OSTEOPROTEGERIN LEVEL IN SAUDI OBESE WITH INSULIN RESISTANCE AND DIABETIC TYPE 2 PATIENTS

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ABSTRACT

Osteoprotegerin (OPG) is a newly identified inhibitor of bone resorption. Recent studies indicate that OPG also acts as an important regulatory molecule in the vasculature. Plasma levels of OPG seem to be elevated in subjects with diabetes as well as in non-diabetic subjects with cardiovascular disease.

In our study we investigate the relationship between serum OPG level in obese with insulin resistance and in type 2 diabetic patients with and without nephropathy.

Methods: Ninety Saudi subjects aged (30-50 years) were included in our study consisting of 15 normal lean, 35obese with insulin resistance and 40 type 2 diabetic patients. Diabetic type 2 patients were further divided to two subgroups according to the presence or absence of microalbuminuria. Blood glucose, serum OPG, urea and creatinine levels were measured for each subject. In addition the microalbuminuria in over-night urine sample was measured in type 2 diabetic patients and blood insulin level in obese subjects.

Results: Serum OPG level was significantly elevated in obese with insulin resistance patients compared to control subjects ($P<0.05$), also serum OPG level significantly elevated in type 2 diabetic patients compared to control and obese with insulin resistance ($P<0.05$). In type 2 diabetic patients the serum OPG level was significantly elevated in type 2 diabetic patient with microalbiminuria compared with type 2 diabetic patient without microalbuminuria ($P<0.05$). In obese with insulin resistance patients there was a positive correlation between blood glucose level and serum OPG level ($r = 0.49$).

In conclusion, our data showed that OPG serum levels increase in type 2 diabetic patients and high level of OPG appear in type 2 diabetic patients with microalbuminuria.

Key words: Osteoprotegerin, Diabetic type 2, Insulin resistace.

BACKGROUND

Coronary artery disease (CAD) is the most important factor in determination of the morbidity and mortality in type 2 diabetic patients, especially in patients with albuminuria. Plasma osteopro-tegerin (OPG) is a predictor of cardio-vascular disease (CVD) in high risk diabetic populations[(1,2)]. Osteo-protegerin is a member of the tumor necrosis factor (TNF) receptor superfamily acting as a soluble decoy receptor for the receptor activator of nuclear factor kb ligand (RANKL) preventing osteoclastogenesis and bone resorption. OPG also a receptor of TNF-related apoptosis-inducing ligand (TRAIL) playing a role in immune regulation and cell survival[(3,4)]. It is also demonstrated at connective tissues such as blood vessels, and play important role for endothelial cell survival and prevent vascular calcify-cation. This idea is based on the observation that OPG knockout mice develop vascular calcifications beside severe osteoporosis, and this may be due to shift of calcium from bone tissue into vasculature[(5,6,7)]. Recently, an elevated plasma OPG level was shown to predict increased mortality in patients with type 1 diabetes and diabetic nephropathy[(8,9)]. The aim of this study is to demonstrate the OPG level in insulin-resistant, diabetic type 2 patients with and without albuminuria.

SUBJECT AND METHODS

This study was done at clinical biochemistry department, faculty of medicine, king Abdulaziz university. The study population consisted of 90 Saudi men aged 30-50 years. They were classified into three groups: The first group consisted of 15 healthy subjects not obese (BMI was < 25), not suffering from diabetes mellitus or any other chronic diseases used as control, the second group consisted of 35 obese BMI > 26.4 with insulin resistance defined by using HOMA-IR model (HOMA-IR= fasting insulin x fasting glucose/22.5, with fasting insulin expressed in µU/ml and fasting glucose expressed in mmol/l). Any subject was defined to be insulin resistant if any one of the three conditions was met (HOMA-IR >4.65, BMI >28.9 kg/m^2, or HOMA-IR >3.60 and BMI >27.5 kg/m^2)[(10)]. The third group consisted of 40 diabetic type 2 patients, these group further classified into two subgroups including 16 diabetic type 2 patients without nephropathy, and 24 diabetic type 2 patients with micro-albuminuria.

For each individual, the questionnaire was collected about age, presence of diabetes mellitus and other chronic diseases, and the duration of diabetes. In addition, the high and weight were measured for each one.

Serum, EDTA-Plasma, and urine samples were collected from each individuals after an overnight fast (10-12 h) after agreement of ethical committee in king Abdulaziz university. The serum was used to determined glucose, creatinine, urea and OPG, while EDTA plasma sample used to determined HbA1c, and urine sample for microalbuminuria determi-nation.

The serum insulin level was determined by sandwich electrochemiluminescence immunoassay (ELYSIS; 2020) (11), while serum glucose, urea, and creatinine were measured by spectro-photometery (Dimension autoanalyzer) [12]. HbA1c was measured by turbidimetric inhibition immunoassay [13], while microalbuminuria was detected by immunoassay (MICRAL test strip)[14].

