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International Journal of Pharmaceutical Research&Allied Sciences, 2019, 8(1):95-101

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Osteoprotegerin Levels among Saudi Cardiovascular Patients

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ABSTRACT

Background: Cardiovascular Diseases (CVD) are considered an overwhelming health burden in Saudi Arabia, greatly aggravated with the increasing rates of Diabetes Mellitus (DM) and obesity. Osteoprotegerin (OPG) is one of the tumor necrosis factor receptors, commonly associated with coronary heart diseases. This study examines the possible correlation among OPG with both DM and CVD. Methods: The study was conducted in Jeddah, Saudi Arabia and included 201 subjects. They were divided into four matched groups: group 1 were patients with CVD only; group 2 were patients with DM type 2; group 3 were patients suffering from DM and CVD, and group 4 was normal control. All groups were divided into male and female subgroups. The following parameters were measured; complete blood picture, lipid profile, insulin, fasting glucose and kidney function and also then relationship to OPG levels. Results: OPG was significantly elevated in males with CVD alone (8.31 ± 4.01 ng/ml), or in association with DM (6.54 ± 3.55 ng/ml). An increase was noted in female patients, but only in the DM and CVD groups (6.75 ± 3.3 ng/ml). A weak positive correlation was detected between OPG concentrations with both fibrinogen and glucose levels in females, while in males OPG levels were positively correlated with waist to hip ratios. Conclusion: OPG could be a valuable biomarker for early CVD appearance.

Key words: *Cardiovascular Diseases, Osteoprotegerin, Obesity, Lipid Profile.*

INTRODUCTION

During the years of the 1980s the global prevalence of Diabetes Mellitus (DM) was estimated at 4.7%, which rapidly doubled in the following decades, with more than 1.5 million related deaths [1]. The global burden of Cardiovascular Diseases (CVD) has markedly increased in the past decade, and namely in association with DM [2]. Similarly, in Saudi Arabia the recent years witnessed a notable increase in the incidence of both conditions [3], and in addition to that the prevalence of Coronary Heart Disease (CHD) has also grown in the kingdom, and is currently the top third cause of hospital mortality after accidents and senility [4]. In a report by the World Health Organization (WHO)about one third of whole non-contagious diseases related deaths solely may be caused by CVD [5]. This growing burden is probably associated to the deficient CVD detection, with the longer than usual status of atherosclerosis, hence there is a necessity for a focused preventative approach, directed by assessments with the appropriate biomarkers [6].

Although the exact mechanism is only partially understood, obesity is attributed with type 2 DM and CVD [7-9]. Osteoprotegerin(OPG), is part of the Tumor Necrosis Factor (TNF) receptor family [10]. It is a protein produced by different cardiovascular tissues [11], and is strongly associated with CHD among type 2 DM patients [12]. Several studies demonstrated a relationship between OPG concentration and the progression of vascular diseases, in particular the severity of coronary artery disease [13]. Till this date no studies examined the

changes of OPG levels in Saudi patients and the state of undergoing pain from DM and CVD. In this research, we assess several parameters of these groups, including OPG levels, and its role as a potential biomarker.

METHODS AND MATERIALS

Study Design

We conducted a prospective cohort study at King Abdulaziz University Hospital (KAUH), in Jeddah - Saudi Arabia. Ethical approval obtained from the Bioethical and Technical Research Committee, at the Faculty of Medicine in KAUH. We approached patients attending the outpatient clinics at KAUH, and then explained the study's objectives and procedures. Informed agreements were obtained from all participants before the study. The methodology applied in this text has been described in detail elsewhere. [14]

Study Participants

Participants were divided into four main groups; the first main group was CVD patients only (mainly atherosclerosis), and the second main group were patients suffering DM type 2 only, the third main group was patients diagnosed with both DM and CVD, and lastly the fourth main group was considered as normal control. All groups were corresponding for age and gender then subdivided by gender. Patients suffering both CVD and DM were followed up at KAUH outpatient clinics. Normal Control was recruited from other clinics, who were non-smokers, with no history of DM, CVD or hypertension nor were on chronic medications. We excluded patients suffering from Cushing's syndrome, DM type 1, or serious disease.

