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RESEARCH ARTICLE

OSTEOPROTEGRIN AS A PREDICTOR OF CORONARY ARTERY DISEASE IN TYPE II DIABETES MELLITUS

Huda M. El Sayed[1], Maha A.Hassan[1], Emad A. Said[1], Mona N.Ali[2], Nagwa A. Mohamed[3] and Hanaa M. Eid[1].
1. Department of Internal Medicine - Faculty of Medicine (Girls) -Al-Azhar University.
2. Department of cardiology - Faculty of Medicine (Girls) -Al-Azhar University.
3. Department of Clinical and Chemical Pathology-National Research Centre (NRC).

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Abstract

Background: Coronary artery disease (CAD)is the most important factor related to increased morbidity and mortality in type2 diabetic patients, particularly in patients with albuminuria.Osteoprotegrin (OPG) is glycoprotein that is present in the arterial wall and increases its serum level reflects the increased OPG content in atherosclerotic arterial tissue. OPG is a mediator of vascular calcification.
Objective: The aim of this study is to investigate the role of OPG as a potential risk marker for identification of early coronary artery disease in asymptomatic diabetic patient.
Materials and methods: This cross section study was conducted on 60 diabetic patients with type 2 DM and were classified into: group (1) 30 diabetic patients without micro-albuminuria and group (2) 30 diabetic patients with micro-albuminuria compared to 30 healthy controls of the same age and sex. Routine laboratory investigations, fasting insulin level from which HOMA-IR was calculated, albumin to creatinine ratio, pro brain natriuretic peptide, serum OPG were measured in both patients and healthy controls. Thallium scan for group2were performed to detect ischemic heart disease
Results: There was significant increase of serum OPG level in diabetic patients when compared to control group, and significant increase of serum OPG level in group2 when compared to group1.
Conclusion: The study concluded that serum level of OPG was higher in type2 diabetic patients compared to control group and it increased in diabetic patients with micro vascular complications. This marker could be used for the early diagnosis of subclinical atherosclerosis.

Introduction:-

DM is considered one of the most serious diseases with its complications. The pathophysiological defects leading to type 2diabetes is much more complex. Reduced insulin sensitivity, increased insulin resistance with enhanced hepatic glucose output and impaired insulin secretion are long-recognized core defects ***[1]***. Micro albuminuria is defined by the presence of urinary albumin above the normal but below the detectable range using conventional urine dipstick technique ***[2]***.It is defined as excretion of 30-300 mg of albumin per 24 hours on 2 of 3 urine

Corresponding Author:-Huda M. El Sayed.
Address:- Department of Internal Medicine - Faculty of Medicine (Girls) -Al-Azhar University.

collections ***[3]***. Albuminuria has considered as a renal damage marker and as a cardiovascular disease (CVD) marker ***[4].***

It is better to understand the mechanism that links diabetes and CVD to treat CVD in diabetic patients, as well as to prevent these complications ***[5]***. Diabetic patients are at a much greater risk (2-4 times) of developing CVD compared with the general population because of the effect of hyperglycemia on their arteries either narrowed or lost its elasticity **[6]**.

Dyslipidemia is one mechanism by which diabetes promotes atherosclerosis; endothelial dysfunction often contributes. Platelet activation, thrombogenesis, blood vessel tone, leukocyte adhesion, and inflammation are regulated by endothelium. Acceleration of atherosclerosis occurred when these mechanisms are defective. Both insulin deficiency and resistance promote dyslipidemia accompanied by triglyceride enrichment of lipoproteins, increased oxidation and glycosylation. In addition to endothelial dysfunction, these factors contribute to the increase in atherogenicity, and thus macro vascular disease, found in patients with diabetes [7]**.**

The first step of atherogenesis is endothelial dysfunction. Hyperglycemia, oxidative stress and low-grade inflammation, were involved in the pathogenesis of endothelial dysfunction, Hyperglycemia-induced early atherogenesis may lead to an increased possibilty of cardiovascular events later in life ***[8].***

Osteoprotegerin (OPG) is a glycoprotein acts as a soluble receptor for the tumor necrosis factor family, also known as Osteoclastogenesis Inhibitory Factor (OCIF) **[9]**. It is produced in many different tissues and also circulates in plasma, although the concentration here is much lower than in bone and arterial tissue. The main arterial source of OPG is vascular smooth muscle cells [10].

