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Assessment of osteoprotegerin as monitor diabetic nephropathy

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The prevalence and complications of diabetes are increasing worldwide. In this work, we aimed at estimating Osteoprotegerin (OPG) as novel factors for controling diabetic nephropathy. The study involved 90 Iraqi volunteers with aged range (35-65) years from both genders. The subjects were subdivided into two groups, (45) with diabetic nephropathy (DN) and (45) control recruited, most assays were measured by enzymic colorimetric methods, serum OPG levels were estimated by ELISA. The results revealed that OPG serum levels were higher compared to control group ($p < 0.00001$). The Pearson's relationship reports OPG level had highly positive with FBG and HBA1C ($r=0.981$, $p<0.00001$), ($r=0.350$, $p=0.020$) respectively. In contrast, strong negatively correlated with insulin level ($r= -0.363$, $p=0.014$) respectively. Moreover, OPG level indicate a significant correlation with parameters of renal function include urea, creatinine, ACR and eGFR ($r=0.629$, $p=0.04$), ($r= 0.426$, $p=0.01$), ($r= 0.074$, $p=0.002$), ($r= -0.612$, $p=0.001$), respectively. These summarized results indicates that OPG is a great biomarker for predicting the occurrence of diabetic nephropathy.

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Introduction

Diabetes Mellitus Type 2 (T2DM) can be described as a metabolic morbidness group or a metabolic disorder that results from different etiologies. Any diabetic individual has hyperglycemia because the pancreas does not make adequate insulin hormones or cells respond to the insulin [1]. As one of the most common diabetes complications, nephropathy develops in roughly 20% of T2DM for ten years. Furthermore, routine medical therapies for diabetes contain adverse side effects. The main clinical characteristic of this disruption is the appearance of a large amount of urinary protein, in particular albumin. Usually, albumin crosses through the glomerulus in small quantities; as a result, raised urinary albumin is represented as one of the most original markers of diabetic nephropathy (DN) [2]. Lately, several factors can perform an essential role in the pathogenesis of DN. One of these factors is Osteoprotegerin (OPG). It is a glycoprotein cytokine that assists with the nuclear factor ligand-receptor activator and tumor necrosis factor (TNF) related apoptosis-induced ligand. Studies have indicated that OPG is expressed in osteoblasts, vascular smooth muscle cells, and endothelial cells. Studies have indicated that circulating OPG derived from endothelial cells is related to active atherosclerosis, including renal

arteries, due to inflammation, which leads to vascular calcification [3,4]. It was reported that OPG and Homeostatic model assessment-insulin resistance (HOMA-IR) correlated positively. There are notable utility impacts of OPG on vascular tissues are associated with endothelial dysfunction and IR. They documented an association between elevated serum OPG levels and cardiovascular disease [5].

Subjects and methods

Subjects

The work was carried out at the Al-Sadder Teaching Hospital and the Centre for Diabetes and Endocrine Glands at the hospital mentioned above in Najaf, Iraq, between November 2020 and May 2021. Demographics and anthropometric measurements (age, body mass index (BMI), waist-to-hip ratio (WHR), blood pressure, gender, duration of disease, and another disease) were collected from all individuals and patients. Samples were collected from 90 study participants with both sexes within the age range (35-65), 45 (24 female and 21 male) patients, and 45 (23 female and 21 male) normal healthy volunteers were taken for control. Patients on insulin, smokers, hypertension, alcoholics, tobacco chewers, abnormal urinary sediment, renal transplantation, urinary tract infection, chronic persistent infection or inflammatory disorders, uncontrolled thyroid disorders, neoplastic disorders, severe liver dysfunction, history of acute myocardial infarction, HIV infections, occlusive peripheral vascular disease, stroke, and congestive heart failure were excluded from this study. According to the World Health Organization, patients are already diagnosed with diabetes type 2 [6].

