Role of Osteoprtegerin levels in a sample of Iraqi diabetic foot patients

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Abstract

Diabetic foot (DF) is serious diabetes (DM) complication leading to delayed wound healing and may result in amputation. Osteoprotegerin (OPG) is a glycoprotein decoy receptor of RANKL(OPGL), involvement of OPG in the pathogenesis of diabetic complications has been proposed. Interactions OPG/OPGL has been proposed to be a major inhibiter of osteoclastogenesis (differentiation of osteoclasts cells),and OPG also has a role in the regulation of the immune response .

This study was done to investigate whether the serum levels of circulating soluble osteoprotegerin, glycoprotein associated with the apoptosis, are altered in patients with type 2 diabetes whom have diabetic foot ulcerations.

Osteoprotegerin was measured with the ELISA method in thirty (30) normal controls (group I), twenty five patients with type 2 DM (duration ranged from 1-32 years (group II) and in twenty five diabetic foot patients with type 2 DM (duration ranged from 2-31 years (group III). Also, serum glucose (Fasting), lipid profile (total cholesterol, triacylglycerol, HDL-c, LDL-c and VLDL-c) and hs-CRP were determined after 12hrs of fasting.

Results: The patients with diabetic foot lesions were found to be poorly controlled and had significantly higher levels of fasting Plasma glucose (FPG) ($p<0.05$,$p<0.001$) when compared to patients whom without diabetic foot lesions and control groups. Also the patients with diabetic foot lesions were found have significantly higher levels of OPG when compared to patients without diabetic foot lesions and healthy control groups ($p<0.01$,$P<0.001$) respectively . Serum levels of total cholesterol, triacylglycerol, LDL-c and VLDL were

significantly higher ($p<0.05$) in diabetic patients (with and without foot ulceration) in comparison with healthy normal control, while HDL-c level was lower in both groups than in the control group but not reach statistical significant. There are no significant different in lipid profile between diabetic foot patients and without diabetic foot patients. OPG serum levels were significantly increased in diabetic foot group as compared with patients whom without diabetic foot and normal control (10.82±2.87 ,6.16±6.18, ng mL□1 Vs 4.89±5.8 ng mL□1, $p<0.001$), also the levels were higher in patients without diabetic foot lesions when compared with that of control but the difference did not reach statistical significant ($p>0.05$).C-reactive protein also was significantly higher in diabetic foot group as compared with patients whom without diabetic foot and normal control(13.96±12.62,4.83±4.02 Vs 3.89±4.21,$p<0.001$).The difference in patients without diabetic foot lesions did not reach statistical significant ($p>0.05$) when compared with that of control. A positive correlation was observed between OPG and FPG, lipids (except HDL-c) and hs-CRP in diabetic foot patients but the correlations did not reach statistical significant.

The aim of this study was designed to investigate the role of OPG in the pathogenesis of diabetic foot ulcerations in a sample of Iraqi patients with type2 diabetes mellitus.

Conclusion: In this study, in diabetic foot patients, we assessed that the development of diabetic foot ulcerations increases by high levels of OPG that associated with apotosis in diabetic foot, and the development of new treatment against apoptosis may play an important role in the management of diabetic foot lesions.

Key words: Osteprtegerin; OPG levels; diabetic foot; type 2 diabetes mellitus

دور مستويات الاوستيوبروتجرين (OPG) في نموذج من مرضى سكري القدم العراقيين

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الخلاصة

مرض سكري القدم هو احد مضاعفات السكري الخطيرة والتي تقود الى تاخير او بطء شفاء الجروح والتي قد تودي الى بتر القدم .ان الاوستيوبروتجرين(OPG) هو بروتين سكري وهو المستقبلة الوهمية (المصيدة) للعامل RANKL (او يدعى ايضاOPGL) والذي يؤدي الى نقض(تأكل) العظم.ان تضمن الاوستيوبروتجرينOPG في تولد مضاعفات السكري قد تم اقتراحه. ان تداخل(OPG/OPGL) قد تم اقتراحه كمثبط رئيس لتولد الخلايا الناقضة للعظم ,كما ان ال OPG ايضا لديه دور في تنظيم الاستجابة المناعية.

