Relations between Interlukin-6 and Some Biochemical Parameters in Diabetic Foot Syndrome

Raid M.H. Al-Salih[1], Zeena M. Ali[2]

[1]Prof., [2]Researcher, University of Thi-Qar–College of Science–Department of Chemistry

Abstract

The aim of this study is to predict the incidence of diabetic foot syndrome in type 2 diabetic patients by measuring some parameters (IL-6, FBS, MDA, CP, BMI). Samples were obtained from (75) type II diabetic patients with and without diabetic complications (diabetic foot and diabetic neuropathy), as well as (25) healthy controls as a control group. They were divided into four groups: Group A (the control group):- It included (25) healthy individuals aged between (35-70 years). Group B (Diabetics type II without complications):- Includes (25) patients, their ages range (35-70 years). Group C (diabetic patients with diabetic neuropathy):- It included (25) patients whose ages ranged from (35-70 years). Group D (diabetic patients with diabetic foot syndrome): - Included (25) patients whose ages ranged from (35-70 years). The results showed a significant increase in the levels of (IL-6, FBS, MDA, CP) in all groups of type II diabetes patients with and without complications compared to the control group ($P \leq 0.05$). There was a significant increase in the parameters measured in the group of diabetic patients with type II diabetic foot syndrome (DF) compared to the group of diabetic patients with type II disease Diabetic neuropathy (DN), and the results also showed a significant increase in group DN compared to group of type II diabetic patients (DM) ($P \leq 0.05$). Our study show Interleukin-6 values, as well as oxidative stress, could predict the future incidence of diabetic foot syndrome.

Keywords: *Diabetic foot syndrome, Diabetes mellitus type 2, Diabetic neuropathy, FBG, IL-6, and oxidative stress.*

Introduction

Diabetes mellitus (DM) describes a group of metabolic disorders characterized by high blood glucose levels. People with diabetes have an increased risk of developing a number of serious life-threatening health problems resulting in higher medical care costs, reduced quality of life, and increased mortality[(1)]. The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than lowincome countries (4.0%). One in two (50.1%) people living with diabetes do not know that they have diabetes[(2)]. Chronic Diabetic Complications Microvascular complications include:

Retinopathy, Diabetic Neuropathy, Diabetic Nephropathy, Diabetic foot[(3)]. Between 13% and 26% of diabetics report painful chronic polyneuropathy. About 50% of patients with diabetes mellitus develop symptomatic peripheral neuropathy within 25 years of disease onset.

Sensory neuropathy is the primary cause of more than 60% of diabetic foot ulcers. Diabetic peripheral neuropathy is a microvascular complication of diabetes mellitus and in type 2 diabetes, not only hyperglycemia but also other metabolic alterations and persistent inflammatory status due to adiposity play a major role in axon injury[(4)]. Diabetic foot is one of the most feared complications of diabetes mellitus. Diabetes and its complications as foot ulcers may develop as a result

Corresponding Author:
Prof. Dr. Raid M.H. Al-Salih
University of Thi-Qar–College of Science–Department of Chemistry
e-mail: raidstry@gmail.com

of polyneuropathy, ischaemic causes, or as a result of all[5]. Hyperglycemia causes tissue injury leading to vascular damage through generation of free radicals and their effect on endothelium in diabetics. oxidative stress plays an important role in the development of Cardio Vascular Diseases (CVD) and diabetes by imbalance of ROS production, and has close relationship with inflammation. It was observed that the lipid peroxidation are increased in patients with MS and it is directly related with the development of atherosclerosis together with establishment of pro-inflammatory state[6,7]. Also, diabetes can be regarded as a pro-oxidant state caused by increased lipid oxidation[8]. Hyperglycemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in secondary complications in diabetes mellitus[9].