Materials

The following materials were used in this study:

The reagent kits for analysis for fasting insulin, fasting blood glucose (FBG), uric acid, fibrinogen and lipid profile were obtained from Siemens Flex® Reagent Cartridge (Newark, DE, U.S.A). The latter were read and analyzed using Dimension Vista® System, at KAUH laboratory.

We used the human serum ELISA kit from Uscn Life Science Inc. Wuhan, China, Cat. No. E0084Hu, which included: Standard (5000 pg/ml) and standard diluents, Detection reaction A and B, Assay diluents A and B (2x concentrate), Tetra methyl benzidine (TMB) substrate, stop-working solution and washing buffer (30x concentrate).

Data collection methods

Anthropometrics and Vitals

In beginning of the study, we recorded the blood pressure and anthropometrics of all participants, namely; height, weight, waist surroundings, hip surroundings, Body Mass Index (BMI) and Waist to Hip Ratio (WHR). Measurements may be taken in the morning, before breakfast, in light clothes collectively and without shoes, using a digital scale and a standardized measuring tape.

Reagents Preparation and Sample Collection

For every patient, we drew a 10 ml whole venous blood sample after an overnight fast, and divided it as follows: 4 ml for chemistry and hormonal analysis and it was centrifuged then the serum was aliquot into a clean, dry Eppendorf tubes, and kept at 80˚C, and also, 3 ml to measure fibrinogen levels, and finely 3 ml to monitor blood glucose levels.

OPG levels were estimated using the above described ELISA kit, with micro plates precoated with a unique antibody. Standard was reconstituted with the diluents, kept for 10 minutes at room temperature, and shaken in a smooth way. We set seven points of this standard; 510 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625ng/ml, 0.312 ng/ml, 0.156 ng/ml, and the last Eppendorf tube was blank. Diluents' A or B concentrate (2X's) were centrifuged, and diluted with distilled water. Wash solution was prepared by diluting the washing solution concentrate with distilled water. The Standards and biological samples were added to the suitable micro titer plate wells, with a biotin-conjugated polyclonal antibody process of making ready. TMB substrate and Avidin conjugated to Horseradish Peroxidase were added to every well and incubated. Only the wells that contained OPG should demonstrate a change in color, which was then measured 450 nm on spectrophotometer.

Procedure and Calculation

All wells were labeled and incubated for 2 hours at 37˚C.The liquid contents of each well was disposed, then 100 µl of reagent was added to the solution and incubated for 1 hour at 37˚C. Solution was aspirated and wells washed with solution for three times. Then, reagent B was added, and plates were incubated for 30 minutes at

37˚C and washed repeatedly for five times. Substrate solution was added to each well, then incubated for 15-25 minutes at 37˚C in dark to color changed to blue, and when stop solution was added, the liquid's color changed to yellow. The liquid was mixed and afterwards cleaned from any drops of water or fingerprints. The micro plate reader was run and the measurements were done at 450 nm immediately using spectrophotometer.

PG concentrations were estimated using the optical density of samples to a standard curve. The readings were averaged for the standard, control, and serum samples, after subtracting the optical density of the zero standards (blank). The standard curve, a four-parameter logistic (4 -PL) curve-fit was created using computer software. Another option or possibility, a curve was drawn by plotting the mean absorbance for every standard on the x-axis against the concentration on the y-axis.

Statistical Analysis

Data were recorded daily, then cross-checked, entered and coded for analysis. Continuous finding was described as standard deviations, and one-way ANOVA was used to test for difference between groups. Associations between OPG levels and other parameters were examined using Pearson relationship. We used the Pearson status arrangement correlation to investigate the relationship among nonparametric differences for the male and female subgroups. A P-value < 0.05 was set as statistically significant.

RESULTS

Two hundred and one subjects were recruited; include 114 males and 87 females constituting 56.7 and 43.3%, respectively as total sample. The first group included 48 patients for CVD patients, the second group included 46 patients DM type 2, the third group consisted of 65 patients suffering from both DM and CVD, and finely fourth group contained 42 healthy individuals considered as normal controls. Gender distribution in each group is shown in Figure (1). The average age for the CVD group was 57.88 ± 10.07, for the DM group was 59.33 ± 10.05, for the DM and CVD group was 56.76 ± 8.56, and finally for the control group was 47.98 ± 12.15.