OPG is involved in the regulation of calcium deposition in vessel wall and in controlling the intensity of the processes of ossification so it can be used predictor of early atherosclerotic lesions of arteries [11]**.** It is considered as a marker of coronary atherosclerosis as increased OPG levels have been associated with the degree of coronary calcification in the general population [12],[13].

Studies suggest that cardiac ischemia may directly influence natriuretic peptide release, independent of left ventricular (LV) function changes. By injecting cardiac imaging isotope we can visualizes blood flow to the heart using myocardial perfusion imaging (MPI). By this technique cardiac ischemia can be visualized and the diagnostic accuracy of cardiovascular stress test is improved. It has the ability of detecting perfusion defects and has advantage of estimating LV function **[14]**. It can evaluate the function of myocardium by calculating the left ventricular ejection fraction (LVEF) of the heart ***[15]***

The Aim of the study: to investigate the role of OPG as a potential risk marker for identification of early CAD in asymptomatic diabetic patient.

Materials and methods:-

This study was conducted on 60 patients with type 2 (DM) diagnosed from history (as polyurea, polydypsia, polyphgia), FBG ≥126 mg/dl and PPG≥200 mg/dl, Hb A1C ≥ 6.5% according to ADA criteria. Their age ranged from (33–70 years), and 30 healthy, age and sex matched subjects who served as control group. Diabetic patients were recruited from internal medicine department in AI-Zahraa University Hospital during the period from December 2012 to April 2014after oral consents and the approval of the ethical committee of the university. They were divided into 3 groups:

Group1:-

Consists of 30 diabetic patients without micro albuminuria, their age ranged between (38 -70) years with mean ± SD (53.37±7.25) years. Duration of diabetes ranged from (3 -10) years with mean ±SD (6.17 ± 1.53) years.

Group 2:-

Consists of 30 diabetic patients with micro albuminuria, their age ranged between (33 - 70) years with mean ±SD (52.40±8.98) years. Duration of diabetes ranged from (5 - 25) years with mean ± SD (8.67 ± 3.84) year. Group 2 patients were classified according to result of MPI results into group (2a): those with positive MPI, and group (2b): those with negative MPI.

Group 3:-
Consists of 30 healthy control persons, their age and sex were matched. From all subjects participating in the study oral consent was taken, also approval of the ethical committee of faculty of medicine, AL-Azhar University was obtained.

Patients with a history of myocardial infarction, angina, stroke, or peripheral artery disease, CKD were excluded from the study.

All patients and control were subjected to the following:-
1. Full history taking and full clinical examination.
2. Complete blood picture.
3. Fasting and post-prandial blood glucose, glycosylated hemoglobin (HbA1c), fasting insulin level from which HOMA-IR was calculated.
4. Lipid profile (total cholesterol, triglyceride, HDL, LDL).
5. Kidney function (urea, creatinine,uric acid), calcium and phosphorous.
6. Liver function (AST, ALT, serum bilirubin, serum albumin, INR).
7. Urine analysis, micro albuminuria detection by albumin to creatinine ratio.
8. Assay of serum levels of Pro BNPand Serum OPG.
9. Thallium scan for group 2 (type 2 DM with micro albuminuria).

Six ml of fasting (12-14Hours) venous blood samples were taken from each subject participating in the study and divided into 3 parts: 1st part was 2ml of venous blood and was put in a an EDTA containing tube for CBC determination. The 2nd part was 1.6 ml and added to a 0.4 Na citrate containing tube, and used for estimation of prothrombin time (PT) immediately on automated blood coagulation analyzer Sysmex CA1500 (Siemens AG, Erlangen, Germany). The 3rd part was 2.4 ml of venous blood was left to clot,and the serum was separated by centrifugation at 3000xg for 10 minutes and the separated serum was stored at -20ºC. For sample preparation for assay of blood sugars, liver & kidney function tests, lipid profile, pro BNP and OPN.