Oxidative stress induced by reactive oxygen species (ROS), which results from hyperglycemia measured with (FBG), which are the first parameters that give the indication of diabetes[10]. Diabetes is characterized by hyperglycemia along with biochemical changes to glucose and lipid peroxidation. Several studies have evaluated free radical and antioxidant-induced lipid oxidation in diabetics[11,12]. Some complications of diabetes are associated with increased free radical-induced lipid peroxidation activity and accumulation of lipid peroxidation products[13]. Abnormally high levels of oxidation and a simultaneous decrease in antioxidant defense mechanisms can damage cellular organelles and lead to oxidative stress[14]. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and the antioxidant defense systems that organisms use to deactivate and protect themselves against free radical toxicity[15]. An imbalance of oxidative/antioxidant balance creates a condition known as oxidative stress. It is well known that oxidative stress is a component of molecular and cellular tissue damage mechanisms in a broad spectrum of human diseases[16]. Interleukin-6 (IL-6), a major cytokine mediator of the acute-phase response, which stimulates acute-phase protein production in the liver and has diabetogenic actions . The highest levels of acute-phase markers and IL-6 were found in those patients with most features of the insulin resistance syndrome (metabolic syndrome X: glucose intolerance, hypertriglyceridemia, low levels of serum high-density lipoprotein cholesterol, [central] obesity, accelerated atherosclerosis, and hypertension)[17]. Through the review, you present from such a series situation view we design this study to evaluate the level of Interleukin-6 as an inflammatory agent, and we see the possible relationship between interleukin levels and levels of (FBG), (BMI) and oxidative stress.

Patients and Method

Design of Study

The study is conducted at the Diabetes and Endocrinology Center in Thi-Qar and in the biochemistry laboratory in college of science, at the period between (November, 2019) to (May,2020)**.** It included (100) cases, (25) control and (75) patients. There were (100) male and female subjects, control and diabetic of type 2 with and without complication diabetic aged (35-70) years were included in this study. They divided into four groups as the following:-

Group A (Control): Included fifty (25) healthy subjects aged (35-70).

Group B (Diabetes type 2): Included (25) patients with diabetes mellitus type 2 aged (35-70).

Group C (Diabetes Type 2 with nerupathy) Included (25) patients with metabolic syndrome aged (35-70).

Group D (Diabetes Type 2 with diabetic foot syndrome) Included(25) patients with diabetic foot syndrome aged(35-70)

Collection of Blood Sample:

After overnight fasting for about 8 hr, (8 mL) of venous Blood samples were collected between 8:00 and 10:00 am from type 2 diabetic patients and controls then divided into the following:

- (2ml) of blood sample was taken to measure ESR.

- (3 ml)of the blood sample was transferred to tubes containing EDTA (Ethylene diamine tetra acetic acid) and used for HbA1c, CRP, hsCRP estimation.

- (3ml) were transferred to a plain tube and allowed to clot at room temperature to get serum by putting it in empty disposable tubes and centrifuged to separate it at 3000 rotor per minute (rpm) for 10 min, the serum samples were separated and stored at (- 20˚C) for later measurement biochemical parameters, unless used immediately.

Determination of Biochemical Parameters

Interleukin 6 (IL-6): Enzyme Linked Immunosorbent Assay (ELISA) technique was used to measure, serum levels of Interlukine-6 (IL-6 ELISA Kit Dublin, Ireland), in diabetic patients with & without complication diabetes type2 and control group[18,19]

Fasting Blood Glucose (FBG): Glucose was determined after enzymatic oxidation in the presence of glucose oxidase . The hydrogen peroxide formed reacts under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator[20].

Ceruloplasmin (Cp): Serum Cp concentration was measured by the method of[21]. It is based on the ceruloplasmin-catalyzed oxidation of colorless para-phenylene diamine (PPD) to blue-violet oxidize form. The reaction is followed photometrically and the blank value is determined after inhibition of the enzyme with sodium azide[22].