Biochemical analysis

A prepared nurse gathered the blood samples after a 10–12 h fasting to measure serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-c), triglyceride TG, and other assays. The low-density lipoprotein-cholesterol (LDL–c) level was estimated using the Fried Ewald formula [7]. The serum Glucose, serum Creatinine, Urine Creatinine, Urea, Uric acid, Lipid profile were measured by enzymic colorimetric methods. Fasting serum insulin level was determined using Cobas e 411 instruments. Insulin resistance (IR) was estimated using the standard equation HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) [8], HOMA-IR = [Fasting insulin (μIU/mL) x Fasting glucose (mg/dL) / 405]. HbA1c by AU480 Beckman coulter analyzer, albumin by use I ChromaII, ACR is determined by dividing the concentration of u. albumin in milligrams over-concentration of u. creatinine in grams, GFR was determined by equation: GFR (mL/min/1.73 m^2) = 186 × Serum Cr-1.154 × age-0.203 × 1.212 (if patient is black) × 0.742 (if female) [9]. BMI was estimated via the standard BMI equation [8]. BMI (kg/m^2) = weight (kg)/height (m^2) Serum OPG level was quantified by using the enzyme-linked immunosorbent assays (ELISA).

Statistical analysis

Study results were analyzed by employing the SPSS software (Version 23), and the values were expressed as "mean ± standard deviation (SD). Pearson's correlation analysis was performed. All calculated strong statistically significant when ($p<0.01$), statistically significant $p<0.05$), and statistically non-significant when ($p>0.05$).

Results and discussion

Anthropometric data and study group's characteristics

One of the most important medical problems is the raised prevalence of diabetic nephropathy patients. The highest incidence of 3% per year is seen in 10 to 20 years of onset diabetes. DN is believed to be a silent

disease with no symptoms for a long time [10]. The general characteristics of all individuals who participated in the present work were given in Table 1, the mean of age, duration, Bp systolic, WHR, Bp diastolic, and weight were highly significant ($p<0.05$) for the diabetic nephropathy group compared with the control group, while non-significant ($p>0.05$) in a mean of BMI.

TABLE 1 The demographic characteristics of the current study

Parameters		DN (m±SD) (n=45)	H.C (m±SD) (n=45)	P-value
Age (years)		52.42±9.33	40.02±4.62	< 0.00001
(35-44 years) No. (%)		8 (17.8%)	34 (75.5 %)	
(45-55 years) No. (%)		25 (55.5%)	11(24.5 %)	a
(56-65 years) No. (%)		12 (26.7%)	0 (0 %)	
sex	Male No%	22(49%)	22 (49%)	a
	Female No%	23(51%)	23 (51%)	
Duration of Disease (years)		10.4±5.94	0.00	< 0.00001
BMI (kg/m^2)		29.11±4.74	28.15±12.1	N.S
WHR		0.965±0.1	0.895±0.05	< 0.05
Bp (systolic) mmHg		133.07±22.	116.51±9.3	< 0.00001
Bp (diastolic) mm Hg		82.56±17.8	74.91±5.70	< 0.05
Weight Kg		81.11±10.9	73.05±11.8	< 0.05

NS = the result is not significant at p-value > 0.05. The significant difference between proportions using Pearson Chi-square test at 0.05 level.
Significant difference among more than two independent means using ANOVA test at 0.05 level

The mean of age duration has the most common intricacy in diabetes since hardening of the glomeruli leads to proteinuria for around 5-10 years with T2DM [11]. Another research assessed that up to 40% of diabetes patients develop renal disease [12]. In contrast, being overweight is one of the reasons insulin resistance may cause diabetes nephropathy. On the other hand, systolic blood pressure impacts the action of glomeruli. The results of a study recorded that the body mass index was higher in (35-44) age group, then (45-55) age group, and the lowest age group (56-65) in patients which can be ascribed to lifestyle changes with diet and medications that can impact the Insulin levels and insulin resistance such as metformin showing to weight loss.