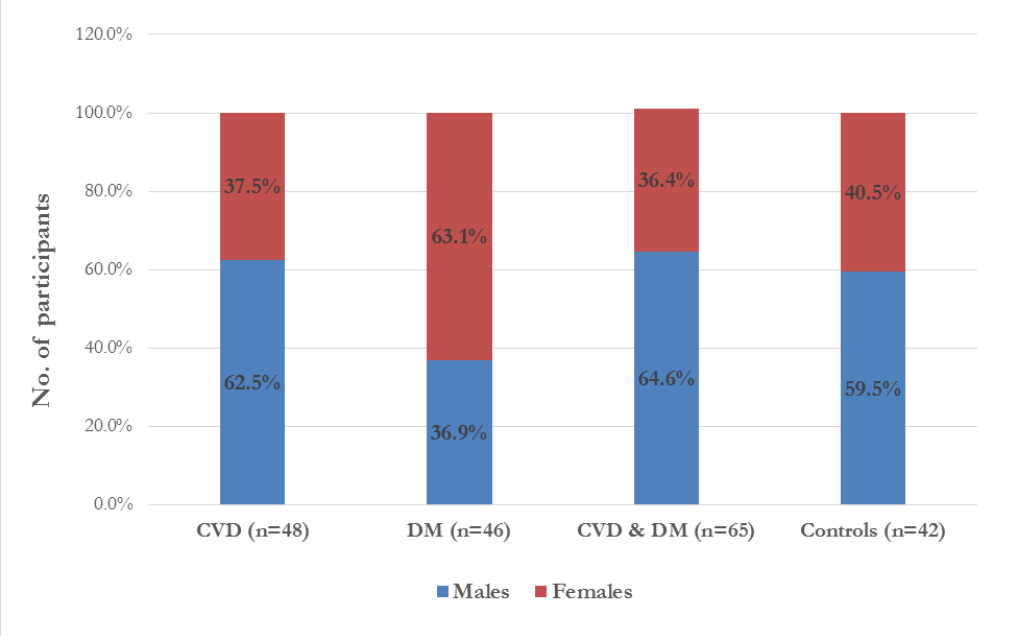

Figure 1: Gender Distribution in the Study Groups

Physical Characteristics of the Study Groups

Details for the mean values of BMI, WHR, systolic (DBP) and diastolic blood pressure (SBP) for each group are reported in **Table (1)**. We detected a significant variation in BMI values when comparing the controls with other groups (P =0.000), however WHR had only a slight difference between these groups (P=0.047). Regarding blood pressure, the results showed that a significant variation was detected in the average SBP values among the healthy control group and all three groups (P=0.000), but none was found between the DBP values **(Table 1)**.

Table 1: Physical Properties and Biochemical Parameters of the Study Groups

Parameter	Study Groups				P - Value
	CVD	DM	CVD & DM	Controls	
Age	57.88 ± 10.07	59.33 ± 10.05	56.76 ± 8.56	47.98 ± 12.15	0.000
BMI	28.29 ± 5.57	29.64 ± 6.96	29.42 ± 5.17	25.17 ± 3.92	0.000
WHR	0.96 ± 0.08	0.95 ± 0.10	0.93 ± 0.14	0.90 ± 0.08	0.047
SBP	143.67 ± 19.36	139.54 ± 21.58	138.78 ± 19.05	123.90 ± 17.09	0.000
DBP	75.67 ± 12.759	77.50 ± 12.171	76.09 ± 11.451	76.74 ± 10.537	0.882
Insulin	12.37 ± 7.95	15.98 ± 21.97	10.69 ± 4.67	10.82 ± 4.78	0.096
FBG	5.99 ± 1.61	8.13 ± 3.35	9.15 ± 3.51	5.45 ± 0.42	0.000
Uric Acid	322.81 ± 76.20	300.37 ± 140.07	298.42 ± 99.83	300.55 ± 116.52	0.646
Fibrinogen	405.61 ± 103.37	447.75 ± 119.81	426.99 ± 127.99	304.40 ± 89.44	0.000
Cholesterol	4.001 ± 1.039	4.751 ± 1.481	4.116 ± 1.150	4.008 ± 2.305	0.054
HDL	2.25 ± 1.07	2.72 ± 1.20	2.08 ± 1.04	2.73 ± 1.80	0.017
LDL	1.18 ± 0.31	1.35 ± 0.48	1.18 ± 0.39	1.06 ± 0.80	0.065
Triglycerides	1.48 ± 0.95	1.95 ± 1.61	1.69 ± 1.11	1.82 ± 1.16	0.286
OPG	6.97 ± 3.85	4.49 ± 0.89	6.61 ± 3.44	4.77 ± 1.17	0.000

