Ann. For. Res. 66(1): 3634-3653, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

THE RELATIONSHIP BETWEEN ADIPOKINES IN DIABETIC FOOT PATIENTS WITH OBESITY IN IRAQ

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ABSTRACT

Background: The World Health Organization (WHO) has classified diabetes mellitus (DM) and obesity as epidemics due to their increased occurrence. Type 2 diabetes (T2D), the most prevalent type of DM in the world, is etiopathogenetically linked to obesity, as are many of its complications. Additionally, there is growing scientific evidence for the connection between type 1 diabetes (T1D) and obesity and overweight. Obesity is a condition marked by an abnormally high level of adipose tissue in the body as well as an increase in the production of adipokines, which are biologically active. Increased amounts of pro-inflammatory adipokines (Leptin) and consequently lower levels of anti-inflammatory adipokines are carried on by excessive proliferation of this tissue (adiponectin). **Aims of this study**: This study aimed to investigate the link between body mass index (BMI) and diabetic microvascular problems as well as the concentration of serum leptin and adiponectin in diabetes mellitus patients. **Material and method**: Clinical specimens were collected from 140 patients with diabetes (serum and blood), at Al-Hussein Teaching Hospital in (Samawa/ Iraq) and Medical city/Baghdad Hospital and private clinics in (Baghdad/ Iraq) from 9/1/2022 to 15/7/2022. Weight (kg), height (cm), BMI (kg/cm), random blood sugar, serum adiponectin and leptin concentration was assessed in 140 with diabetes and 70 healthy controls. The diabetic patients were classified depending on the type of diabetes and according to sex into male group and female group, use of oral hypoglycemic medication or insulin, mean duration of diabetes Serum adiponectin and leptin levels were measured by sandwich ELISA. Gene expression was performed by Real-time polymerase chain reaction. **Results:** Random blood sugar for patients was 226.99 mg/dL. The results revealed that diabetic foot ulcers factors were: 93(66.4%) are male and the rest 47(33.6%) are females, high 79(56.40%) are within (31-60) year, 50 (35.7%) of patients with DFUs were current smoker, 55 (39.3%) were overweight, 131(93.6%) were T2DM, 62(44.3%) were of duration between (15-30) years, both drugs using were 55 (39.3%) were factors statically associated with diabetic foot ulcer (P value < 0.05). The results also showed a significant increase in the levels of (leptin and adiponectin) in diabetic foot patients compared to the control serum group (P≤ 0.05). The result of the current study showed that there was a relation between DFUs and gene expression of adiponectin gene. **Conclusion:** Results of this study reported that found statistically relationship between age, sex, smoking, BMI, type of DM, DFUs grade, DM duration, DFUs duration and drugs using.

Keywords: Diabetes Mellitus; Obesity; adipokines; leptin; adiponectin; Sandwich ELISA and RT-PCR.

1. Introduction

The primary cause of insulin resistance, which manifests early in the disease and is mostly reversed by hyperinsulinemia, is obesity. Obese children with excessive weight, height, and waist circumference are more likely to have insulin resistance. The early adiposity rebound at the age of three years, which was demonstrated to result in an increased BMI while adolescent, is one factor in the occurrence of obesity. T2D is caused on by obesity and insulin deficiency together (Chobot *et al*., 2018;Jaid *et al*., 2022).

Adipokines are a class of bioactive molecules produced by adipose tissue that act as paracrine and endocrine hormones. These chemicals play a role in appetite and satiety, fat distribution, inflammation, blood pressure, hemostasis, and endothelial function, among other things. They affect a variety of organs, including adipose tissue, the brain, the liver, muscle, and blood vessels. Adiponectin, leptin, TNF-α, IL-6, resistin, IL-1, MCP-1, and others are some of the adipokines (Lendeckel *et al*., 2022). The pattern of adipokine secretion can indicate adipose tissue activity, and this pattern is crucial in determining an individual's risk of developing metabolic and cardiovascular comorbidities associated with obesity. Adipokine secretion is considerably shifted toward a diabetogenic, proinflammatory, and atherogenic pattern when adipose tissue inflammation and dysfunction are developed (Al-Attaby and Al-Lami, 2019;Chait and Den Hartigh, 2020).

Obesity-induced inflammation differs from other types of inflammation, such as infections and autoimmune illnesses. Because obesity is a chronic illness, it causes a low-level activation of the innate immune system, which can disrupt homeostasis over time. It's worth noting that adipose tissue macrophages (ATM) might also be regarded as a significant source of proinflammatory cytokines (Freitas *et al*., 2015).

The obesity (*Ob*) gene produces leptin, a 16-kDa adipokine produced by adipocytes. Leptin affects neutrophil growth, phagocytosis, chemotaxis, and oxygen radical emission through activating macrophages/monocytes and natural killer cells. Leptin is mostly generated in mature white adipose tissue (WAT) cells. Leptin biosynthesis and secretion are influenced by WAT mass and reflect the state of energy reserves (Hsu *et al*., 2015; Sadiq *et al*., 2022). Fat tissue mass and adipocyte size are the two key parameters that influence leptin levels in the blood. These measurements reveal a link between leptin manufacturing in adipose tissue and its level in the blood (Zorena et *al*., 2020).