تهدف هذه الدراسه لاكتشاف فيما اذى كان هناك تغير في مستويات الاوستيوبروتجرينOPG(وهو بروتين سكري) والذي يرتبط بالموت المبرمج للخلايا والCRP في دم مرضى السكري المصابون بقروح القدم السكري.

تشمل هذه الدراسه ثمانين شخص تراوح معدل اعمارهم بين (36-86) سنة . ثلاثون شخص (30) اصحاء اختيرو كمجموعه ضابطه وخمسون شخص (50) مصابون بمرض السكري النوع الثاني :(25) شخص منهم كانوا مصابون بتقرحات القدم السكري تم تعين درجه اصابتهم وفقا لتصنيف واكنر, (25) مريض الباقون من مرضى السكري كانوا بدون مشاكل القدم السكري . لقد تم تقدير مستويات الاوستيوبروتجرين (OPG) وكذالك مستويات البروتين الفعال سي(CRP) بواسطه تحليل الايلايزة (ELISA) للمرضى والاصحاء باستخدام الكتات التجارية المتوفرة . بالاضافة الى ذلك تم تعيين مستوى السكر (FPG) ومستوى انماط الدهون في الدم بعد الصيام لمدة 12 ساعة , ومعرفة مدة الاصابة بالسكريDuration of DM ودالة كتلة الجسم BMI والعمر والجنس .

اظهرت **النتائج** ارتفاع معنوي ذو قيمة في تراكيز ال OPG والCRP ($P<0.05$) في دم مرضى القدم السكري مقارنة الى مرضى السكري بدون قدم سكري والاصحاء .كما اظهر مرضى القدم السكري ارتفاع معنوي ذو قيمة في مستويات سكر الدم عن مرضى السكري بدون قدم سكري وعن الاصحاء. اضهر مرضى السكري جميعهم ارتفاع في مستويات الكوليستيرول, والدهون الثلاثية,والدهون واطئة الكثافة (LDL,VLDL) ($p<0.05$) مع بقاء الدهون العالية الكثافة عند مستويات اعتيادية مقارنة بالاصحاء($p>0.05$).لم تكن هناك تغيرات في مستويات انماط الدهون بين مرضى سكري القدم ومرضى السكري غير المصابين بسكري القدم . معظم مرضى سكري القدم كانوا مع مدة اصابة بالسكري طويلة تصل اكثر من عشر سنوات(معدل الاصابة مع انحراف قياسي يساوي 7.73+13.28).كما اضهرت الدراسة ارتفاع في مستويات OPG لمرضى القدم السكري من الذكور مقارنة بالاناث , مع انخفاض في مستويات CRP للذكور مقارنة بالاناث.لقد كان هناك ترابط موجب بين ال FBS وOPG ولكنه لم يبلغ القيمة الاحصائية المعنوية.ولكن هناك ترابط سالب OPG والCRP لقد كان **الاستنتاج** من هذة الدراسة بان مستويات ال FBS وOPG يكون لها دورفي تولد قروح القدم السكري والCRP يزداد نتيجة التهاب هذة القروح, وان تطوير علاج جديد ضد مسار الموت المبرمج للخلايا قد يؤدي الى شفاء قروح القدم السكري.

الكلمات المفتاحية: الاوستيوبروتجرين : مستويات OPG : القدم السكري : مرض السكري النوع الثاني.