The determination of CBC was performed on Coulter Counter T890 (Coulter Counter, Harpenden, UK). PT determination was determined using tissue thromboplastin method on automated blood coagulation analyzer (Siemens AG, Erlangen, Germany).

The determination of fasting & post prandial blood glucose, serum urea, creatinine, uric acid, calcium, phosphorous, SGOT, SGPT, albumin, bilirubin (total & direct), total cholesterol and triglyceride were performed on Hitachi 912 auto analyzer (Roche Diagnostics GmbH, D-68298 Mannheim, USA) by colorimetric techniques. For determination of HDL-cholesterol, phosphotungestic acid and magnesium ions are used for precipitating all lipoproteins except HDL fraction that was present in the supernatant and measured by Hitachi 912 auto analyzer.LDL cholesterol was measured by Friedwald formula **[16].**

Fasting serum insulin was determined using radio immuno assay **[17].** Insulin resistance was calculated as HOMA-IR using the following equation: HOMA-IR=fasting blood glucose (mg/dl) x fasting serum insulin (µIU/ml)/405 **[18].**

Serum pro-BNP was determined using ELISA kit **[19]** supplied from Kamiya Biomedical Company (12779 Gateway Drive, Seatle,USA). Osteoprotegerin is determined using sandwich enzyme immunoassay kit ***[20]*** and the kit was supplied from Bio Vendor GmbH (ImNeuenheimer Feld 583, Heidlberg, Germany).

Complete urine analysis was done to detect the presence of active urinary sediment (protinuria, pyuria, RBCs or RBCs casts, granular cast). Albumin in urine was estimated by immunoturbidimetric method using Boerhinger reagents (Germany). We compared albumin in the sample against its creatinine concentration (measured by Jaffe reaction) and albumin/creatinine ratio was calculated **[21].**

Statistical method:-
Data was analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 10. Parametric data was expressed as mean ± SD and non-parametric data was expressed as number and percentage of the total. Comparing the mean ± SD of 2 groups was done using the paired and unpaired student's t test.

Determining the extent that a single observed series of proportions differs from a theoretical or expected distribution was done using the Chi square test. Correlation between different studied parameters was done using Pearson correlation coefficient, P value > 0.05 is considered non-significant, P value < 0.05 is considered significant, and P value < 0.01 is considered highly significant.

Results:-

This study was conducted on 60 diabetic patients with type 2 DM and 30 apparently healthy persons who served as a control group. They were 31 (34%) males and 59 (66%) females, their ages ranged from (33 - 70) years with mean ± SD of (52.88 ± 8.11) years. Duration of diabetes ranged from (3 - 25) years with mean ± SD (7.42 ± 3.16) years (table 1).

Table (1): Demographic Data of group 1, group 2 and group 3

Parameters	group 1 Mean±SD	group 2 Mean±SD	group 3 Mean±SD
Age(Year)	53.37 ± 7.25	52.40 ± 8.98	52.50 ± 8.78
Body weight(Kg)	84.73 ± 13.18	82.47 ± 11.55	84.50 ± 9.16
Duration of DM(Year)	6.17 ± 1.53	8.67 ± 3.84	-