Biochemical Parameters of the Study Groups

The mean serum levels of all chemical parameters for each group are detailed in Table (1). The serum levels of FBG and fibrinogen were significantly increased for all study groups in comparison to the control group (P =0.000). Yet, both insulin and uric acid levels showed no difference across groups. For the participants' lipid profile, only HDL concentrations were found to significantly increase for the three study groups when compared to the normal controls (P=0.017). Serum OPG concentrations were significantly increased for the study groups when compared to control groups (P =0.00); Table (1). Within the subgroups, we noted a marked increase of OPG levels in males with CVD alone or in association with DM, while in females only those in the DM and CVD group showed similar increase of OPG levels.

Correlation of OPG Levels to Anthropometric and Biochemical Parameters in Subgroups

Within the males and females' subgroups we tested for correlations between OPG concentrations and those of the other measured parameters, using two variables Pearson rank order attachment. In all males' groups, we detected a positive relationship among OPG levels and the WHR measurements (P=0.038); **Figure (2)**. On the other hand, for females, positive correlations were observed between OPG concentrations with the levels of both FBG (P= 0.026) and fibrinogen (P=0.010); Figures (3 & 4).

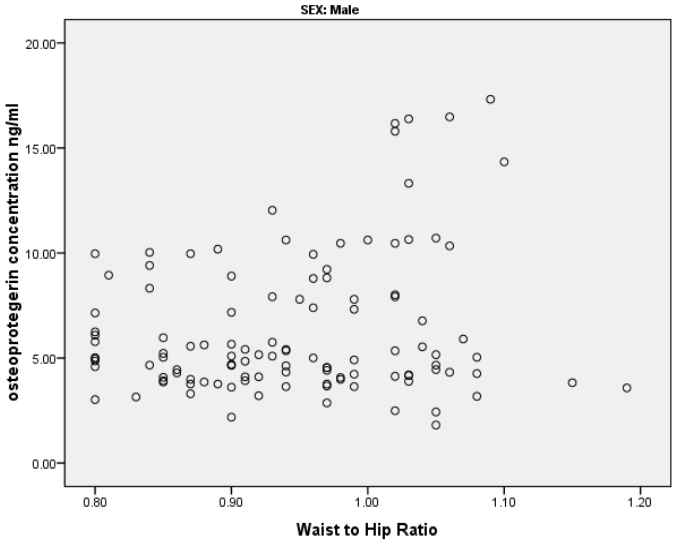

Figure 2: Weak positive correlation between OPG levels & WHR in males

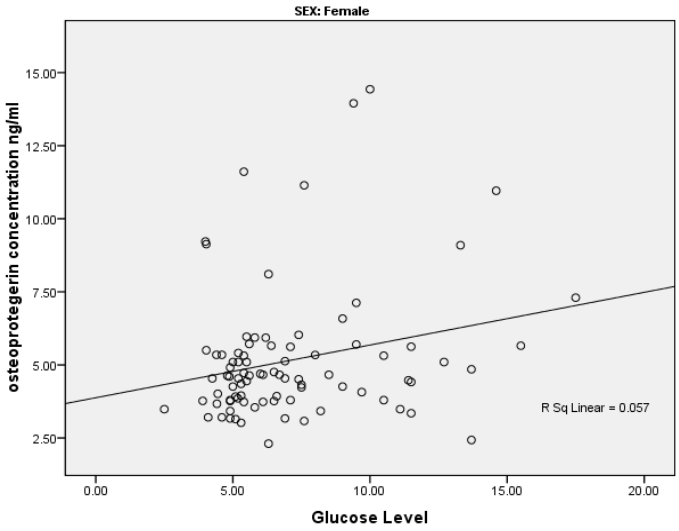

Figure 3: Weak positive correlation between OPG & FBG levels in females

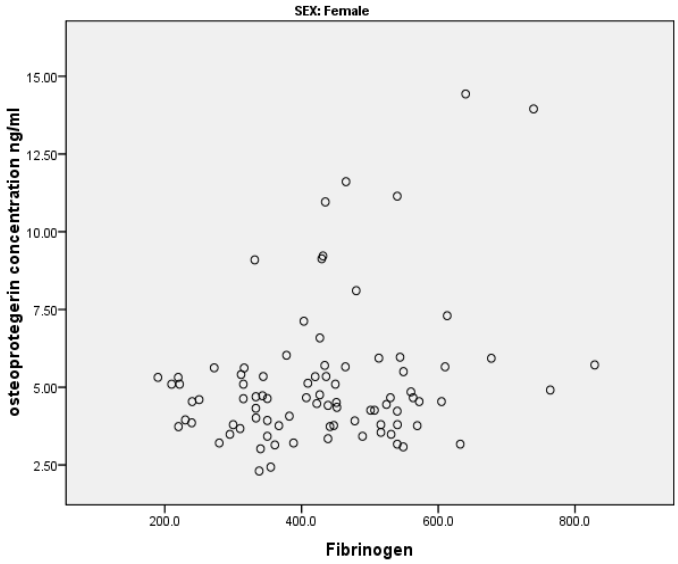

Figure 4: Weak positive correlation between OPG & fibrinogen levels in females

DISCUSSION

Obesity is markedly associated with DM, hypertension, dyslipidemia, and CVD. More than 30% of the Saudi population is overweight, and despite governmental efforts to raise public awareness this percentage is increasing. Females usually suffer the most, and thus are more prone to those diseases [15]. CVD are among the top causes of mortality [16], and diabetics suffer till to six folds' higher risk of developing CVD [17], and the latter is recognized as a main cause of morbidity and mortality among DM patients [18]. The study focused on assessing different parameters for DM & CVD patients in Saudi Arabia, including the determination of OPG levels, and then comparing these levels with the values of normal individuals, to evaluate its use as potential biomarker for DM and CVD. For this sample, we noted a significant increase of OPG in males with CVD alone or in association with DM, when compared to other groups. On the other hand, only females in the DM and CVD group showed a similar OPG increase in comparison to females from other groups.