In addition, leptin has been demonstrated to improvement glucose uptake and oxidation in skeletal muscles while also improving insulin sensitivity in peripheral tissues. Also, leptin influences thermogenesis by regulating mitochondrial proteins specific to brown adipose tissue (Czech, 2020). It plays a role in lipid and glucose metabolism, as well as immune system response, blood pressure regulation, blood coagulation, and fertility. Leptin is thought to be a possible indicator of obesity-related problems. Atherosclerosis and neuropathy are linked to elevated leptin levels, but not diabetic retino- and nephropathy (Salloom *et al*., 2013;Zorena *et al*., 2020).

Adiponectin has been identified as an anti-inflammatory cytokine in numerous investigations (Mihalopoulos *et al*., 2020). The altered activity of TNF is one of the reasons for adiponectin's anti-inflammatory properties. TNF inhibits adiponectin gene expression via suppressing adiponectin-induced nuclear factor NFκβ, according to in vitro research (Mirada *et al*., 2020). In human studies, patients with high adiponectin mRNA have lower TNF secretion in adipose tissue, whereas increasing insulin resistance and body fat mass upregulate TNF expression, resulting in lower adiponectin levels (Mihalopoulos *et al*., 2020).

Adiponectin has also been demonstrated to promote IL-10 production by macrophages while decreasing proinflammatory cytokines TNF-α and IL-6 production. Adiponectin suppresses inflammatory processes in the early stages of atherosclerosis and microangiopathy by inhibiting the expression of adhesion molecules in vascular endothelial cells and the production of cytokines in macrophages. Adiponectin levels in the blood are thought to rise in response to vascular endothelial damage. Reduced levels of adiponectin, which are linked to fat, are also connected with incident hypertension. Authors, on the other hand, have found that patients with diabetic nephropathy have higher blood and urine adiponectin levels. A link between adiponectin levels and the severity of diabetic retinopathy has also been discovered in T2DM patients (Abood *et al*., 2014;Zorena *et al*., 2020).

2. Materials and Methods

A. Study subjects and experimental design

This a cross-sectional study was included patients suffering from diabetes mellitus disease at Al-Hussein Teaching Hospital in Samawa/ Iraq and Baghdad Medical city/Baghdad Hospital in Baghdad/ Iraq from 9/1/2022 to 15/7/2022. 140 patients from (29-87 years) of both sexes suffering diabetes disease. Also, healthy individuals (control) were included. 5 ml of blood was collected from (210) samples of study groups: (140) diabetic with foot ulcer and (70) samples for each control groups (healthy persons), 3ml transferred into a gel tube for serum separation and kept frozen at -70°C until analysis. The remaining blood sample: 2ml placed in sterilized Ethylene diamine tetra acetic acid (EDTA) tube and stored at -80°C for gene expression experiment.

The control group consisted of 70 healthy volunteers (51 women and 19 men), aged 18–55 years. The absolute criterion of choice was normal BMI value (16.0–24.9 kg/m^2) and lack of symptoms of inflammatory condition. Body weight in kg, height in meters, and BMI (kg/m^2) were calculated. Following the application of a mathematical calculation that divides the weight in kilograms by the square of height in meters to determine body mass index for all participants, and the results were measured as follows: BMI (kg/m^2) = weight (kg) / height (m^2): Underweight ≤ 18.5(kg/m^2), Normal weight between 18.5 - 24.9(kg/m^2), Overweight between 25-29.9(kg/m^2), Obese ≥30(kg/m^2) and severe obesity >40.0 (kg/m^2) (Zhou *et al*., 2017).

B. Demographic data

The following health and demographic data was grouped: sex, age, education, smoking, BMI, type of diabetes, DM duration, sugar value, type and treatment: whether oral anti-diabetic, insulin, both or no receives any treatment. The type of DM was decided on the standard World Health

Organization criteria for diagnosis of diabetes type (Malta *et al*., 2019). Laboratory investigation included random blood glucose.

C. Biological material preparation

Blood specimens for serum Leptin and Adiponectin levels were centrifuged at 4000 rpm for 10 minutes after storage for 30–60 minutes. Serum samples obtained were stored at −70°C in a deep freezer until adipokines assays were performed. Leptin and adiponectin concentrations of particular adipokines were measured in the collected sample. 2ml of blood in sterilized EDTA tube and stored at -80°C for RT-PCR experiment.

D. Methods

The evaluation of selected adipokines serum concentrations: Serum Leptin and Adiponectin levels were measured using Human LEP (Leptin) and ADP/Acrp30 (Adiponectin) sandwich ELISA (Elabscience, USA) method according to manufacturer suggestions. Results were reported as ng/ml, pg/mL for leptin and adiponectine respectively.

