative CRP.

Anthropometric

Body mass index (BMI) was used to define obesity and calculated from the formula: $BMI (kg/m^2) = \text{Weight (kg)} / (\text{Height})^2 (m^2)$. In the present study we will use the WHO classification of underweight ($BMI < 18.5 kg/m^2$), normal weight ($BMI = 18.5-24.9 kg/m^2$), overweight ($BMI 25.0-30.0 kg/m^2$) and obese ($BMI > 30.0 kg/m^2$) for adults.

Biochemical Investigations

- **Blood samples**

Every patient and control had a 5mL venous blood sample an overnight fast (8 -12 hours) collected using single-use needle plastic syringes. Clean tubes were used to transfer the samples. Hemolysis samples were thrown away. The blood was allowed to clot at room temperature for a 15 min. before being centrifuged at 3000 rpm for ten minutes. The serum was then separated and transferred to a new disposable plain tube, which was then frozen (-20 °C) until analysis.

- **Experimental Apparatus:** ELISA, spectrophotometer, Centrifuge, Gel-Tubes, Anti-coagulant tubes, Micropipettes, Deep freezer.
- **Chemicals:** IL-31, IL-31R, Insulin, HbA1c, Blood Glucose, Total cholesterol (TC) Triglyceride (TG), High density lipoprotein (HDL) and CRP kits.

Statistical Analysis

The Kolmogorov-Smirnov test was used to examine the distribution types of the results group. A statistical distribution divided variable results into two types; nonparametric variables and normally distributed variables. The results were expressed for the variable normally distributed, like (mean \pm standard deviation). The control and patient groups and subdivided groups were compared by use Pooled t-test in the measured parameters. The distinction among groups is considered like different of statistically when $p < 0.05$. SPSS Statistics base 26

and IBM-USA performed all statistical analysis. While the numbers were structured by use Excel Microsoft Office 2016.

Results and Discussion

Comparison of demographic and clinical

The demographic and clinical data of T2DM patients and Controls are presented in Table 3.1.

Table 3.1
Demographic and clinical data of T2DM patients and healthy controls

Variables	Patients (60)	Controls (30)	P- value
Age (year)	53.18±9.57	50.27±7.5	0.13
Hight cm	163.11±9.04	163.8±7.29	0.7
Weight Kg	76.48±10.62	77.13±11.78	0.801
BMI (Kg/m ²)	28.83±4	28.9±5.2	0.929
FBG mM	11.17±3.42	5.46±0.31	0.001<
HbA1c %	9.97±1.98	5.54±0.6	0.001<
Sex Male/Female	31/29	12/18	0.1802
Smoking Yes/No	18/42	7/23	0.501
CRP P/N	0/60	0/30	-

There are no significant differences between patients and controls in both age and BMI. This result to exclude the influence of body mass and age on the results. There is an expected significant increase in fasting blood glucose (FBG) ($p < 0.001$) and glycated hemoglobin (HbA1c) in T2DM compared with healthy group. During the course of treating diabetes, measuring HbA1c is regarded as a crucial diagnostic tool for evaluating diet control and therapy regimens (Rodas et al 2022). High FBG adversely affects lipid profile, adipocytokines and liver function that may have desirable effects on metabolic markers in the patients (Ghadge, et al, 2017). Adipose tissue serves as an important endocrine organ, releasing mediators (adipokines) that can affect insulin signaling, and it also produces free fatty acids that contribute to insulin resistance (IR), according to mounting evidence (Morigny et al 2016). Another study showed that many diabetic individuals had insufficient and subpar levels of glycemic control. And this was linked to advanced age, a longer duration of diabetes, insulin therapy, poor diet compliance, and a failure to establish control targets (Rodas et al 2022). There is a relationship between obesity and macrophages, where macrophages are aggregated in adipose tissues. Several obesity-related diseases, including IR, are largely attributed to this adipose tissue inflammation (Guilherme et al 2008).

Comparison of lipid profile parameters

The results of lipid profile parameters in healthy controls and T2DM patients are presented in Table 3.2.