Lipid Peroxidation Marker (Serum MDA): Lipid peroxidation was determined by using the thiobarbituric acid method. MDA concentrations were calculated, using the molar extinction coefficient of MDA (ƐMDA) equal to 1.56 x105 mol-1. cm-1[23]

Statistical Analysis: The statistical analysis proceeded in all groups of study, descriptive statistics analyzed by using one-way analysis of variance (ANOVA) were performed using mean and standard deviations (SDs) with least significant difference (LSD) test for continuous variables (p value ≥0.05) was considered to be significant. All analyses were performed with the Statistical Package for the Social Sciences SPSS for Windows (version 23.0, SPSS Inc, Chicago, III).

Results and Discussions

Clinical and Characteristic Features of the Studied Groups: There are 100 case studies included in this study, with a difference between the groups of type 2 diabetes patients and the health control group in each of them (IL-6,FBG), oxidative stress (MDA,CP), and without difference in each (age, gender, and body mass index).

Interleukin 6 (IL-6): Table (1) shows the statistically significant increase in the levels of (IL-6) in patient groups compared to the control group (P≤0.05), and there is a significant increase in group (DF) compared to groups (DN) and (DM), and also a slight difference was found between group (DN) and group (DM)(P≤0.05). The results of this study agree with A previous study like[24-26].

The most important factor in the development of foot ulcer is peripheral neuropathy. Diabetic peripheral neuropathy is one of the most serious complications of type 2 diabetes mellitus (T2DM) that decreases the quality of life of T2DM patients[27]. T2DM is a metabolic pro-inflammatory disorder characterized by chronic hyperglycemia and increased levels of circulating cytokines suggesting a causal role of inflammation in its etiology. Interleukin (IL)-6, is an important proinflammatory cytokine, that plays a potential pathological role in Diabetic foot disorder. .Proinflammatory cytokines are involved in the pathogenesis of Diabetic foot disorder. Interleukin-6 (IL-6) plays an important role in the inflammatory and autoimmune processes[28].

IL-6 levels are closely related to insulin resistance in type 2 diabetes patients and its complications[29]. IL-6 level is associated with slow flow/microvascular dysfunction[30]. Local levels of inflammatory mediators are associated in the uncontrolled type 2 diabetic patients[31].Type 2 diabetes has been identified as an immune disease that leads to weak insulin signals and selective destruction of insulin-producing beta cells in which cytokines play an important role[32] as well as this study[33] showed higher values of (IL-6) for patients with type II diabetes compared to the control group. And it was proven through the study that (IL-6) reduces insulin sensitivity in the body[34] and thus the concentration of (IL-6) independently predicts the future risks of developing type 2 diabetes and its complications[35] On the other hand, we know that type II diabetes is associated with an mainly with an increase in the waist circumference clinically, and people who suffer from obesity fat tissue represents 50% of the body weight and is the first part of the immune system, as well as the (IL-6) secreted from many tissues, including fat tissue[36] and this explains for us a difference in levels The (IL-6) between study groups according to different body mass index.

Fasting Blood Glucose Concentration: Table (1) shows a significant increase in the concentration of (FBG) in all patient groups compared to the healthy group (P≤0.05). We observed a significant increase in

the concentration of (FBG) in group (DF) and (DN) compared to group (DM) (P≤0.05). This result is consistent with the results of the study[37].

The fasting blood glucose level, which is measured after a fast of 8 hours, is the most commonly used indication of overall glucose homeostasis, largely because of disturbing events. A persistent elevation in blood glucose leads to glucose toxicity, which contributes to cell dysfunction and the pathology grouped together as complications of diabetes[38]. Glucose can be transported from the intestines or liver to other tissues in the body via the bloodstream. Long-term hyperglycemia causes many health problems including heart disease, cancer,eye, kidney, and nerve damage[39].There is a significant increase in the concentration of (FBG) in group (DF) and (DN) because all patients suffering from diabetic foot and neuropathy are patients with type 2 diabetes and obese and their period of diabetes has exceeded 10 years or more. Self-management (controlling the fasting blood sugar level) of diabetes is an essential element to prevent or mitigate complications of diabetes, as[40] it was stated that (FBG) levels increased in diabetic foot patients compared to diabetic patients, because the chronic elevation (FBG) leads to diabetic neuropathy. Which is the gateway to diabetic foot syndrome, as revealed in this study[37]. There is a positive correlation between IL-6 and FBG levels in each of DF(r = 0.29), DN (r= 0.33) and DM (r = 0.21) as shown in figure(1).