Gene expression: total RNA were extracted from blood samples by using TRIzol® reagent kit and done according to company (Bioneer/Korea) instructions. The quantitative Real-Time PCR used in quantification of target genes (adiponectin-qPCR and GAPDH-qPCR) expression analysis that normalized by housekeeping gene (GAPDH) in patient and healthy blood samples by using Real-Time PCR technique and this method was carried out according to method described by (Yadav *et al*., 2020).

The primers for adiponectin target genes and housekeeping gene (GAPDH) were designed by using NCBI-Gene Bank data base and Primer 3 design online**.** These primers were provided by (Scientific Researcher. Co. ltd) in Iraq as following table (1).

Table (1): Sequence of the primers used with the name and size of the product.

Primer	Sequence (5'-3')		Product Size	NCBI Reference
Adiponectin gene	F	TCCAAGGCAGGAAAGGAGAAC	107bp	NM_001177800.2
	R	AAAGCGAATGGGCATGTTGG		
GAPDH	F	AATTCCATGGCACCGTCAAG	104bp	NM_001256799.3
	R	ATCGCCCCACTTGATTTTGG		

RT-PCR master mix was prepared by using GoTaq® qPCR Master Mix (Promega/USA) kit based on SYBER green dye detection of target and GAPDH gene amplification in Real-Time PCR system and include according to table (2). RT-PCR Thermocycling conditions set at one cycle of 95°C for 5 min, followed by 45 cycles of 95°C for 20 sec and 60°C for 30 sec. The data results of q RT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) (The ΔCT Method Using a reference gene) that described by (Dar *et al*., 2019).

Table (2): RT-PCR reaction components for detection of genes in this study.

NO.	RT-PCR master mix	volume
1	cDNA template (100ng)	5µL
2	Forward primer(10pmol)	1 µL
3	Reverse primer (10pmol)	1 µL
4	qPCR Master Mix	12.5 µL
5	DEPC water	5.5 µL
6	Total	25 µL

Statistical tests

Statistics were done using SPSS version 15. All data were expressed as mean ±standard deviation in the different groups. The results were considered significant whenever p values <0.05 and highly significant when p values <0.001. Z –score was used to assess weight-for-age, height-for age, and weight-for-height for each participant. Means of anthropometric data as well as biochemical concentrations were compared by Student`s "t" test. Relationships between different quantitative parameters were assessed by simple linear regression analysis ,and Pearson correlation coefficients (r) were presented (McDonald, 2014).

Ethical Approval

This study involving human participants was reviewed and the patients/participants were provided their written informed agreement to participate in this paper.

3. Results and Discussion

a. Demographic data and clinical characteristics of patients

A cross-sectional study involved 140 patients with diabetic foot ulcers was conducted at Al-Hussein Teaching Hospital (Samawa/ Iraq), private clinics and Medical city/Baghdad Hospital (Baghdad/ Iraq) from January to July, 2022. A total of 70 healthy controls were used. We evaluated the demographic data including age, sex, education, smoking, BMI, type of DM, DM duration, DFU grade, duration of DFU, and drug using. Random blood sugar for patients was 226.99 mg/dL.

The distribution of diabetic foot ulcers patients in the current study according to age groups as the following: 2(1.40%) are ≤30 year, 79(56.40%) are within 31-60 year, and 59(42.10%) are ≥61 year. The age groups of 31-60 and ≥61 are greater than ≤30 year, Table (4-1). Also, there is statistically significant difference (P value < 0.05) which indicates that the incidence rate of DFUs increased with the age, table (1).

Table (1): Frequency of diabetic foot ulcers patients according to age groups

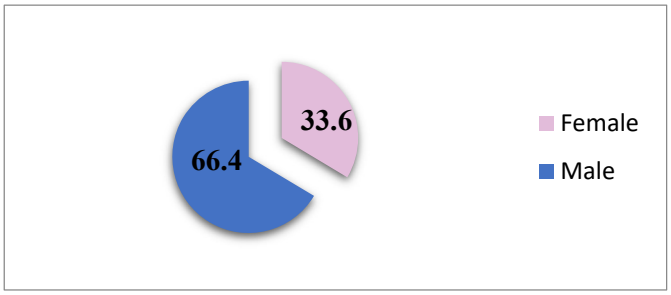
Age Groups(years)	No.	%	*P value*
≤30	2	1.40	<0.0001*
31-60	79	56.40	
≥61	59	42.10	
Total	140	100	

* represents a significant difference at p<0.05.

This result of the high incidence rate with the old ages agreed with the study that described about 68% of them aged above 50 years (Fawzy *et al*., 2019). Older age was associated with higher frequency of diabetic foot occurrence it is likely to be associated with a poorer outcome probably because of a slower immune response to infection and the presence of other comorbidities that delay healing such as an impaired vascular blood flow (Musa *et al*., 2018).