INTRODUCTION

In diabetic patients neuropathy with vascular diseases in the legs contributes to the risk of diabetes-related foot problems, such as diabetic foot ulcers and infection that can be difficult to treated and occasionally lead to (end with) amputation. Recently, many researches were focused on proteins regulating metabolic pathways in human organism. Increase in knowledge about the role of OPG/RANKL/RANK (OPG:–Osteoprotegerin, RANK – receptor activator of nuclear factor-β; L-ligand) .Osteoprotegerin (OPG) is a protein belonging to superfamily of tumor necrosis factor (TNF). Osteoprotegerin (OPG), a secreted member of the tumor necrosis factor receptor superfamily of cytokines, has been associated with endothelial dysfunction. OPG plays an important role in regulation of bone resorption rate [1]. OPG role is to protect RANK receptor against interaction with RANKL [2] .In the absence of OPG, RANKL cytokine connects to RANK localized on entire osteoclasts cells line leading to bone tissue resorption process [3]. OPG is RANKL antagonist so it protects bone tissue against resorption. OPG gene TNFRSF11B polymorphisms are related to the pathogenesis of many diseases. T245G polymorphism in TNFRSF11B has proven relation with prevalence of osteoporosis, Paget's disease, idiopathic hyperphosphatemia and is linked with increased risk of ischemic heart disease occurrence [4-9].Recent researches prove a linkage between TNFRSF11B gene T245G and C1181G polymorphisms with one of diabetic foot type (DF) [10]. DF is a polyetiological diabetes complication leading to delayed wound healing and lower limbs amputation in consequence [11]. Due to dominating etiology, we marked out DF of neuropathic origin where sensorimotor neuropathy dominates, DF of angiopathic origin, where the etiological factors are atherosclerotic changes in lower limbs vessels, and DF of mixed origin where both mentioned factors coexist. The most common is DF of neuropathic origin. On basis of coexisting autonomic neuropathy, neuroarthropathy may develop. Neuroartropathy is a consequence of small arteries blood flow disregulation. There is an increased flow through denervated vessel that passes through distal tissues supplied by small arterioles. Blood reaches venous system through avoiding shunts. This increased blood flow leads to severe bone tissue resorption in foot. Only early correct medical intervention during the acute phase of Charcot neuroarthropathy may lead to decrease of bone tissue resorption. In many patients, resorption changes lead to foot curve alignment and foot tissue ulceration.

Therefore, Charcot neuroarthropathy diagnosed at chronic stadium usually leads to lower limb amputation. Therefore, OPG seems to be a factor of significant importance in Charcot joint and DF pathogenesis generally.

The **aim** of the study was to specify if OPG levels are DF risk factor in DM type2. The premise to the study was a role of OPG levels in pathogenesis apoptosis pathways leading to DF of ischemic and neuropathic origin

MATERIALS AND METHODS

Materials:

This cross sectional study population included 80 subjects. All subjects were divided into 3 groups:

Group I; Control group: comprising 30 apparently healthy subjects which matched with age and sex as the diabetic patients. They had no recognizable diseases or previous history of endocrine disturbances. They were clinically free from any abnormality. They were not receiving and medications.

Group II; Diabetic group: including 25 patients with type 2 diabetes (diagnosis according to criteria of the ADA, 2006). Diabetes duration ranged from 1 to 32 years. The age ranged from 46 to 68 years.

Group III; Diabetic foot group: including 25 diagnosed type 2 diabetic patients with diabetic foot lesions are selected. Diabetes duration ranged from 2 to 31 years. The age ranged from 36 to 77 years. .

All patients and participants gave their informed consent for the study, which was approved by National center of diabetes, Al-Mustansirya university, Baghdad, Iraq. The following variables were recorded: age, BMI, gender . BMI was calculated as weight divided by height squared (kg m^{-2}). The cutoff point of abnormal BMI was 25 kg m^{-2} (WHO, 2004)[12] .Waist Circumference (WC) was measured, with the subject standing, at the level midway between the lower rib margin and the iliac crest [13].

In all cases blood samples were taken after 12 h overnight fasting. Venous blood was collected in vacutainers without additive, allowed to clot for 30 min at room temperature and centrifuged at 3000 rpm for 10 min to get serum for immediate measurement of glucose

(fasting) and lipid profile. Hemolysed samples were excluded. The remaining serum of the control subjects, diabetic patients were separated from their whole blood, divided into aliquots and were stored at-80°C until the measurement of OPG.

Methods:

Glucose, total cholesterol, triacylglycerol and HDL-c were determinated using the methods

By Barham and Trinder (1972)[14] ;Allain et al(1974)[15]; Fossati and Prencipe (1982)[16] and Finley *et al*. (1978)[17] respectively. LDL-c was calculated by the Friedewald *et al*. (1972)[18] formula.VLDL concentration is calculated as one – fifth of the serum TG[18].

OPG concentration was assessed using the Enzymelinked Immunosorbent Assay (ELISA) kit (Cusabio biotech co.,LTD,China), with a sensitivity less than 0.078 ng mL□1 in serum. sFasL concentration was assayed using the hs-CRP ELISA kit (Demeditecdiagnostics Gmbh.Lise-Meitner-StraBe2, Germany). All procedures were performed according to the manufacturer's instructions.