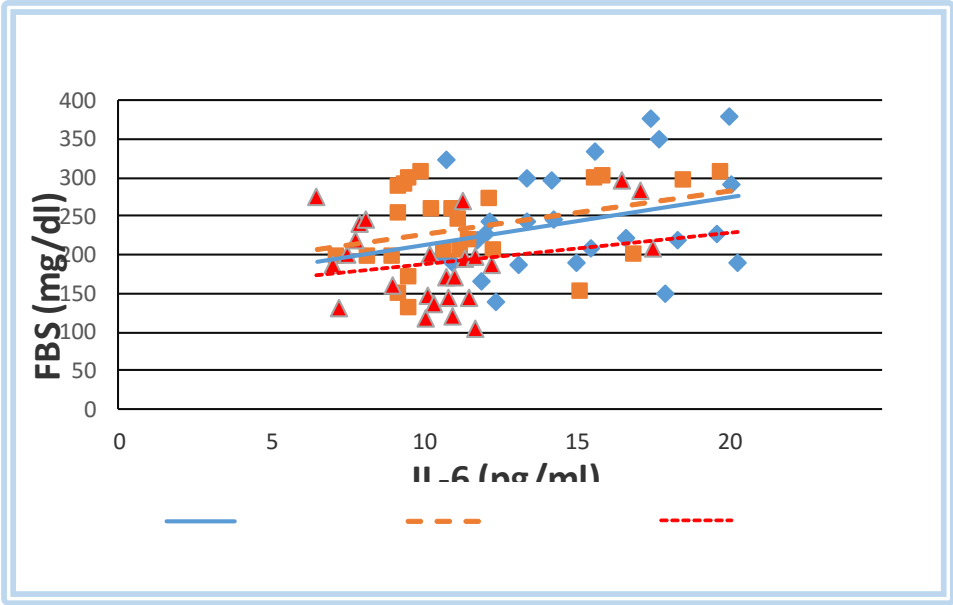

Fig (1): Correlation between IL-6 and FBG

Body mass index (BMI): In all groups, we observed a general increase in BMI due to obesity, but the results for all age groups showed an increase in BMI in all patient groups compared to the control group (p≤0.05). Table (1) show increase simple significance in group DF compared to DN and DM (P≤0.05). These results are consistent with the results of[41]. Obesity is one of the variables that can be changed to reduce the possibility of foot ulceration, and this is especially important because patients who suffer from diabetic foot ulcers often suffer from obesity. Therefore, weight reduction helps relieve pressure on the feet, and also reduces the incidence of diabetic neuropathy. Thus, mitigating the potential strong effect of BMI through appropriate dietary and metabolic control measures the complications of type 2 diabetes in general and of morbidity and the presentation of diabetes in particular[41]. There is a positive correlation between IL-6 and BMI levels in each of DF (r =0.37), DN (r= 0.19) and DM (r = 0.19) as shown in figure(2).