Guo *et al*., reported the positive relationship between age and the risk of DFU recurrence (Guo *et al*., 2022). Current results differed with Chinese study that found no significant difference was found in age (Huang *et al*., 2019). The varying age identity criteria used by the involved studies may be the cause of the variable outcomes. As people become older, the wound healing process is impaired, due to many factors such as peripheral arterial disease, decreased defense mechanisms, and impaired immunity (Marzoq *et al*., 2019).

According to the sex, the patients in the present study distributed as follow: 93(66.4%) are male and the rest 47(33.6%) are females. This result suggests that incidence of DFUs is significantly (P value <0.05) associated with the sex factor, Figure (1).



Figure(1): Frequency of DF ulcers patients according to sex groups

There is a Turkish study agreed with current study by showing the diabetic foot frequency is significantly different by the sex (Korkmaz *et al*., 2018). Also, the majority patients were 42 male (56.0%) from 75 participants (Kagwa *et al*., 2018). But, present result disagreed with Saudi Arabia study which showed that the occurrence that 70% of the DFUs patients were females (Fawzy *et al*., 2019). Two studies shown that sex was independent risk factors of DFU development, but the author did not give any explanation for this negative result, possibly because of the small sample size (Khalifa, 2018);(Yazdanpanah *et al*., 2018).

According to this as well of other previous studies, moreover, DF syndrome is a strongly sexoriented complication being much more prevalent among males (Crawford *et al*., 2015);(Seghieri *et al*., 2019). They argued that females could have a lower risk relative to males partly because of less severe neuropathy, increased joint movement, and lower foot pressure (Navarro-Flores and Cauli, 2020).

The present study showed that 50(35.7%) of patients with DFUs were current smoker, whereas the rest 90(64.3%) were not as showed in table (2). The result revealed that there is significant relationship (P value < 0.05) between smoking behaviors and DFUs. This is like a study that found a statistically significant relationship between smoking and DFU (Eltilib, 2021).

Table(2): Frequency of diabetic foot ulcers patients according to smoking groups

Smoking	No.	%	*P value*
Yes	50	35.7	0.001*
No	90	64.3	
Total	140	100	

* represents a significant difference at p<0.05.

Studies by Mariam *et al*., (2017) and Zhang *et al*., (2017) also agreed with current study by showing the diabetic foot is significantly different by smoking. Current result inconsistent with Brazil study that report smoking was not associated with diabetic foot that lead to lower limb amputation or death in this diabetic population (Costa *et al*., 2017).

Previous studies have identified smoking as a risk factor for diabetic foot ulcers because daily tissue hypoxia may cause vascular and neuropathic disorders in the lower extremities of diabetic patients (Obaid *et al*., 2015);(Zhang *et al*., 2017). Its pathogenesis is reduced capacity to transport oxygen in the blood due to harmful by-products of cigarette smoking and results in tissue hypoxia and arteriospasm. This damage leads to compensatory erythrocytosis that increases blood viscosity and decreases tissue perfusion. Decreased tissue perfusion and oxygenation inhibit healing of diabetic ulcer, which can increase the risk of lower extremity amputation LEA (Lee *et al*., 2022). Regarding to the BMI, the distribution of DFUs patients came as the following: 2 (1.4%) were under weight, 41(29.3%) were normal, 55(39.3%) were overweight, and 42 (30.0%) were obesity. The finding of this study showed that overweight diabetic group greater than normal and underweight. Further, Obese diabetic patients were more likely to develop diabetic foot ulcer as compared to diabetic patients with normal body mass index, Figure (2).

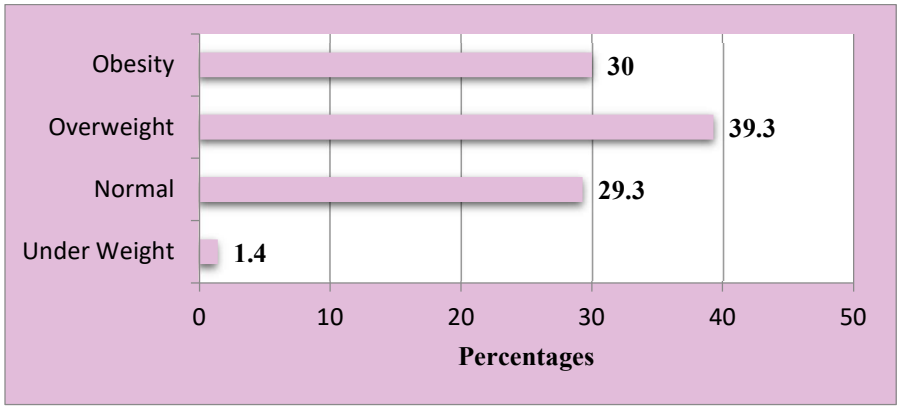


Figure (2): Frequency of DFUs patients according to BMI groups

This statistically significant difference (P value <0.05) which indicates that the occurrence rate of diabetic foot increased with BMI consistent with the study conducted in Ethiopia (Mariam *et al*., 2017). The possible reason could be due to the presence of higher foot pressure in those heavily weighed and with higher body mass index (BMI) diabetic patients as well obesity and overweight might decrease intensively the normal blood circulation pattern at the lower extremities; as a result, this might lead them to develop diabetic foot ulcer.