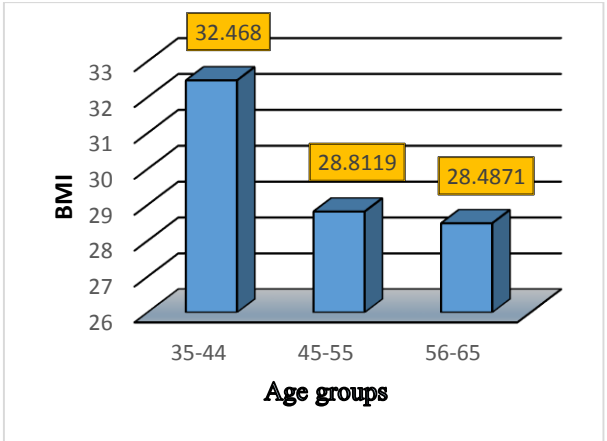

FIGURE 1 Distribution BMI according to the age groups

Glycemic state of study

All individual results of glycemic state are displayed in Table 2, including fasting insulin (μIU/mL), HOMA-IR, fasting Serum glucose (mg/dl), and HbA1c (%), which showed significant values by using the ANOVA test ($p<0.05$).

TABLE 2 Summary statistics of glycemic state results in patients and controls

Parameters	DN (m±SD) (n=45)	H.C (m±SD) (n=45)	P value
FBG (mg/dl)	184.61±49.9	99.619±13.9	< 0.00001
Insulin (μIU/ml)	15.576±6.29	10.791±4.29	< 0.00001
HOMA-IR	6.846±3.03	2.652±1.395	< 0.00001
HbA1c (%)	7.926±1.768	4.889±0.524	< 0.00001

Our determinations found both FBG, Insulin (μIU/mL), HOMA-IR, and HBA1C have a highly significant rise in the mean values of patients ($p < 0.00001$) compared with healthy control. These results agree with other recent studies [12]. The level of HbA1c reflects the level of serum glucose over the previous 3 months, is predictive for high glucose level, and avoids changes in blood glucose. Uncontrolled diabetes can be seen from the HbA1c values. Poor control of T2DM unhurriedly leads to the glomeruli's destruction, leading to chronic renal disease. The current outcomes are consistent with the study conducted by Chiu et al. [13] that HbA1c was highly associated with the development of DN [13].

The leptin hormone plays a critical role in regulating energy balance and body weight. Additionally, the necessary action of glucose homeostasis, the association between diabetic nephropathy and leptin has been broadly researched. Leptin levels were found to be a risk factor for kidney function diminished. [14]. The raised insulin level in the body because of insulin resistance leads to it converting glucose into fat that collects around the liver and abdominal areas, leading to an increase in the waist circumference and an elevated mean of WHR, where FBG level is high. Further, one of the functions of insulin is to present potassium into the cells. This ion is not introduced into the cell in the possibility of insulin resistance. Therefore, sodium increases, ensuring high pressure, which illustrates the increase in SBP and DBP [15, 16].

Relationship between insulin and various variables in the study

There were positive and negative connections between insulin concentration and some variables via bivariate statistical analysis, the results showed the statistical analysis of the correlation coefficient of insulin with other parameters, it showed a high significantly positive correlation of Insulin with HOMA, DN ($r=0.720$, $p<0.00001$), H.C ($r=0.947$, $p<0.00001$), also, it was found negative significantly with glucose in a patient ($r= -0.406$, $p = 0.006$), while it was positively significant in healthy subjects group ($r=0.499$, $p=0.002$). Also, means of insulin have been negatively correlated with the age of volunteers ($r = -0.614$, $p= 0.04$) whereas, non-significant in individuals. Insulin displays a strongly positive correlation with U.A ($r=0.513$, $p<0.00001$) and a nonsignificant correlation in healthy control groups ($r=0.254$, $p=0.092$).

The above outcomes were consistent with other research, which found that patients with high glucose and HbA1c levels are associated with development nephropathy. Moreover, glycemic control limits the evolution of nephropathy to ESRD. Our outcomes supported a mechanism between glucose levels and incident albuminuria [17]. The

insulin shows nonexistence of any correlation with BMI, blood pressure (SYD and DIA), WHR, duration, HBA1C, ACR, U. Creatinine, S. Creatinine, urea, lipid profile in patient and control groups. On the other hand, OPG levels have a significant adverse correlation with insulin levels (r=-0.363, p=0.014), whereas in the control group the correlation has positive significance (r=0.468, p=0.001).