Table (2): laboratory data of group1 and group2

Parameters	group1 Mean±SD	group2 Mean±SD	P	S
Duration of DM(Year)	6.17 ± 1. 53	8.67 ± 3.84	0.002	HS
Urea (mg/dl)	29.23 ± 12.06	32.37 ± 9.71	0.272	NS
Creatinine (mg/dl)	0.66 ± 0.21	0.72 ± 0.22	0.348	NS
Uric acid (mg/dl)	5.17 ± 1.05	5.97 ± 1.73	0.034	S
FBG(mg/dl)	189.80± 63.93	222.47± 83.14	0.094	NS
PPBG(mg/dl)	236.73 ±67.06	291.70± 98.87	0.015	S
HbA_1C %	7.64 ± 1.26	8.74 ± 1.48	0.003	HS
Cholesterol(mg/dl)	190.50 ±85.36	188.47± 42.89	0.908	NS
TG (mg/dl)	184.83 ±98.96	168.37± 51.55	0.423	NS
LDL (mg/dl)	87.29 ± 34.71	86.97± 17.89	0.964	NS
HDL (mg/dl)	47.31 ± 14.13	43.37 ± 7.91	0.644	NS
Albumin/creainine ratio(mg/g)	19.77±4.98	262.23 ±45.00	0.000	HS
OPG (ρmol/l)	8.88 ± 1.80	13.82 ± 2.28	0.000	HS
HOMA IR	5.68 ±1.15	20.67±8.81	0.000	HS
pro BNP (ng/l)	61.51±20.36	145.55±35.50	0.000	HS

P value > 0.05 is considered non-significant.
P value < 0.05 is considered significant.
P value < 0.01 is considered highly significant.

Table (3): Laboratory data of group 1 and group 3

Parameters	group 1	group 3	P	S
FBG(mg/dl)	189.80 ±63.93	93.73±9.22	0.000	HS
PPBG(mg/dl)	236.73±67.06	127.60 ±11.54	0.000	HS
HbA_1C (%)	7.64 ± 1.26	5.33 ± 0.22	0.000	HS
Cholesterol(mg/dl)	190.50±85.36	146.87± 24.70	0.011	S
TG (mg/dl)	184.83±98.96	138.77± 27.01	0.019	S
LDL (mg/dl)	87.29 ± 34.71	39.53 ± 9.15	0.000	HS
HDL (mg/dl)	47. 31 ± 14.13	49.70 ± 7.48	0.091	NS
Albumin/creatinine ratio(mg/g)	19.77±4.98	16.60±3.58	0.007	NS
OPG (ρmol/L)	8.88 ± 1.80	4.30 ± 0.85	0.000	HS
HOMA IR	5.68 ±1.15	2.55 ± 0.49	0.000	HS
pro BNP (ng/L)	61.51±20.36	36.73 ± 9.86	0.000	HS

Table (4): Laboratory data of group 2 and group 3

Parameters	group 2	group 3	P	S
Urea (mg/dl)	32.37±9.71	22.01±4.22	0.000	HS
Creatinine (mg/dl)	0.72±0.22	0.73±0.21	0.899	NS
Uric acid (mg/dl)	5.97±1.73	4.68±1.07	0.001	HS
FBG(mg/dl)	222.47±83.14	93.73±9.22	0.000	HS
PPBG(mg/dl)	291.70±98.87	127.60±11.54	0.000	HS
HBA_1C (%)	8.74±1.48	5.33±0.22	0.000	HS
Cholesterol(mg/dl)	188.47±42.89	146.87±24.70	0.000	HS
TG (mg/dl)	168.37±51.55	138.77±27.01	0.008	HS
LDL (mg/dl)	86.97±17.89	39.53±9.15	0.000	HS
HDL (mg/dl)	43.37±7.91	49.70±7.48	0.002	HS
Albumin /creatinine ratio(mg/g)	262.23±45.00	16.60±3.58	0.000	HS
OPG(ρmol/l)	13.82±2.28	4.30±0.85	0.000	HS
HOMA IR	20.67±8.81	2.55 ± 0.49	0.000	HS
pro BNP(ng/l)	145.55±35.50	36.73±9.86	0.000	HS

Table (5): Comparison between group (2a) and group (2b) according to the results of MPI

Parameter	Group 2a Mean ± SD	Group 2b Mean ± SD	P value	Significance
OPG(ρmol/l)	15.98 ±1.63	12.74 ±1.74	0.001	HS
Pro BNP (ng/l)	164.66±27.3	136 ± 35.80	0.023	S

Group 2a: positive MPI. Group 2b: Negative MPI
MPI (Myocardial perfusion imaging)