But, current findings disagreed with a study that discussed the association between BMI and DFU. The result showed that there is no statistically significant association between BMI and DFUs (Guo *et al*., 2022).

Out of the total population, the present study showed that only 9(6.4%) patients presented with type 1 DM and the rest 131(93.6%) were type 2 DM. Statistically there is significant relationship between DFUs and type of DM, Table (3).

Table(3): Frequency of diabetic foot ulcers patients according to type of DM groups

Type of DM	No.	%	*P value*
Type one	9	6.4	<0.0001*
Type two	131	93.6	
Total	140	100	

* represents a significant difference at $p<0.05$.

Similar results were which indicated type 2 DM diabetes mellitus was significantly associated with the occurrence of diabetic foot ulcer (Mariam *et al*., 2017);(Zhang *et al*., 2017). Unlike current result, Hussein *et al*., (2022) found that type of diabetes and development of foot ulcers was not associated.

However, the principal mechanisms have not been elucidated. The possible explanation could be in type 2 diabetic patients; there are related complications of the disease, such as mechanical changes in the conformation of the bony construction of the foot, peripheral neuropathy, and atherosclerotic peripheral arterial disease; as a result, the patient may have less tissue epithelisation, consumption of oxygen, nutrient transportation, and cell detoxification resulting in ulceration in the extremities (Mariam *et al*., 2017). Also, there was limited evidence about diabetic foot ulcer epidemiology in type 1 diabetes.

We were using Wagner's classification (Pitocco *et al*., 2019) in determine DF grades and done by physicians. In present study, diabetic foot grades of ulcers as following: 35 (25.0%) were grade 1, 32 (22.9%) were grade 2, 25 (17.9%) were grade 3, 28 (20.0%) were grade 4 and 20 (14.3%) were grade 5. This result suggests that incidence of diabetic foot is not significantly (P value > 0.05) associated with the DF grades, figure (3).

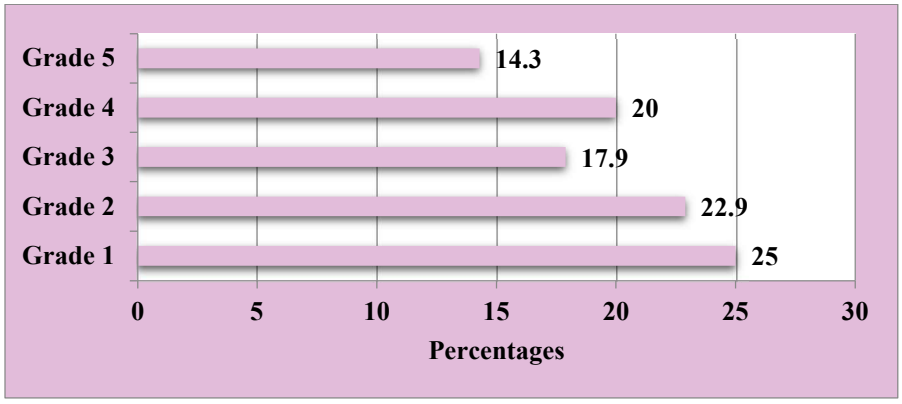


Figure (3): Frequency of DFUs patients according to DF grade groups

According to the DM duration, 57 (40.7%) of them had diabetes less than15 years, 62(44.3%) were of duration between 15 and 30 years, 21 (15.0%) were of duration more than 30 years. This result suggests that incidence of DFUs is significantly (P value <0.05) associated with the duration factor, Table (4).

Table(4): Frequency of DFUs patients according to type of DM duration groups

DM duration	No.	%	*P value*
<15	57	40.7	<0.0001*
15-30	62	44.3	
>30	21	15.0	
Total	140	100	

* represents a significant difference at p<0.05.

The result of current study is supported by studies reported that long duration of diabetes was significantly associated with DFUs (Saleem *et al*., 2017);(Khalifa, 2018). Furthermore, developing diabetes for a longer period of time is likely to be linked to additional diabetic problems, such as micro and macrovascular disorders, which are likely to be significant in the development of the skin breach and the spread of the ulcer (Musa *et al*., 2018). However, current result disagreed with a study found that there was no statistical significance between the duration of diabetes and the recurrence of DFUs (Huang *et al*., 2019).

Similar study conducted by Musa *et al*., (2018) in which gradation made no statically difference to DF ulcers. Unlike current result, a study reported that found relationship between the depth of the ulcer and severity of DFU measured with the Wagner's grades (Jalilian *et al*., 2020).