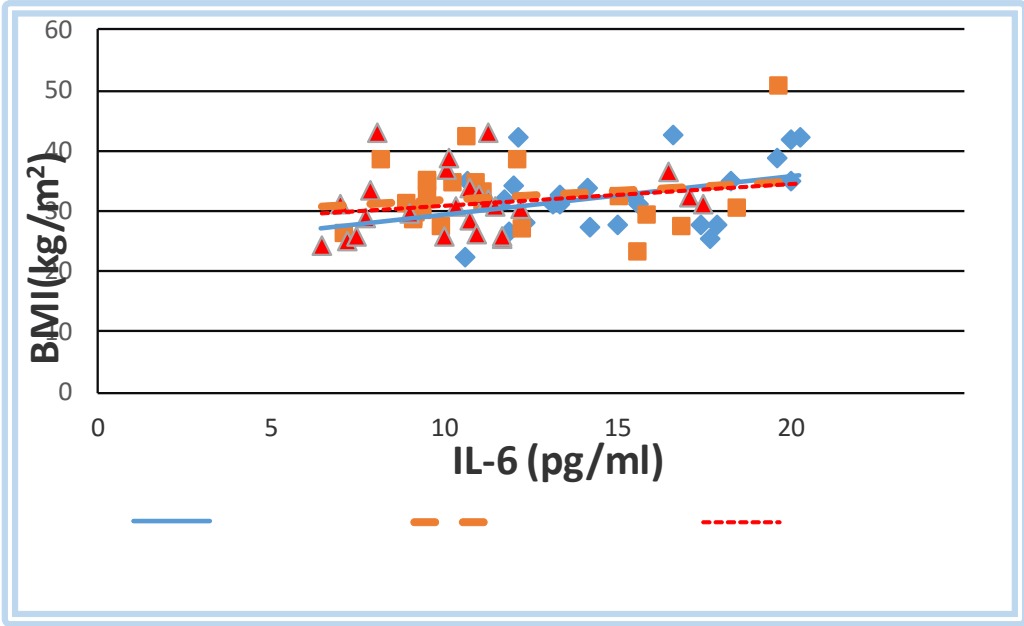

Fig (2): Correlation between IL-6 and BMI

Serum Malondialdehyde (MDA) Concentration: The table (1) shows a statistically significant significant increase in the concentration of (MDA) in all patient groups compared to the control group (P≤0.05). We noticed a significant increase in the concentration of (MDA) in group (DF) and (DN) compared to group (DM) (P≤0.05). We did not notice a statistically significant significant difference between Both groups (DF) and (DN)(P≤0.05). However, we found slight significant differences in the serum concentration between groups (DF) and (DN) .. The results of this study were consistent with the results of a study such[42].

Oxidized lipids have a signaling function in pathological situations, are proinflammatory agonists and contribute to neuronal death under conditions in which membrane lipid peroxidation occurs[43]. Results indicate that lipid peroxidation has a role in the pathogenesis of several such as inflammatory[44].A level of MDA indicates the degree of lipid oxidation resulting from the oxidative degradation of polyunsaturated fatty acids due to increased production of free radicals and impaired antioxidant defenses. Excess fat during each oxidation process may damage various body tissues[45]. Elevated levels of (MDA) may indicate oxidative stress associated with disease activity and its treatment. Diabetic neuropathy may arise from a combination of nerve and microvascular deficits. Oxidative stress can greatly contribute to these deficiencies and may be a direct result of hyperglycemia. Short postprandial peaks in plasma glucose are sufficient to generate the oxidative stress of hyperglycemia. In contrast, acute glucose deprivation also induces apoptosis of peripheral neurons through a mechanism that at least partially involves oxidative stress[46]. Treatments like antioxidants that target oxidative stress are the best solution to control blood glucose levels and thus prevent neuropathy as well as other complications of diabetes[47]. There is a positive correlation between IL-6 and MDA levels in each of DF (r =0.15), DN (r= 0.11) and DM (r = 0.23) as shown in figure(3).