Table (6): Correlation between serum OPG and some parameters of group 1

Parameters	OPG	
	r	P
Cholesterol	0.521	<0.01
TG	0.705	<0.01
LDL	0.615	<0.01
HDL	-0.091	>0.05
Albumin/creatinine ratio	0.424	<0.05
HOMA IR	0.457	<0.05
pro BNP	-0.078	>0.05

Table (7): Correlation between serum OPG and some parameters of group 2

Parameters	OPG	
	r	P
Cholesterol	0.331	>0.05
TG	0.163	>0.05
LDL	0.533	<0.01
HDL	-0.208	>0.05
Albumin/creatinine ratio	0.665	<0.01
HOMA IR	0.396	<0.05
pro BNP	0.576	<0.01

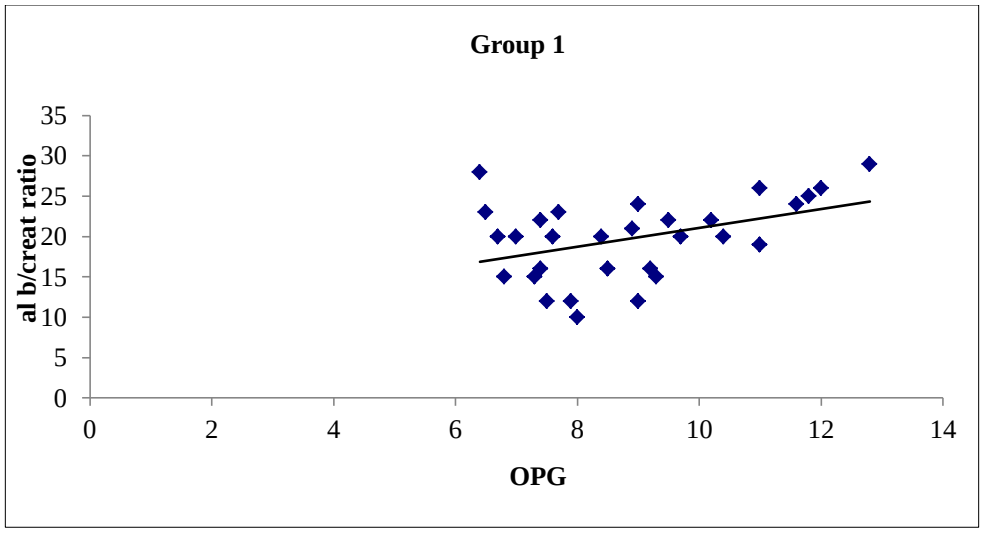

Figure (1):- Linear regression analysis shows positive correlation between serum OPG and alb/creat ratio of group 1

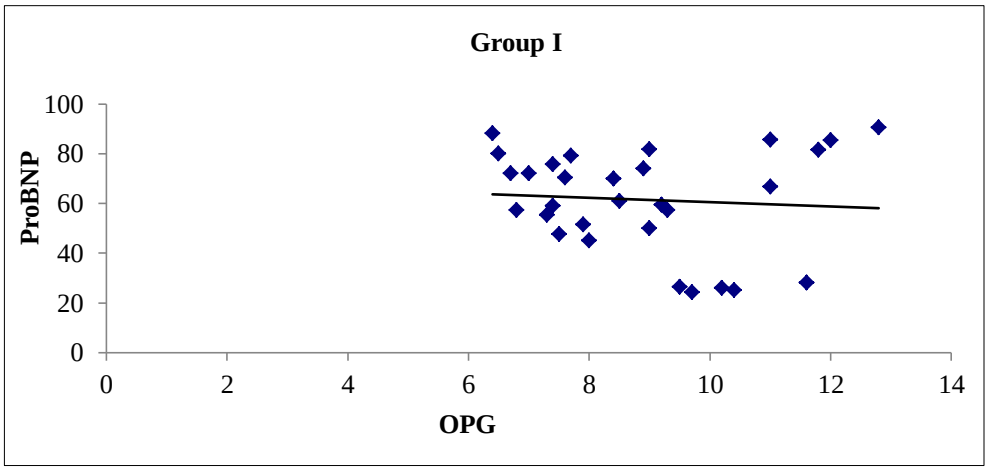

Figure (2):- Linear regression analysis shows negative correlation between serum OPG and Pro BNP of group1