In this study, the distribution of DFUs according to duration of DFU came as the following: 20 (14.3%) were weeks, 97 (69.3%) were months, and 23 (16.4%) were years. Moreover, there is statistically significant difference (P value < 0.05) which indicates that the incidence rate of DF ulcers increased with the duration of DF ulcers, Table (5).

Table(5): Frequency of DFUs patients according to Duration of DFU groups

Duration of DFU	No.	%	*P value*
Weeks	20	14.3	<0.0001*
Months	97	69.3	
Years	23	16.4	
Total	140	100	

* represents a significant difference at p<0.05.

Current results presented that patients with duration of DFUs >4 weeks are at a higher risk of DFU. This is similar Chinese study revealed that found statistically significant between duration of ulcers and DFUs (Huang *et al*., 2019). Also, there is a study in Iran showed that the statically relationship between DFU and duration of the DFU (Jalilian *et al*., 2020). Similarly, the effect of duration of DFU on severity of foot ulcers can be due to the cumulative effect of foot ulcers, and prolonged duration of DFU can be a sign of severity of DFU and the failure of therapy (Moeini *et al*., 2017).

According to drug using, the present study showed that 40 (28.6%) of patients were insulin using, 43 (30.7%) were oral antibiotic using, 55 (39.3%) were both drugs using, and 2 (1.4%) were no drug using. Statistically, there is significant relationship (P value < 0.05) between DFUs and drug using, figure (4).

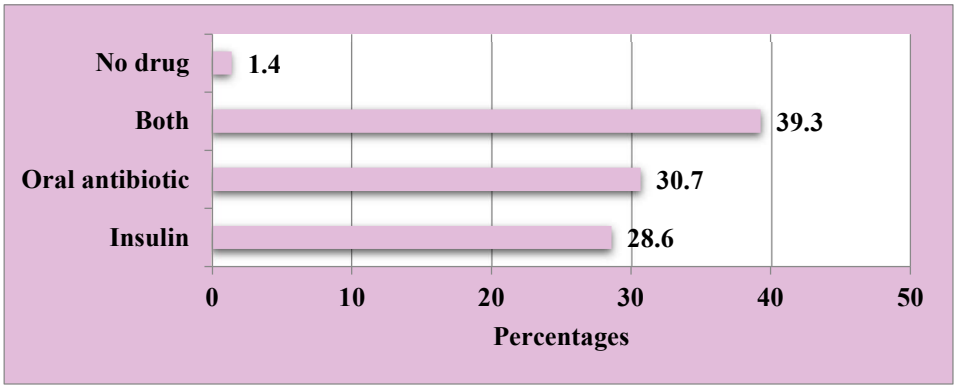


Figure (4): Frequency of DFUs patients according to drug using groups

Current result shown high percentage 55 (39.3%) of patients were using insulin combined with other oral antibiotic drugs. This significantly association between DFUs and drug using agreed with an Iranian study that stated a significant relationship between medication use and severity of DFU. Also, same a study reported a relation between both oral medication and insulin injections with severity of DFU (Madmoli *et al*., 2019). Turkish study disagreed with current result by report that no significant relationship between insulin treatment, antibiotic use and DFU (Sen *et al*., 2019). Although there is no clear reason for this relation, consider that insulin injection is more related to severity of DFU and can be due to the inflammation reaction in the body (Jalilian *et al*., 2020);(Welty *et al*., 2016).

b. Serum Adipokines (Adiponectine and Leptin):

Estimation of the Adipokines (Adiponectin and Leptin) among diabetic foot patients serum has been calculated in this study. Adiponectine and Leptin amounts were studied in each study groups (controls and patients), and shown the statistical differences among study groups, and comparison between each two groups, as shown in table (6).

Table (6): Comparison of study parameters (Adiponectine and Leptin) among studied groups (control and patient).

Parameters	Patient n=140	Control n=70	*P value*
Adiponectine (pg/ml)	1.455±0.38	0.887±0.22	<0.0001*
Leptin (ng/ml)	1.154±0.35	0.096±0.015	<0.0001*

The current study revealed that amounts of Adiponectine and Leptin among study groups (control and patient) serum is statically significantly different (P value <0.05), table (6). The average levels of Adiponectine and Leptin were found to be higher in subjects with diabetic foot (1.455±0.38 pg/ml and 1.154±0.35ng/ml, respectively) serum levels compared to controls

(0.887±0.22 pg/ml and 0.096±0.015 ng/ml, respectively) serum levels, which is in line with the a Italian study by Tuttolomondo *et al*., (2015) that found adiponectine levels in patients with diabetic foot showed significant different compared to controls. But, dissimilar to a study reported that serum leptin showed no significant increase in diabetic patients compared to controls (Hassn, 2017).