Lipid profile assays

All results of the lipid profile are presented in Table 3. A high significant variation in HDL, TG, and VLDL (p>0.00001). In contrast, a non-significant difference in cholesterol and LDL level is found.

TABLE 3 Mean and standard deviation of lipid profile and p-value

Parameters	DN (m±SD) (n=45)	H.C (m±SD) (n=45)	P value
TG (mg/dl)	186.42±94.05	83.080±18.72	< 0.00001
Chol.(mg/dl)	148.04±36.00	134.82±36.60	NS
HDL (mg/dl)	34.826±13.91	48.719±7.45	< 0.00001
LDL (mg/dl)	75.90±38.50	70.873±36.20	NS
VLDL(mg/dl)	36.479±17.79	16.614±3.748	< 0.00001

The mean of TG and VLDL were high in patients with DN, while HDL levels had a low mean. Obesity is correlated with dyslipidemia diagnosed by elevated triglycerides and reduced HDL levels. This dyslipidemia is highly correlated with increased risk of diabetes mellitus type two, and after spending extended periods of diabetes, had been suffering from complications such as diabetic nephropathy [18]. The abnormal lipoprotein in diabetic patients related to glomerulosclerosis and renal disease. In type 2 diabetes patients, elevated cholesterol levels are associated with the development of hard urine albumin secretion.

Moreover, lower Cholesterol and TG levels in diabetic patients assist in developing urine albumin secretion from moderate to normal [19]. In our finding, the raised blood lipid levels correspond to those observed the relationship between lipid and renal dysfunction identified by higher serum creatinine levels in T2DM. Patients with raised Triglyceride and lower HDL have a higher creatinine level. The present results have been supported by other extensive investigations in T2DM that found a strong correlation between improved dyslipidemia and kidney disease [20].

Renal function assays

The results of Urea, Creatinine, Uric acid, eGFR, and ACR have been shown a significant difference compared with the control group as depicted in Table 4.

TABLE 4 Mean ±SD values of Urea, Creatinine, Uric acid, eGFR, and ACR

Parameters	DN (m±SD) (n=45)	H.C (m±SD) (n=45)	P-value
Urea (mg/dl)	39.367±10.99	22.827±4.311	< 0.00001
Creatinine (mg/dl)	1.177± 0.264	0.598±0.052	< 0.00001
Uric acid (mg/dl)	5.638±1.199	4.803±0.677	< 0.05
eGFR	63.773±19.48	139.782±27.2	< 0.00001
ACR	235.01±95.43	21.040±4.947	< 0.00001

These results are similar to recent findings study [21]. Increased creatinine is an extremely sensitive marker compared to urea employed in the early detection of kidney defects. It is a primarily excretory creatinine metabolite, almost all of which is in the muscle

of the skeleton. The liver and kidneys are the main places of creatinine synthesis in the human body. Thus, blood creatinine can be used to compute glomerular filtration, and different investigations have shown that raised serum levels of creatinine and urea are associated with increased blood glucose [22]. Previous work suggests that urea, uric acid, and creatinine are likely to be involved in the pathogenesis of DN Compared with T2DM patients [23].

Serum OPG level assays

Although renal function tests are widely utilized to diagnose DN, their predictive capability is finite. Therefore, it is critical to find more accurate and sensitive markers specialized for nephropathy diagnosis in the early stage of kidney disruptions in T2DM.

OPG is a novel biomarker utilized as a powerful detector to predict glomerulopathy and diagnostic diabetic nephropathy. The results illustrated (mean + standard deviation) of OPG (pg/ml) (395.522±101.5) for patients and (187.00 ± 23.6) of normal people, and p= < 0.00001.