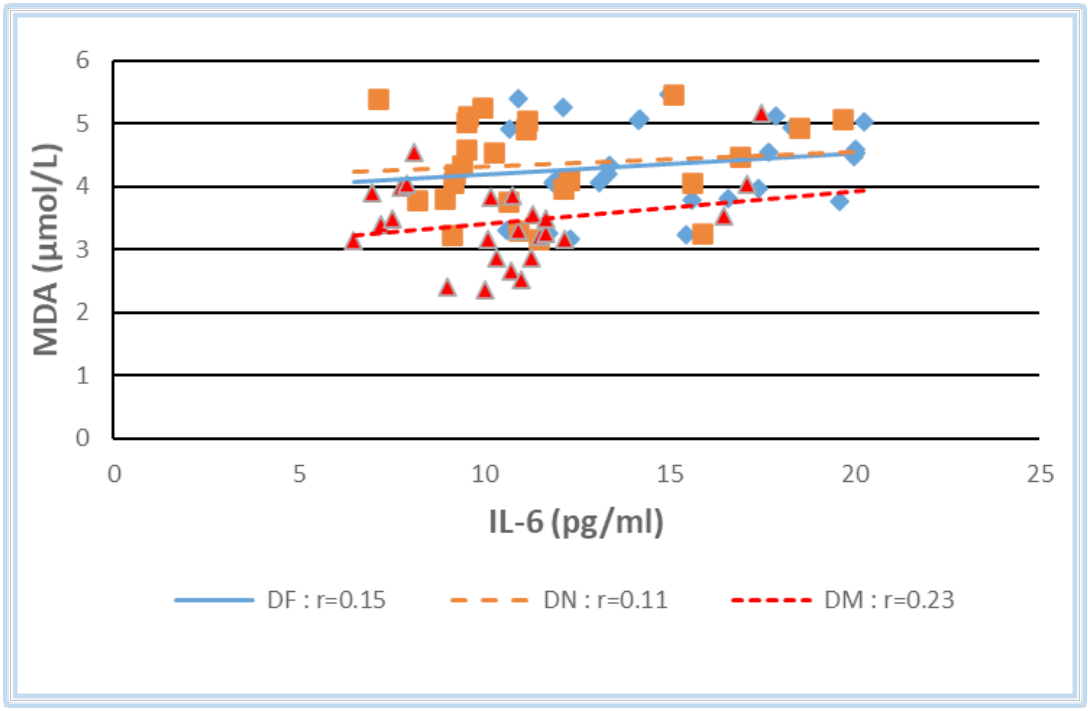

Fig (3): Correlation between IL-6 and MDA

Serum Ceruloplasmin (Cp) Concentration: The table (1) indicates a significant increase in the concentration of (CP) for all groups of patients compared with the control group (P≤0.05). There was a statistically significant increase in group (DF) compared to group (DM) (P≤0.05). We did not notice significant differences in groups (DN). When compared with the two groups (DF) and (DM) (P≤0.05), the results of this study were identical to that of[48].CP plays a role in many biological functions including copper transport, coagulation, angiogenesis, feroxidase activity, and defense against oxidative stress[49]. Coagulation and angiogenesis have a role in diabetes complications. The antioxidant properties of CP are attributed to its ability to inhibit lipid oxidation, scavenge superoxide, and isolate free copper ions[49,50] .CP a protein with specific domains capable of facilitating cellular energy production and preventing the formation of oxygen radicals, and its role in iron metabolism[51]. When there is an abnormality in secretion CP, the excess iron is deposited in the liver, pancreas, and retina, leading to cirrhosis and endocrine abnormalities such as diabetes and vision loss respectively[52,53]. The mechanism behind the positive association between ceruloplasmin levels and the development of diabetic complications is largely unknown. One plausible explanation is that ceruloplasmin might act as a pro-oxidant under conditions of increased oxidative stress, such as in type 2 diabetes mellitus[53]. Although ceruloplasmin possesses antioxidant properties due to its ferroxidase activity, increased generation of ROS disrupts the binding of copper from ceruloplasmin[54]. Ceruloplasmin is an acute phase reactant,and the serum concentration increases during inflammation, infection, and trauma largely as the result of increased gene transcription in hepatocytes mediated bythe inflammatory cytokines[55]. This explains the positive relationship between levels of (CRP,ESR and HS-CRP) that appeared in our study and (CP), as well as the positive correlation between it and (IL-6). There is a positive correlation between IL-6 and CP levels in each of DF (r =0.33), DN (r= 0.52) and DM (r = 0.26) as shown in figure (4).