The adipose tissue releases proteins known as adipokines. Hormones, cytokines, growth factors, vasodilators, and various other chemicals, including critical signal molecules, constitute adipokines (Zorena *et al*., 2020). Adiponectin, leptin, and other adipokines are the proteins that are most studied. The roles and molecular mechanisms behind the actions of adipokines are also not fully understood. The control of energy and food, lipid and glucose metabolism, insulin function, endothelial cell function, inflammation, blood pressure, hemostasis, atherosclerosis, metabolic syndrome, etc. are just an among the numerous processes that adipokines are involved in and can affect (Fasshauer and Blüher, 2015).

The cytokine adiponectin is released from adipose tissues (adipokine). Adiponectin is thought to have a role in a number of physiological processes, molecular and cellular activities, including lipid metabolism, energy regulation, immunological response and inflammation, and insulin sensitivity, according to the available research. Neural stem cells and neurons are both protected by it (Khoramipour *et al*., 2021).

Current result that found adiponectin levels in diabetes patients with DFUs more than diabetes without DFUs this may be due to the increased amount of body fat in those patients. This is consistent with previous studies presented that adiponectin total levels were higher in type 1 diabetic patients with than without microvascular complications (Al Saeed, 2013;Grzelak *et al*., 2019). This finding increases the possibility that adiponectin actually speeds up the onset and progression of diabetic nephropathy. After kidney transplantation, other investigations showed that high serum adiponectin levels reduced, indicating that renal insufficiency may have affected adiponectin clearance or stimulated adiponectin synthesis (Fazeli *et al*., 2018).

A Czech study inconsistent with current result which report that patients with T2D have significantly lower levels of adiponectin (Spurná *et al*., 2018). Also, adiponectin levels have been reported to be negatively correlated with cancer, cardiovascular disease, and diabetes, and shown to be affected (i.e., significantly increased) by suitable healthy nutrition (Prates *et al*., 2016).

Leptin is a protein hormone that controls appetite. It is also produced by adipocytes. Leptin has an effect on insulin control because high levels inhibit insulin secretion while low levels increase insulin synthesis. Leptin has the ability to control cardiometabolic conditions through influencing peripheral adiposity and the central nervous system (CNS). Disruption of CNS leptin signaling causes metabolic disorders such as obesity, type 2 diabetes, and hypertension (Farkhondeh *et al*., 2020).

Current result that found leptin levels in diabetes patients with DFUs more than diabetes without DFUs. This is consistent with findings indicating obese type 2 diabetic patients had considerably higher leptin levels than healthy controls (Kocot *et al*., 2017), which could be

attributed to the patients' increased body fat. Another study disagreement with present study by shown that patients with foot ulcers had lower leptin levels ($p = 0.052$) (Saydam *et al*., 2021).

c. Detection of adipokine genes by RT-PCR

Adipokine gene expression in DFUs patients and controls. Real-time PCR was used to examine the quantitative changes in adipokine gene expression.

1. RNA Extraction

RNA was extracted from the selected adipokine (adiponectin) from both patients and controls. Total RNA of samples was extracted by using TRIzol reagent, and the concentration was ranged between 43 to 328 ng/µl.

2. Adiponectin expression in study groups

To evaluate the difference in expression of the adiponectin gene between DFUs patients and controls, quantitative real-time PCR was carried in a two-step RT-PCR procedure using SYBR green. Using livak equation 2-CT, a simple approach for evaluating relative changes in gene expression in real-time quantitative PCR studies, the results revealed an increase in the expression level (up regulation) of the adiponectin gene in patients compared to controls (Maren *et al*., 2023; Flatschacher *et al*., 2022).

The result of RT-PCR in this study revealed that the expression of adiponectin gene in DFUs patients in 34 blood samples was 5.6947 compared to control group, the expression of the adiponectin gene in controls in 70 blood samples from was 0.958 as shown in table (7) and figures (5) and (6). Adiponectin gene expression was observed to be considerably higher in DFUs patients than in the control group. We found that patients had a fivefold greater expression of adiponectin DFUs than the control group.

The results of RT-PCR showed that found a statically relationship between study groups (P value < 0.05).

Table (7): Gene Expression among Control and Patients versus the reference gene (GAPDH)

Groups	N	Expression levels (2^-(ΔΔCt))		
		Mean	SD	SE
Control	70	5.6947	5.46	0.937
Patient	34	0.958	0.716	0.085
P value	<0.0001*			

* represent a significant difference at p≤ 0.05.