Table 3.2
Lipid profile parameters in T2DM and healthy group

Variables	T2DM	Controls	P- value
T.C mM	6.39±1.62	6.31±1.52	0.8
TG mM	2.79±1.08	1.51±0.44	0.001<
HDLc mM	0.58±0.17	0.46±0.13	0.8
LDLc mM	4.57±1.6	4.97±1.49	0.7
VLDLc mM	1.27±0.49	0.724±0.2	0.001<

The results showed a significant increase ($p < 0.001$) in serum TG, VLDLc in T2DM as comparison with the control group, while There are no significant differences among T2DM patients and control group in TC, HDLc and LDLc. This result may the effect of diabetic drug on lipid profile where some drugs such as metformin that reducing cholesterol. Alalwan et al found fasting plasma glucose (FPG) was significantly associated with TC and HDL while no significant associated with LDL and TG (Alalwan, et al. 2020). Other studies found that individuals with poor glycemic control had considerably lower HDL-c levels and significantly higher TC and LDL-c levels than those in the group without poor glycemic control (Carey et al 2013). In clinical practice, TG may be useful for predicting T2DM. It may act as a mediator in the relationship between the development of T2DM and BMI (Low, et al., 2018). Patients with type 2 diabetes are more likely than people without type 2 diabetes to develop advanced metabolic dysfunction-associated fatty liver disease (steatohepatitis, fibrosis, cirrhosis, etc.) (Kwok et al 2016).

Additionally, there is a strong bidirectional association between advanced metabolic dysfunction-associated fatty liver disease and cardiovascular disease (Eslam et al 2021). Having good glycemic control can be difficult for diabetic patients who have fatty liver disease with metabolic dysfunction (Afolabi et al 2018). The enhanced insulin sensitivity observed after short-term exercise training was associated with a marked decrease in TG content in patients with T2DM (Bruce et al 2004). The TG index was significantly associated with risk of incident diabetes and could be a valuable biomarker of developing diabetes (Chamroonkiadtikun, et al 2020). Another study discovered that patients with good and poor glycemic management did not have significantly different TG levels. The removal of lipids from peripheral cells by functioning HDL may reduce inflammation.

There is some biological proof that raising HDL levels can improve peripheral tissues' sensitivity to insulin (Carey et al 2013). The management of lipid profiles and glycemic control are closely related where, good glycemic control contributed to control of lipid profiles for patients with T2DM. On the one hand, one of the key elements influencing glycemic control in T2DM patients was maintaining good control of lipid profiles (Artha, et al. 2019). In T2DM patients, HDL infusion therapy enhanced plasma HDL levels and decreased plasma glucose levels through raising plasma insulin and turning on AMP-activated protein kinase in skeletal muscles (Drew et al 2009). A cording Fujita et al, demonstrated increasing lipid metabolism by short-term, intense glycemic management could considerably lower levels of TC (Fujita, et al 2008).

Comparison of Interleukin-31 (IL-31)

The graphical presentation of the IL-31 results in T2DM patients and the control groups are shown in Figure 3.1.

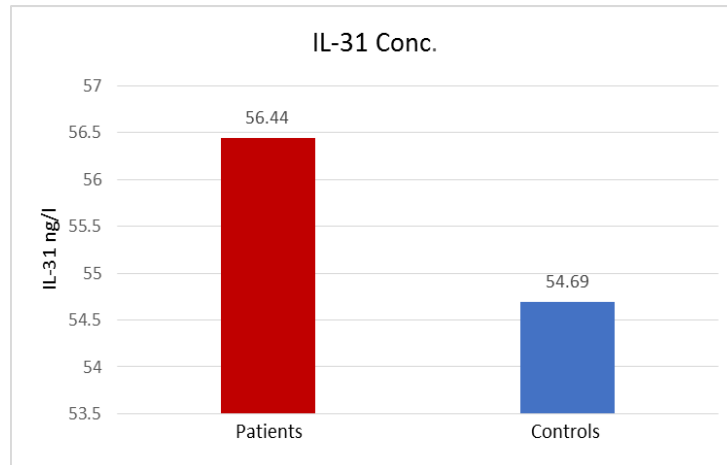


Figure 3-3. Serum IL-31 in T2DM patients and Controls in bars plot

There is a significant increase ($p=0.032$) in serum IL-31 level in T2DM patients as compared with the controls. Interleukins, which have a variety of biological roles and interact with the body's cells and tissues, are crucial for preserving immunological homeostasis and are also specifically engaged in the progression of many disorders (Mertowska et al 2022) regarding autoimmune disorders, diabetes, as well as neoplastic and neurological conditions (Ortega et al 2010). IL-31 consider inflammatory interleukin that increases within any inflammation (Lissoni et al. 2020) therefore, increase this interleukin result from inflammation in patients with T2DM therefore, the elevated this interleukin as a marker for inflammation in patients with T2DM. Numerous biological processes and immunomodulatory effects are regulated by IL-31 signaling, including the release of chemokines and pro-inflammatory cytokines as well as the control of cell proliferation, which results in the itching sensation (Zhang et al 2008). IL-31 has been discovered in several studies to have a significant involvement in common inflammatory skin conditions including atopic dermatitis, particularly in relation to pruritus (Gibbs et al 2019). IL-31 enhanced the expression of IL-6, which causes inflammation (Kuzumi et al 2021).