Finally, serum OPG level was measured by sandwich enzyme immunoassay using Human osteoprotegerin ELISA kit (BioVendor Laboratory Medicine, Inc. Cat. No. RD194003200)[15].

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software version 16 (SPSS Inc., Chicago, IL, USA). The correlations were tested by Spearmans test. Comparisons were performed by the t-test, and ANOVA (analysis of varia-nce). Comparisons and correlations were considered statistically significant when $P<0.05$.

RESULTS

Ninety Saudi male subjects were classified into three groups; control, obese with insulin resistance, and type 2 diabetic patients. The age of control subjects was (30±3), while the age of obese and type 2 diabetic patients was (42±8 and 46±5) respectively. Blood glucose level and HbA1c for control, obese and type 2 diabetic patients were (103±6, 3.4±0.3, 119±24, 4±0.7, 196±73 and 7.7±0.9) respectively. The OPG level of control, obese and type 2 diabetic patients was (3.74±0.7, 4.47±0.97, and 6.00±0.99) respectively, while urea and creatinine levels for these groups were (24.8±4.6, 0.92±0.19, 29.2±8.1, 0.99±0.28, 35.1±107 and 1.10±0.24) respectively. By using t-test the serum OPG, blood glucose, HbA1c and BMI were significantly elevated in obese with insulin resistance patients compared to control subjects ($P<0.05$), Also the serum OPG, blood glucose, HbA1c, urea, and creatinine levels were significantly elevated in type 2 diabetic patients compared to control subjects ($P<0.05$) (table. 1). The serum OPG, blood glucose, HbA1c, and urea levels were significantly elevated in type 2 diabetic patients compared to obese with insulin resistance patients ($P<0.05$) in the same table. By using ANOVA all three groups show statistical significant differences in serum OPG, blood glucose, HbA1c, and urea levels, with higher level in diabetic type 2 patients (table. 2). In type 2 diabetic patients the serum OPG level was significantly elevated in type 2 diabetic patient with microalbiminuria compared with type 2 diabetic patient without micro-albuminuria ($P<0.05$) by using t-test (table.3). Table. 4 showed a significant higher serum OPG level in type 2 diabetic with microalbuminuria than diabetic type 2 patients without nephropathy by using ANOVA ($P<0.05$).

Table 1: Comparison of BMI, blood glucose levels, HbA1c, OPG levels, urea and creatinine levels in control, obese, and type 2 diabetic subjects (Mean±SD)

Variables	Control n = 15	Obese with insulin resistance n = 35	Type2 diabetic n = 40
BMI	22.2 ± 1.6	36.1 ± 7.2 *	29.9 ± 4.4 **
Glucose (mg/dl))	103 ± 6	119 ± 24 *	196 ± 73 * **
HbA1c%	3.4 ± 0.3	4 ± 0.7 *	7.7 ± 0.9 * **
OPG (pmol/l)	3.74 ± 0.70	4.47 ± 0.97 *	6.00 ± 0.99 * **
Urea level (mg/dl)	24.8 ± 4.6	29.2 ± 8.1	35.1 ± 10.7 * **
Creatinine level (mg/dl)	0.92 ± 0.19	0.99 ± 0.28	1.10 ± 0.24 *

BMI (body mass index), OPG (osteoprotegerin).

Values are expressed as (mean±SD)

* Comparison with control subjects using t-test (P < 0.05).

** Comparison with obese subjects using t-test (P < 0.05).

Table 2: Comparison of BMI, blood glucose levels, HbA1c, OPG levels, urea and creatinine levels in control, obese and type 2 diabetic patients.

Variables	Control n = 15	Obese with insulin resistance n = 35	Type2 diabetic(s) n = 40	P value
BMI	22.2 ± 1.6	36.1 ± 7.2	29.9 ± 4.4	3.022E – 09 *
Glucose (mg/dl)	103 ± 6	119 ± 24	196 ± 73	3.840E – 08 *
HbA1c%	3.4 ± 0.3	4 ± 0.7	7.7 ± 0.9	0.00E + 00 *
OPG (pmol/l)	3.74 ± 0.70	4.47 ± 0.97	6.00 ± 0.99	1.187E – 10 *
Urea level (mg/dl)	24.8 ± 4.6	29.2 ± 8.1	35.1 ± 10.7	3.00E – 03 *
Creatinine level (mg/dl)	0.92 ± 0.19	0.99 ± 0.28	1.10 ± 0.24	0.0796

BMI (body mass index), OPG (osteoprotegerin).
Values are expressed as (mean±SD).
Comparison was done using one way ANOVA (analysis of variance).
* P<0.05 is considered significant.