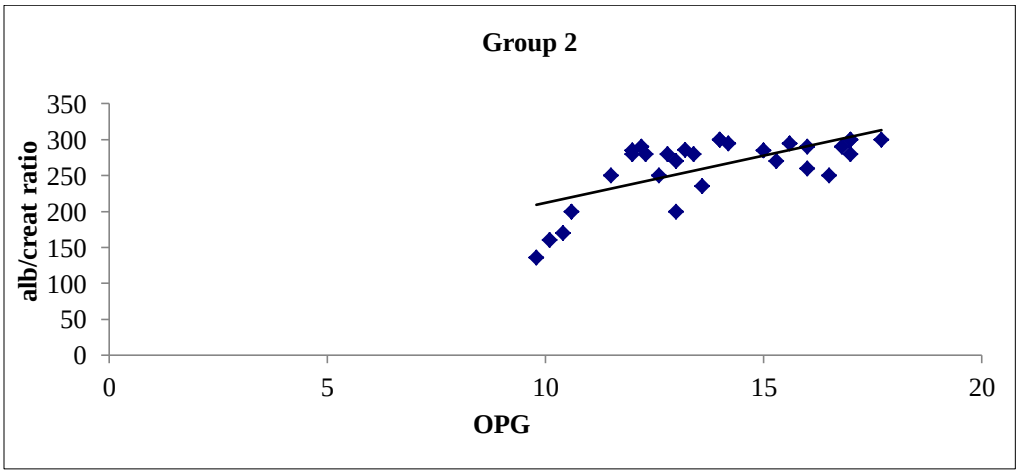

Figure (3):- Linear regression analysis shows positive correlation between serum OPG and alb/creat ratio of group 2

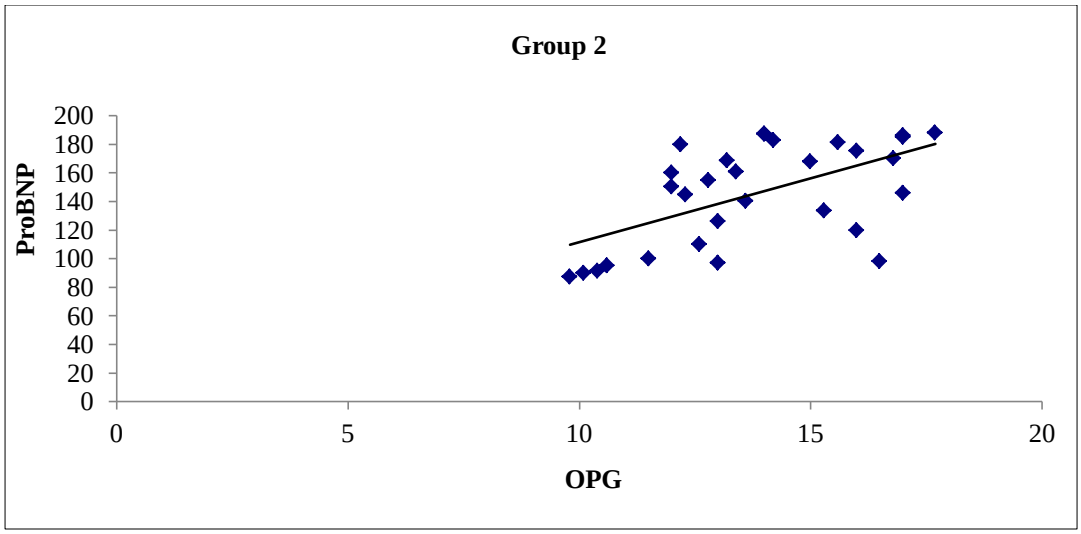

Figure (4):- Linear regression analysis shows positive correlation between serum OPG and pro BNP of group2