A strong correlation was found between WHR and OPG concentrations in males, and between both FBG and fibrinogen levels with OPG concentrations in females. The relation of OPG with glucose metabolism has been the subject of several studies, where OPG levels were higher in DM patients, and thus matching our findings [19, 20]. Yet, the exact association remains unclear. Two previous reports, one focusing on obese individuals

[21] and the other on elderly men [22] found a positive relationship between OPG levels and insulin sensitivity, while a third one among females alone found no relationship [23]. A fourth study showed that OPG was positively correlated with Homeostatic Model Assessment (HOMA) index, which have been used to quantity insulin resistance in diabetic subjects [24], and hence the findings of our study are consistent with the former. Unlike FBG levels, the relationship between OPG and lipid profile has been the subject of only few and controversial studies. A study including Korean females reported that obese subjects with hyper-cholesterolemia had higher OPG levels [23]. Yet, another study conducted in 2003 reported an inverse correlation between triglycerides and OPG levels in males with CVD, although OPG was associated with the disease severity [25]. For our sample, no relationship was found between OPG and any of the parameters included in the clinical lipid profile. Finally, we detected a direct correlation between OPG and fibrinogen levels ($P = 0.010$), and although fibrinogen is associated with CVD risk, serum OPG levels stayed as a danger agent, which may be cause of CVD, vascular mortality, atherosclerosis, and coronary calcification as was reported elsewhere after accounting for fibrinogen effect into consideration [26]. In fact, many clinical studies consistently showed that higher OPG levels which relationship with cardiovascular complications involved vascular calcification, advanced atherosclerosis, heart failure, abdominal aortic aneurysm, other diabetic complications and fatality. [27].

The next in time limitations must be acknowledged. Due to resources constraints, we were only able to recruit relatively small sample size, and a larger one is needed to confirm the predictive value for OPG in DM and CVD patients. Secondly, and since only the total amount of OPG was measured, we could not differentiate between the effects of free and complex forms of OPG. Lastly, the study lacked an estimate of the body fat mass distribution and the in vivo insulin action, both of which may have enhanced its results.

CONCLUSION

OPG was significantly higher in the CVD groups, for both males and females, and could be attributed as CVD biomarker. More follow-up studies are needed to investigate OPG association with DM, and its role in the early detection of CVD.

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