Comparison of Interleukin-31 RA (IL-31RA)

The graphical presentation of the IL-31RA results in T2DM patients and the control groups are shown in Figure 3-4.

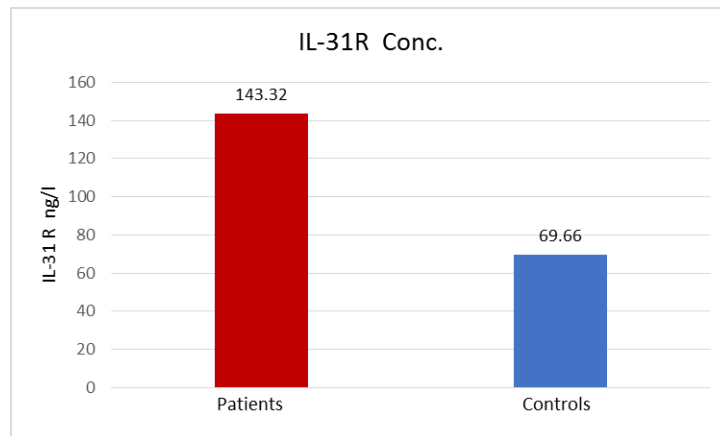


Figure 3-4. Serum IL-31RA in T2DM patients and Controls in bars plot

There is a significant increase ($p < 0.001$) in serum IL-31RA level in T2DM patients than controls. This increase may result from increase IL-31 level in T2DM. When IL-31RA binds to the IL-31 ligand, signaling is activated and proinflammatory cytokines and chemokines are expressed (Zhang et al 2008) leading to inflammation and tissues remodeling (Edukulla et al 2015). Therefore, research on the relationship between IL-31/IL-31RA may have practical significance for the management of IL-31-related diseases (Edukulla et al 2015). Other studies suggested that increase IL-31RA result from lack of the IL-13R2 gene may have augmented IL-13-driven IL-31RA expression during allergic disease (Horejs-Hoeck et al 2012). Kuzumi, *et al* showed the overexpression of IL-31 and IL-31RA in dermal fibroblasts from systemic sclerosis (SSc) patients (Kuzumi, *et al* 2021).

Comparison of T2DM with and without DFU

The demographic and clinical data of DFU (5 male and 5 female) and T2DM (26 male and 24 female) are presented in Table 3-3.

Table 3-3
Demographic and clinical data of DFU and T2DM patients

Variables	T2DM With DF Ulcers	T2DM Without DF Ulcers	P-value
Age (year)	53.1±9	53.2±9.74	0.97
BMI (Kg/m ²)	30.1±4	28.57±4	0.29
FBG mM	11.39±3.6	11.12±3.37	0.833
Duration of Dis.(year)	9.6±3.3	10	0.74
HbA1c %	11±1	9.8±2	0.008

There are no significant differences between patients (T2DM and DFU) in both age and BMI. With the use of age matching, variations in parameter results that could be the result of a large age difference are reduced, but there is significant increase in HbA1c. This agrees with Iraqi study by Mariam et al There is no significant difference in age between DFU and non-DFU group (Mariam et al 2019). Iraqi

study found there was significant differences in both age and duration of disease in both DFU and T2DM. Increase age and duration of disease causes complications and foot ulcers (Mohammed et al 2016). Also, Saudi Arabia studied, there are no significant differences between patients in both age and BMI and also duration of disease but significant increase in HbA1c in DFU than T2DM (Fawzy et al 2019).

While Bilal et al. found there was significantly higher in age, duration of disease and HbA1c with DF ulcers as compared to without DFU on the other hand, found there was no significant differences in BMI between patients (Bilal et al 2018). Although a strong positive relationship between complication of diabetes and poor glycaemic control, discrepancies were shown in the results regarding HbA1c values, fasting or postprandial blood and plasma glucose concentrations, where found for HbA1c, a positive association with complication was shown in several studies (Sarfo-Kantanka et al 2019; Bilal et al 2018; Robinson et al 2016). Moodley et al found, there was no significant differences in age, random blood glucose (RBG) and HbA1c between T2DM patients and DFU patients. In addition, the RBG level was not significantly associated with DFU and this result is not consistent with numerous studies in the literature, where age and RBG are significant risk factor of DFU development (Moodley et al 2021). Others suggested it is well known that the use of many medications decrease patient compliance to therapy, which further mean losing glycemic control and increasing DFU risk to the patient (Mohammed et al 2016). According to several research, higher HbA1c levels are linked to a higher risk of developing foot ulcers (Mohieldein et al 2008; Boyko et al 2006).

Comparison of lipid profile parameters of T2DM with and without DFU

The results of lipid profile parameters of T2DM with and without DF Ulcers are presented in Table 3-4.