There were highly significant increases in duration of diabetes, albumin/creatinine ratio, HbA_1c, HOMA IR, serum OPG and serum pro BNP in group 2 patients compared with group 1. There was significant increase in uric acid and PPBG in group 2 compared with group 1 **(table 2).**

There were highly significant increase in FBG, PPBG, HbA_1c, LDL, serum OPG, HOMA IR, and serum Pro BNP in group 1 patients compared with group 3.There was significant increase in serum cholesterol and serum triglyceride in group 1 compared with group 3 **(table 3).**

There were highly significant increase in FBG, PPBG, HbA_1c, urea, uric acid, cholesterol, triglyceride, LDL, albumin/creatinine ratio, serum OPG, HOMA IR, and serum Pro BNP with highly significant decrease in HDL in group2 compared with group 3 **(table 4).**

There was highly significant increase in serum OPG and significant increase in serum pro BP in group 2a compared with group 2b patients **(table 5).**

There were significant positive correlation between serum OPG and serum cholesterol, triglyceride, LDL, albumin/ creatinine ratio and HOMA IR in group 1 **(table 6).**

There were significant positive correlation between serum OPG and serum LDL, albumin/creatinine ratio, HOMA IR and serum Pro BNP in group 2 **(table 7).**

Discussion:-

Vascular complications of diabetes mellitus are not completely clinically evident in diabetic patients except late in course of the disease. However, subclinical vascular involvement in the form of impaired endothelial function may present so markers of micro and macro vascular diseases are needed in diabetes in order to identify patients at risk of severe complications ***[22].***

CAD is the most important determinant of the increased morbidity and mortality in type2 diabetic patients, particularly in patients with albuminuria **[23].**

It is postulated that plasma OPG reflects its arterial OPG content and so it is considered as surrogate marker of arterial damage. Plasma OPG has been associated with angiographic disease severity and its expression is increased in the atherosclerotic arterial wall **[24].**

The increased circulating level of OPG may reflect injuries in arterial wall, as seen in diabetes and in situations with wide spread vascular calcifications, as a result of the influence of pro-inflammatory molecules on arterial cells ***[25].***

Several studies found that plasma OPG is a strong prognostic cardiovascular risk marker in diabetic and non-diabetic populations **[26].** OPG has been implicated in various inflammations and has been related to diabetes mellitus, as a strong correlation of OPG levels and angiopathy was established **[27].**

The aim of this study is to investigate the role of OPG as a potential risk marker for identification of early coronary artery disease in asymptomatic diabetic patient.

In the present study, there is a highly significant increase in plasma OPG among group2 (type2 diabetic patients with micro albuminuria) when compared to both of group 1 (type2 diabetic patients without micro albuminuria) and control group.

To detect the presence of IHD in diabetic patients without history suggestive of ischemia (as chest pain) or evidence of ischemia by ECG or Echocardiography, MPI was done for group 2 (type2 DM with micro albuminuria) in home did not had symptoms of ischemia, and there were positive MPI (ischemic change in 10 patients(33) from 30 patients).The present study is in agreement with **[28]** who found that, in 70 of 133 (53%) high risk patients (according to OPG level), significant CAD was demonstrated by MPI.

There was a highly significant increase in serum OPG among group 2a (positive MPI) when compared to group 2b (negative MPI).

The explanation for these results is that there is vascular endothelial damage in group 2 DM with micro albuminuria so, there will be more increase in OPG level as it is produced from vascular endothelial smooth muscle cell.

These results agreed with **[25]** who observed significantly higher concentrations of OPG in arteries in diabetic patients compared with non-diabetics. Also agree with **[29]** who reported increased OPG levels diabetic patients with micro vascular complications compared to those patients without complication.

Similar findings were reported by **[30]** who found that increased OPG plasma levels in patients with vascular damage by inhibition of vascular calcification, so who suggested that OPG can act as vascular protective factor in these patients.