Obesity is usually attended by a condition of systemic inflammation, hyperglycemia, hyperinsulinemia, and insulin resistance, which may gradually cause T2DM and after a while DN disease.

In addition, the output of advanced glycation end products (AGE), which are oxidative stress inducers, causes the change of vascular smooth muscle cells into osteoblast-like cells that form nodules, spontaneously mineralize and ultimately produce hydroxyapatite.

It seems possible that bone mineralization and vascular calcification are energetic processes concerned with common pathophysiological and biochemical properties. It has been suggested that the Osteoprotegerin molecule participates in bone homeostasis play essential role in vascular calcification [24].

TABLE 5 The correlation between OPG and other parameters

Parameter	OPG			
	Controls		DN	
	r	P-value	R	P-value
Age	0.056	0.715	0.120	0.433
Duration	a		0.104	0.495
BMI	-0.019	0.901	0.068	0.658
WHR	0.242	0.109	-0.099	0.516
SYS	0.093	0.544	0.050	0.744
DIA	0.063	0.679	-0.009	0.955
Glucose	0.819**	<0.00001	0.981**	<0.00001
Insulin	0.468**	0.001	-0.363*	0.014
HOMA	0.594**	<0.00001	0.226	0.135
HbA1c	0.333*	0.025	0.350*	0.020
Urea	0.260	0.084	0.629	0.04
Creatinine	-0.087	0.570	0.426**	0.01
Uric acid	0.157	0.304	-0.213	0.160
T. Protein	0.011	0.942	-0.087	0.569
TG	0.254	0.093	0.013	0.933
CHOL.	0.130	0.394	0.169	0.267
HDL	-0.128	0.403	-0.062	0.684
LDL	0.092	0.548	0.173	0.255
VLDL	0.255	0.091	0.044	0.776
eGFR	0.137	0.370	-0.612**	0.001
ACR	0.260	0.084	0.532**	0.002

*.Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed), a. cannot be computed because at least one of the variables is constant.

OPG is the chronic inflammatory sensitive marker. Also, it has been reported that OPG values significantly correlate with the mean of glucose, HbA1c, Insulin, urea, creatinine, ACR, and eGFR. OPG is an indication of atherosclerotic disease, including the arteries and blood vessels of the renal because it correlated with vascular calcification as a result of inflammation [25].

ROC area under curve

The results of ROC analysis as shown in Figure (2) with sensitivity =%97.3, specificity =%100, AUC=0.990. Also, this proves that OPG is a promising biomarker for this diagnosis.

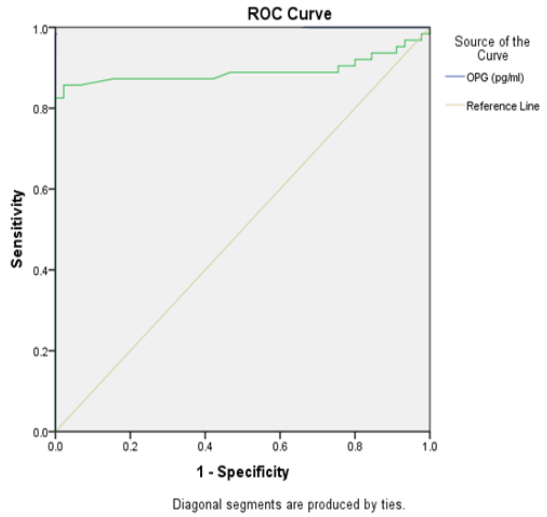

FIGURE 2 ROC for the different DN and control group parameters

Conclusion

From this research it is concluded that Serum OPG is increased with the progression of diabetic nephropathy. Therefore OPG is considered useful to indicate patients of diabetes at risk of nephropathy previous onset microalbuminuria or the lower GFR, decreasing the mortality and morbidity. Also, poor glycemic control is a characteristic of diabetes patients with macro and microalbuminuria. On other hand, the ROC curve analysis of the specificity and sensitivity displays that of markers. Serum OPG can be proposed as a potential biomarker for the early designation of diabetic nephropathy.

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