Table 3-4
Lipid profile parameters in DFU and T2DM patients

Variables	T2DM With DF Ulcers	T2DM Without DF Ulcers	P-value
TC mM	6.78±1.47	6.18±1.78	0.27
TG mM	2.62±0.78	2.83±1.1	0.5
HDLc mM	0.25±0.05	0.45	<0.001
LDLc mM	5.33±1.6	4.23±1.75	0.077
VLDLc mM	1.19±0.35	1.28±0.51	0.5

The results showed a significant decrease in serum HDLc in DFU than T2DM, while no significant differences in TC, TG, LDLc and VLDLc between patients. This result agrees with Dai et al where found, there was no significant differences in serum TC, TG, LDL and VLDL, while a significant decrease in HDL in DFU than T2DM (Dai et al 2020). In contrast Iraqi study was found a significant increase in TC, TG and LDL while no significant in HDL and VLDL (Mariam et al 2019). On the other hand, studied have significant increase in lipid on patient with DFU in T1D (Hamri et al 2021).

When glucose cannot be digested by the cells, fats are mobilized, resulting in excessive levels of fatty acids in the bloodstream and leading to dyslipidaemia, which is frequently linked to T2DM. Four studies that examined this possible risk factor, only one cross-sectional case-control research identified a positive connection between dyslipidaemia and DFU, suggesting that the condition is not related to DFU problems (*Tuttolomondo et al. 2017*), whereas in a different study, a protective effect for lower extremity amputation (LEA) was shown with hyperlipidaemia (*Lai et al. 2015*). No correlation with DFU and dyslipidemia was found in two further investigations (*Sarfo-Kantanka et al 2019*). The findings of the research are quite inconsistent for abnormal levels of HDLc and LDLc, whereas for low levels of HDLc, two studies revealed a favorable relationship, one study found a negative relationship, and two studies found no association. One study revealed a beneficial correlation between elevated LDLc levels and the result LEA, while no correlation was found in the other two investigations (*Zhao et al. 2019*).

Comparison of Interleukins with and without DFU

The graphical presentation of the IL-31 and IL-31RA results in DFU patients and T2DM are shown in Figure 3-5.

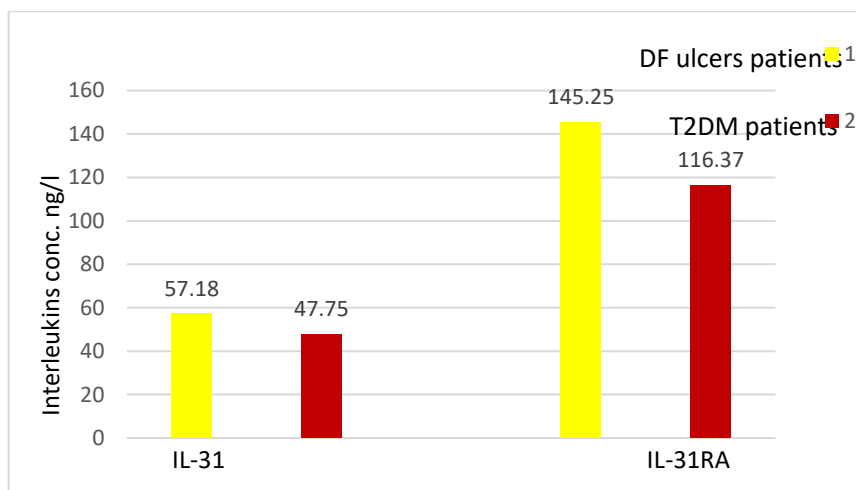


Figure 3-5. Serum IL-31 and IL-31RA in DFU patients and T2DM in bars plot

There is no significant difference in serum IL-31 level in DFU patients and T2DM patients, while a significant increase ($p= 0.0019$) in serum IL-31RA in DFU than T2DM. Multiple inflammatory skin diseases have recently been linked to elevated levels of IL-31(*Sonkoly et al 2006*). Other study suggested excessive inflammatory response: During the inflammatory stage of a typical wound, neutrophils and monocytes move to the area of damage and release a number of cytokines and growth factors (*Schilreff et al 2022*). Some studies in both humans and mice have suggested a link between IL-31–IL-31R expression and skin inflammation (*Jacqueline et al 2007*). It's interesting to note that mice that overexpressed IL-31 had severe itching, baldness, and skin lesions. Anti-IL-31 therapy dramatically decreased scratching in mice in an animal model of atopic dermatitis (*Grimstad et*

al 2009). This appears to be true for people as well, as evidenced by studies showing a correlation between IL-31 levels and the severity of atopic dermatitis (Raap et al 2012). Greater IL-31RA was found in lesional skin (Gibbs et al 2019).

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