Statistical analysis

All results were expressed as the mean ± SD. Statistical analysis was performed with Statistical Package for the Social Science for Windows (SPSS) whichversion. The data were analyzed by one-way Analysis Of Variance (ANOVA). To compare the difference among the groups, post hoc testing was performed by the Bonferroni test. The p value less than 0.05 were considered statistically significant (Dawson and Trapp, 2001)[19].

RESULTS

The baseline characteristics of all the groups included in the study were summarized in Table 1. The diabetic studied groups and control group were comparable to each other.The patients and controls were age matched ($p>0.05$). A significant difference was detected between diabetic group and control group in WC ($p<0.05$). It was seen that, no significant difference detected between diabetic groups and control group in BMI ($p>0.05$). FPG was

significantly higher in the studied groups compared to control group as represented by $p<0.05$.It was also seen significant difference between diabetic foot patients and without diabetic foot patients($p<0.05$).

Table 2 demonstrates the changes of lipid profile of diabetic patient's type 2. Total Cholesterol (TC), triacylglycerol (TG), LDL-c and VLDL manifested significant elevations ($p<0.05$) in diabetics compared to normal control subjects, while HDL-c level showed no significant difference ($p<0.05$) among the study groups. TC, TG and LDL-c represented pronounced increases in diabetic patients with long duration compared to diabetic patients of short duration. It was seen no significant difference in lipid profile between diabetic foot patients and without diabetic foot patients ($p>0.05$). Table 3 showed the levels of OPG, and hs-CRP in the diabetic groups and controls. OPG, and hs-CRP was significantly higher in patients of diabetic foot compared to patients without diabetic foot and controls.

Table 1: Baseline characteristics of the subjects studied,

Parameters	**Group III**	**Group II**	**Group 1**	**P value**
Subjects (n)	25.0	25.0	30.0	
Age (years)	58.12±9.29	59.60±9.27	53.13±8.61	0.072
Duration of DM (years)	13.28±7.73	14.24±11.07		0.268
BMI (kg/ m^{2})	28.84±3.88	31.79±5.84	29.44±5.11	0.563
WC (Cm)	106.1±11.56	110.0±12.10	104.9±12.07	0.009*
F.P.G (mg/dl)	229.84±92.38	193.68±62.53	96.23±9.03	0.0001*
-The values expressed as mean ± S.D *Significant using ANOVA test at 0.05 level				

Table 2: Serum lipid profile in different studied groups

Parameter		Group III	Group II	Group 1	p value
Cholesterol (mg/dl)	Mean ± SD	181.44±63.47	188.60±43.28	144.13±12.42	0.0001*
	Range	(100.0-307.0)	(100.0-250.0)	(125.0-170.0)	
Triglycerides (mg/dl)	Mean ± SD	157.88±104.55	158.68±78.98	113.47±9.37	0.035*
	Range	(88.0-600.0)	(90.00-400.0)	(101.0-140.0)	
HDL (mg/dl)	Mean ± SD	43.20±7.97	42.88±7.52	43.00±1.97	0.983
	Range	(30.0-60.0)	(30.0-60.0)	(40.0-47.0)	
LDL (mg/dl)	Mean ± SD	120.12±74.39	120.24±42.98	86.30±5.55	0.012*
	Range	(28.0-297.0)	(37.0-190.0)	(75.0-96.0)	
VLDL (mg/dl)	Mean ± SD	25.88±7.97	29.04±11.08	21.97±1.81	0.004*
	Range	(17.0-43.0)	(18.0-56.0)	(19.0-26.0)	
-Data were presented as Mean ± SD (Range)					
*Significant using ANOVA test at 0.05 level					

Serum OPG, and hs-CRP in patients and control.

OPG, and hs-CRP levels were found to be significantly higher in diabetic foot group than the patients whom without diabetic and control ($p < 0.001$, $p < 0.05$, $p < 0.01$, $p < 0.001$) respectively as shown in table (3).

Table (3) Mean ± SD and range value of OPG, and hs-CRP in three groups.