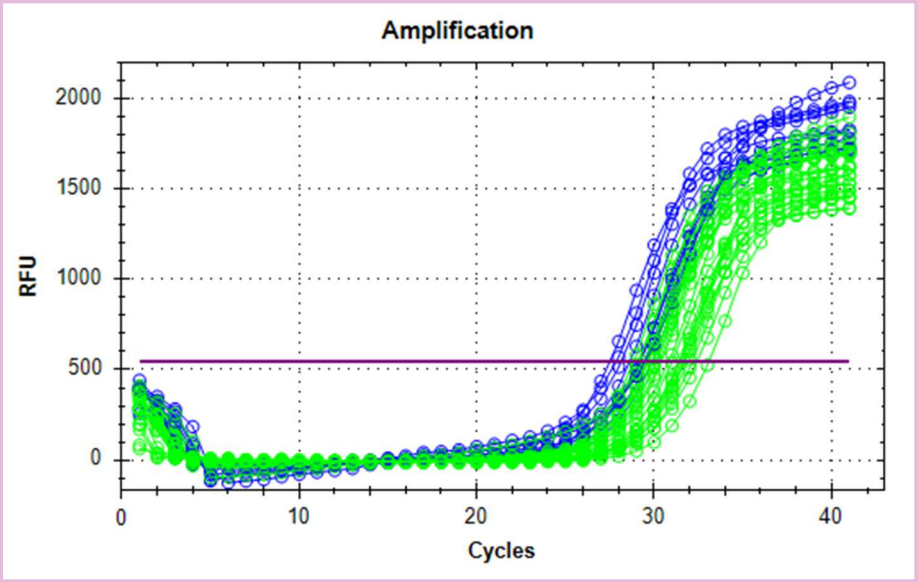


Figure (5): The Real time amplification plots of (adiponectin) gene in patient and healthy control blood samples. Where, the blue plots (patients samples) and the green plots (healthy control)

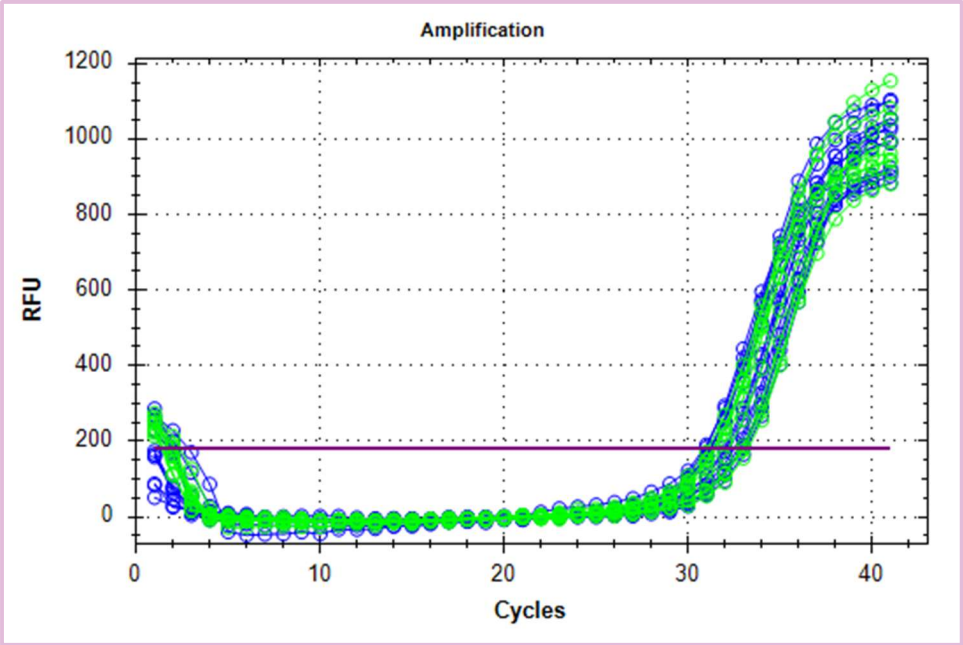


Figure (6): The Real time amplification plots of (housekeeping GAPDH) gene in patient and healthy control blood samples. Where, the blue plots (patients samples) and the green plots (healthy control)

The result of the current study showed that there was a relation between DFUs and gene expression of adiponectin gene. Current results like a Polish study that found despite higher adiponectin mRNA expression, its protein content in EAT was lower than in SAT (Toczylowski *et al*., 2019). In different to current results, Bambace *et al*., reported lower adiponectin mRNA levels in EAT than in SAT (Bambace *et al*., 2014).

However, due of the disparity in protein and mRNA expression, these findings should be regarded with caution. This gap could be explained by the complex process of protein synthesis. Post-transcriptional, translational, and protein breakdown processes all influence protein abundance (Beaulieu, 2019). Tissue mRNA levels were shown to explain just 40% of tissue protein variability. As a result, gene and protein expression may differ between tissues (Buccitelli and Selbach, 2020).

4. Conclusion

ELISA results show the amount of adipokines (adiponectin and leptin) were found to be higher in subjects with diabetic foot serum levels compared to controls serum levels.

Recommendations

Estimation the serum levels of adiponectin and leptin in patients with diabetes mellitus as biomarkers for obesity. Using liquid and tissue biopsy for the immunological evaluations.

Acknowledgements

The authors would like to thank the editors and the anonymous reviewers for their valuable comments and suggestions, which have helped immensely in improving the quality of the paper, and anyone else who contributed to the completion of this work.

Conflict of Interest Statement

We have no conflicts of interest to disclose.

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