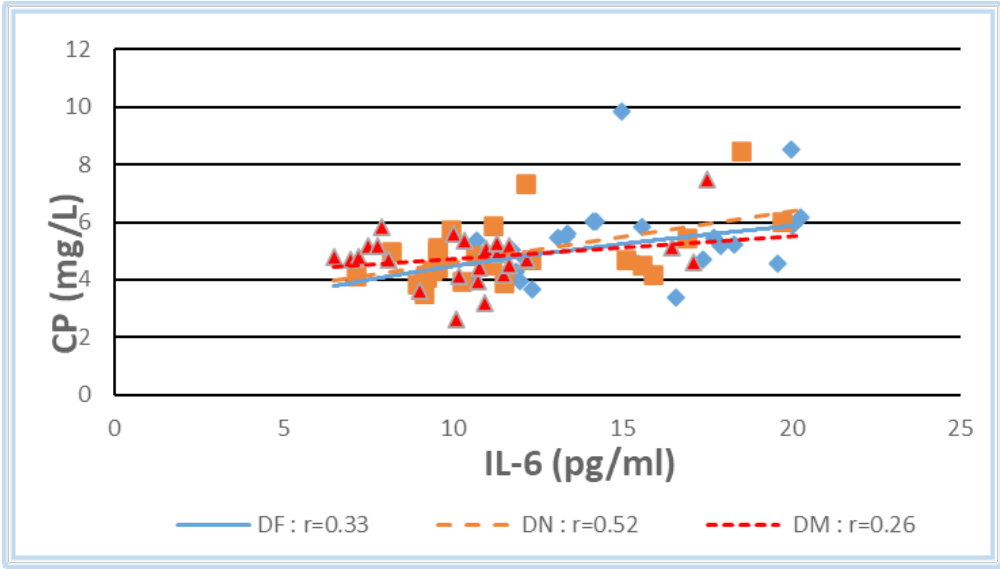

Fig (4): Correlation between IL-6 and CP.

Table 1: Characteristic data for all studied groups.

Parameter	Groups	N	Mean	Std. Deviation
IL_6 (pg/ml)	Control	25	8.89^{c}	1.02
	Diabetes	25	10.63^{b}	2.93
	Neuropathy	25	11.66^{b}	3.34
	Diabetic foot	25	14.97^{a}	3.19
L.S.D	1.30			
FBG (mg/dl)	Control	25	101.96^{c}	13.23
	Diabetes	25	190.0^{b}	54.76
	Neuropathy	25	236.44^{a}	55.19
	Diabetic foot	25	244.52^{a}	68.66
L.S.D	24.54			
CP (mg/L)	Control	25	4.08^{c}	0.78
	Diabetes	25	4.76^{b}	0.92
	Neuropathy	25	4.89^{ab}	1.13
	Diabetic foot	25	5.34^{a}	1.39
L.S.D	0.50			
BMI (Kg/m^2)	Control	25	30.43^{b}	4.510
	Diabetes	25	31.26^{ab}	5.26
	Neuropathy	25	32.13^{ab}	5.79
	Diabetic foot	25	33.70^{a}	8.92
L.S.D	2.98			
MDA (µmol/L)	Control	25	1.89^{c}	0.44
	Diabetes	25	3.43^{b}	0.65
	Neuropathy	25	4.32^{a}	0.72
	Diabetic foot	25	4.36^{a}	0.71
L.S.D	0.30			

Conclusions

There are oxidative stress and a disturbance in the balance of oxidants and antioxidants in diabetic foot syndrome, neuropathy, and diabetes, which are associated with high levels of glucose. Diabetes and its complications can be controlled by correcting this disorder. IL-6 Positive correlation with (FBG,MDA,CP,BMI).

Ethical Clearance and financial support: Lastly the ethical approval for this study was issued by the ethical commettee of college of science of Thi-Qar university. Moreover there was a financial support from college of science in Thi-Qar university.

Conflict of Interest: Nil

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