Parameter		Group			P value
		Diabetic foot	Diabetic	Control	
OPG (ng/ml)	Mean±SD	10.82±2.87	6.16±6.18	4.89±5.80	0.001*
	Range	4.65-16.54	0.05-26.27	0.05-24.38	
hs-CRP (mg/l)	Mean±SD	13.96±12.62	4.83±4.02	3.89±4.21	0.0001*
	Range	0.87-35.37	0.54-12.34	0.27-13.03	
*Significant using ANOVA test at 0.05 level					

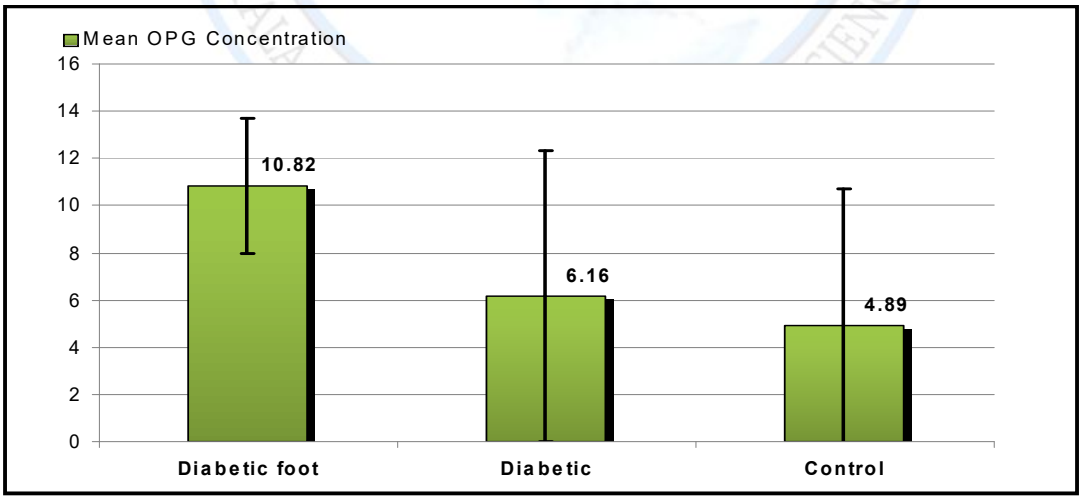
Serum Human Osteoprotegerin (OPG)

Table (4) and figure (1) below showed that mean OPG found to be significantly elevated ($p<0.05$) in diabetic foot patients and without diabetic foot patients when compared with healthy control. In the same table mean OPG concentrations in diabetic foot patients was found to be significantly elevated ($p<0.05$) when compared with diabetic patients without diabetic foot ulcers.

Table (4) mean ± SD and range values of serum OPG in patients and control.

Groups	Serum OPG (ng/ml)		P value in comparison to	
	Mean ± SD	Range	Diabetic	Control
Diabetic foot	10.82±2.87	4.65-16.54	0.001*	0.0001*
Diabetic	6.16±6.18	0.05-26.27		0.436
Control	4.89±8.80	0.05-24.38		
* significant using ANOVA test at 0.05 level.				

Figure (1) mean ± SD and range values of serum OPG in patients and control.



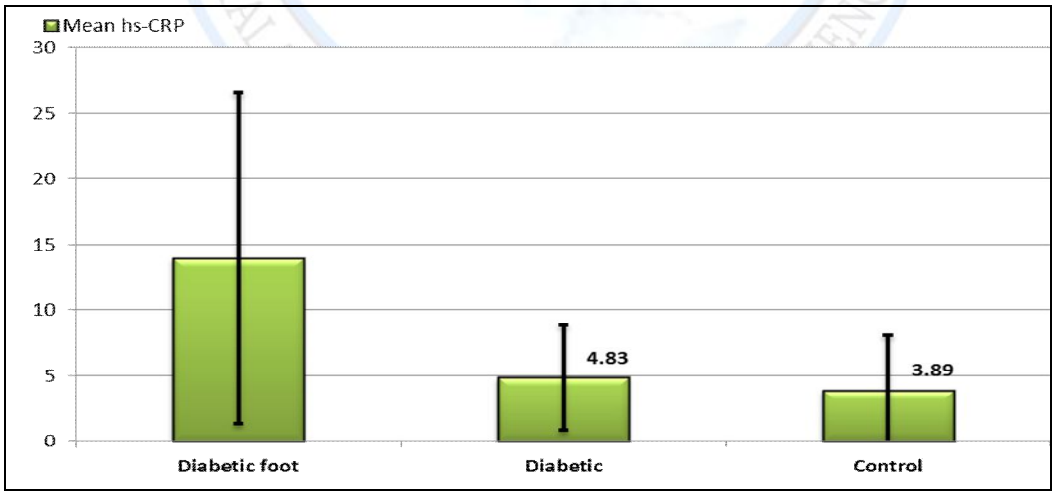
Serum Human high sensitive C-reactive protein (hs-CRP)