These results agreed with **[31]** who reported that increased OPG levels were significantly associated with endothelial dysfunction in diabetics especially type2. So OPG may act as an important organizer in the development of vascular dysfunction in diabetes.

These results disagreed with study performed by **[32]** who concluded that, OPG levels are lower in subjects with arterial calcification in CAD than those without arterial calcification. This may be due to the mean OPG level of subjects with arterial calcification is greater than that of the previous studies which is matching with our results.

In the present study, there is higly significant positive correlation between serum OPG and micro albuminuria of group 2 patients.

AS micro albuminuria is usually the first sign of nephropathy in diabetic patients. It is a marker of inflammation and an independent risk factor for cardiovascular mortality; Forty to fifty percent of type2 diabetics with micro albuminuria eventually die of cardiovascular disease ***[33].***

The present study agreed with Knudsen **[29]** who found that OPG plasma levels were significantly increased in patients with micro vascular complications with micro albuminuria rather than without micro albuminuria .Who suggested that OPG plasma levels may reflect micro vascular damage among diabetic patients.

In the present study, there is a significant positive correlation between OPG and serum cholesterol, triglyceride and LDL of patients of group 2 and no significant correlation between OPG and HDL.

The strong relation between bone pathologies and atherosclerosis lead to recognition of common molecular mediators linking the skeletal and the vascular systems, including OPG and RANKL, were shown to be expressed

within atherosclerotic plaques ***[34].*** Some studies suggested that OPG may act as predictor of CVD due to its association with vascular calcifications ***[35], [36]***

These results agreed with [***37]*** who found a positive weak correlation between OPG and both total cholesterol and triglycerides, but not correlated with HDL and cholesterol.

In the present study, there is a significant correlation between OPG and HbA_1C of patients of group 2 (type2 DM with micro albuminuria).This was partially explained by the fact that high HbA1c levels are associated with increased risk of diabetic macro vascular and micro vascular complications***[38].***

The present study is in agreement with ***[39]*** who concluded that a significant correlation between HbA1c and OPG was shown in patients with type 2 diabetes mellitus.

In the present study, there is significant positive correlation between OPG and HOMA-IR of patients of both patient groups. These results agreed with ***[40]*** who found a positive correlation between OPG and HOMA-IR. This may be due to insulin decreases the production of OPG, this direct effect of insulin on vascular cells**[25].**

As insulin resistance is associated with increased coronary heart disease and mortality***[35]*** it is interesting that some studies have suggested plasma OPG as a predictor of cardiovascular disease. Many authors assume that this connection is due to an association between OPG and vascular calcifications***[36].***

In this study, there is a significant positive correlation between OPG and pro BNP of patients of group 2.This is in agreement with ***[28]*** who demonstrated association of OPG with CAD independent of NT-proBNP. Who concluded that OPG may be considered as an additional marker of atherosclerosis.

Since all diabetic patients should benefit from screening for silent myocardial ischemia especially in those with cardiovascular risk factors. This helps to identify asymptomatic subjects who might present coronary lesions susceptible to benefit from revascularization.

MPI cannot be generalized to the whole diabetic population, because of its cost although it has been shown to be effective in detecting CAD in asymptomatic diabetic patients.

As OPG is a powerful predictors of CAD so it might represent useful and cost-effective means to select patients require stress testing. OPG can be used as potential predictors of silent CAD in asymptomatic type 2 diabetic patients.

Conclusion:-

The study concluded that serum level of OPG was higher in type2 diabetic patients compared to control group and it increased in diabetic patients with micro vascular complications more than diabetic patients without micro vascular complications.OPG was considered to be an independent risk factor for progressive atherosclerosis and cardiovascular disease in the population. OPG is a strong prognostic cardiovascular risk in diabetic and non-diabetic populations.

Recommendation:-

It is very important to find a noninvasive methods (laboratory marker) for monitoring vascular changes such as OPG due to high cardiovascular morbidity in diabetic patients.. This marker could be used for the early diagnosis of subclinical atherosclerosis, to reduce the cardiovascular event rate in these patients.

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