The results in table (5) and figure (2) revealed that mean hs-CRP were found to be significantly elevated ($p<0.05$) in diabetic foot patients compared with healthy control, while mean hs-CRP in patients without diabetic foot ulcers was found to be no significantly different compared with healthy control. In the same table mean hs-CRP in patients with diabetic foot was found to be significantly ($p<0.05$) compared with diabetic patients whom without diabetic foot ulcers.

Table (5) mean ± SD and range values of serum hs-CRP in patients and control.

Groups	Serum hs-CRP (mg/L)		P value in comparison to	
	Mean ± SD	Range	Diabetic	Control
Diabetic foot	13.96±12.62	0.87-35.37	0.0001*	0.0001*
Diabetic	4.83±4.02	0.54-12.34		0.099
Control	3.89±4.21	0.27-13.03		
*Significance using Student-t-test for two independent means at 0.05 level of significance.				

Figure (2) mean ± SD and range values of serum hs-CRP in patients and control.



CORRELATION : between Serum OPG level and Other parameter

The correlations between OPG and other parameters in all groups included in the study were summarized in table 6. There was positive, with no significant, correlation between sFas and age (r =-0.025, p>0.05) in diabetic foot patients. There was significant negative correlation between OPG and BMI (r=-0.463,p<0.05),and negative correlation with Waist(r=-0.277,p>0.05) in diabetic foot patients. There were positive correlation between OPG and duration and FPG(r=0.063,p>0.05),(r=0161,p>0.05) respectively in diabetic foot patients. Also there were positive correlation between OPG and triglyceride and VLDL (r=0.215,p>0.05) ,(r=0.131,p>0.05) respectively but there was negative correlation with cholesterol, HDL, LDL(r=-0.111,p>0.05) (R=-0.249,P>0.05),(R=-0.133,P>0.05) respectively diabetic foot patients, and also there was negative correlation between OPG and hs-CRP(r=-0.03,p>0.05).

Table (6) Correlation between OPG and some other variables in three groups.

Parameter		OPG (ng/ml)		
		Diabetic foot	Diabetic	Control
Age (years)	r	0.092	-0.203	-0.318
	p	0.660	0.330	0.086
BMI (kg/m^2)	r	-0.463*	0.038	0.156
	p	0.020	0.856	0.410
Waist (cm)	r	-0.277	0.051	0.279
	p	0.180	0.810	0.135
Duration (years)	r	0.063	0.127	-
	p	0.764	0.544	-
FBS (mg/dl)	r	0.161	0.289	0.290
	p	0.443	0.161	0.120
Cholesterol (mg/dl)	r	-0.111	0.079	-0.008
	p	0.599	0.707	0.969

Triglycerides(mg/dl)	r	0.214	-0.148	0.217
	p	0.304	0.480	0.249
HDL(mg/dl)	r	-0.249	0.123	-0.087
	p	0.231	0.558	0.649
LDL(mg/dl)	r	-0.133	0.008	0.144
	p	0.526	0.971	0.447
VLDL(mg/dl)	r	0.131	-0.080	-0.005
	p	0.533	0.703	0.981
hs-CRP(mg/l)	r	-0.030	-0.020	0.199
	p	0.886	0.926	0.293
*. Correlation is significant at the 0.05 level.				

DISCUSSION

Type 2 diabetes is characterized by hyperglycemia and dyslipidemia which associated with a cluster of risk factors forming the metabolic syndrome [20] and leads to serious complications . High levels of glucose (and cholesterol) leads to macro and micro vascular diseases. Cardiovascular diseases are the cause of death up to 80% of patients with type 2 diabetes[21].Retinopathy is damage of small blood vessels, in the retina of eyes, can cause blindness. The high hyperglycemia(carbohydrate metabolic disorder) in diabetic patients leads to neuropathy. How the nerves are injured is not entirely clear but research suggests that high blood glucose changes the metabolism of nerve cells and causes reduced blood flow to the nerve .Neuropathy and vascularopathy alone or together can cause diabetic foot ulcerations. In this study, the levels of blood glucose was significantly higher in the diabetic foot patients than blood glucose of diabetic patients without diabetic foot and control(fig.1),and that showed that the diabetic foot patients often with very poor(bad) glycemic control[22].

Also lipids(except HDL) were significantly higher in diabetic patients(with and without diabetic foot) as compared with control subjects (Fig. 2), but the levels were with no significant different between diabetic foot patients and diabetic patients without diabetic foot ,this may attributed to no other reasons can increase lipids in diabetic foot patients over

without diabetic foot patients. The dyslipidemia which observed in the present diabetic patients elucidated by significant elevation in plasma total cholesterol, triacylglycerol, LDL-c and VLDL-c, while the plasma level of HDL-c was not chanched markedly in diabetic groups compared to control one. These results agree with those of [23,24]. These lipoprotein abnormalities which are due to reduction of lipoprotein lipase activity are held to be responsible for considerable cardiovascular diseases related morbidity and mortality [25]. Osteoprotegerin (OPG), a secreted member of the tumor necrosis factor receptor superfamily of cytokines, has been associated with endothelial dysfunction and cardiovascular risk. Serum OPG levels were higher in both diabetic foot patients and in without diabetic foot patients, but the elevation was significantly higher in diabetic foot patients. This result agreement with Sibel Guldiken (2009) study who showed similar result, and the result revealed that the OPG levels are associated with the apoptosis in diabetic foot[26].This result can explained by Schopped M, et. al.,(.2007) study who showed that OPG expression by dendritic cells increases with maturation and the first step in activation adaptive response is maturation of dendritic cells by phagocytosis and pinocytosis[27]. Jayshree Swain et al.(2012) showed that increases OPG in diabetic patients leads to vascular calcification and it association with peripheral vascular diseases of diabetic foot[28].

High levels of CRP(C-reactive protein) in diabetic foot patients agreed with fact that most of lesions are infected because wounds are an ideal place for bacteria to colonize and proliferative since raw tissue and serous exudate provide an excellent medium for bacterial growth. This result similar to the observations of (Sibel Guldiken et al) who showed resemble finding. But the correlation of OPG with hs-CRP in our study not agreed with this of (Sibel Guldiken et al) who showed positive significant correlation between OPG and hs-CRP ($r=0.46, p<0.05$) in his study. It seems, OPG/RANKL/RANK system is involved in DM complications pathogenesis, including DF[29]. In our study correlation of OPG levels with DF ulceration occurrence in patients with DM irrespectively of DF type was proved. Study of Piotr Nehring *et al.*(2012) has shown that genetic predisposition for DF occurrence in patients with DM may be a consequence of TNFRSF11B gene variability [30]. Moreover, they proved the role of rs2073617 allele A in prevention of DF occurrence in females. Italian researchers' study from 2009 showed significant correlation between rs2073618 and rs3134069

TNFRSF11B gene polymorphisms and Charcot neuroarthropathy [10].Importance of OPG/RANKL/RANK system was also proved in studies showing correlation between DF frequency and OPG serum concentration in patients with DM [25,30] .Recent study performed by Korzon-Burakowska in (2012) that included 54 individuals with Charcot joint, 35 patients with neuropathy without Charcot joint and 95 healthy control showed that OPG were more often present in patients with Charcot join comparing to control group [31]. Piotr Nehring *et al* (2012) presume the existence of genetic background for both DF generally and DF of neuropathic origin in patients with diabetes and this showed OPG/RANKL/RANK system is involved in DM complications pathogenesis, including DF, but not every polymorphism in gene TNFRSF11B shows frequency variations in population with DF and DF of neuropathic origin. Still the greatest importance in prevention of diabetes-related complications has good metabolic control and patient self- awareness about factors leading to diabetic foot development[32,33].

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