Table 3: Comparison of BMI, blood glucose levels, HbA1c, OPG levels, urea and creatinine levels in type2 diabetic patients classified according to the presence or absence of microalbuminuria

Variables	Without nephropathy n = 16	Micro Albuminurea n = 24
BMI	30.0 ± 3.3	28.8 ± 5.8
Glucose level (mg/dl)	189 ± 81	180 ± 58
HbA1c %	7.5 ± 0.9	7.7 ± 0.9
OPG level (pmol/l)	5.53 ± 0.69	6.40 ± 1.30 *
OPGc	0.19 ± 0.04	0.22 ± 0.03
Urea (mg/dl)	33.3 ± 12.2	34.4 ± 8.4
Creatinine (mg/dl)	1.04 ± 0.27	1.14 ± 0.24

BMI (body mass index), OPG (osteoprotegerin).
Values are expressed as (mean±SD).
* Comparison of type 2 diabetic patients without or with microalbuminuria, using t-test (P < 0.05).

Table 4: Comparison of BMI, glucose levels, HbA1c, OPG levels, urea and creatinine levels in type 2 diabetic patients classified according to the presence or absence of microalbuminuria

Variables	Without nephropathy n = 16	Micro Albuminurea n = 24	P value
BMI	30.0 ± 3.3	28.8 ± 5.8	0.6496
Glucose level(mg/dl)	189 ± 81	180 ± 58	0.3644
HbA1c %	7.5 ± 0.9	7.7 ± 0.9	0.3708
OPG level(pmol/l)	5.53 ± 0.69	6.40 ± 1.30	0.0147 *
Urea (mg/dl)	33.3 ± 12.2	34.4 ± 8.4	0.4052
Creatinine (mg/dl)	1.04 ± 0.27	1.14 ± 0.24	0.3719

BMI (body mass index), OPG (osteoprotegerin).
Values are expressed as (mean±SD).
Comparison was done using one way ANOVA (analysis of variance).
* P<0.05 is considered significant.

DISCUSSION

OPG is produced by cells of the cardiovascular system, including coronary artery smooth muscle cells and endothelial cells, and OPG represents a protective factor for the vascular system. In the vascular system, increased OPG production may indicate endothelial damage, intimal hyperplasia, smooth muscle cell hypertrophy, or advanced plaque calcification. Several studies support a role of OPG in vascular homeostasis. In animal models, *opg*-deficient mice, which have no measurable OPG in their blood, develop premature arterial calcification (mainly in the media of large vessels), that is preventable by restoration of the gene. The protective vascular effects of OPG are also evident from a study in which parenteral administration of OPG prevented vascular calcification induced by treatment with warfarin and supra physiological doses of vitamin D in rats[(1)].

The high level of osteoprotegerin found in obese with insulin resistance and diabetic type 2 patients compared to the control group in our study is in accordance with those reported by **Dhakshinamurthy *et al*., (2006), and Jong *et al*., (2011)**, who also reported a higher level of OPG in insulin resistance and diabetic type 2 compared with a normal one. Also, our results are in agreement with those reported by **Henrik *et al*. (2011)** who found that, the level of OPG is positively associated with diabetic type 2 patients, and elevated in patients with albuminurea more than the one without this complication. Our finding of high level of osteoprotegerin in patients with microvascular complication (microalbuminuria) is supported by the finding of **Knudsen *et al*. (2003)** who found, increased plasma concentration of osteoprotegerin in type 2 diabetic patients with microalbuminuria. They found that plasma values of OPG were significantly increased only in patients with microvascular complications, suggesting that elevated plasma levels of OPG may reflect microvascular damage among patients with diabetes rather than the diabetic state. They also found a strong correlation between the presence of diabetic maculopathy and incipient diabetic nephropathy. And the elevated OPG levels in patients with maculopathy well represent an increased production of this molecule by endothelial cells and smooth muscle cells in diseased microvessels not only in the retina but in the entire microcirculation of these patients.

Elevated HbA1c is a known cardio-vascular risk factor with hyperglycemia, **Jong *et al*., (2011)**, found a strong correlation between plasma OPG and HbA1c in diabetic type 2 patients, this finding was not clear in our study as in **Henrik *et al*., (2010)** study, and this may due to the small size of our study.

Henrik *et al*., (2010), Gitte *et al*., (2009) and Knudsen *et al*., (2003) all reported high level of osteoprotegerin in diabetic patients which go along with our results, they suggested that increased serum OPG levels have been interpreted as an insufficient compensatory self-defensive response to prevent further bone loss and the progression of atherosclerosis.. Our study limitations are the measurement of total OPG, because the detection system used cannot discriminate between free OPG and OPG complex to its ligand, RANKL. Therefore, increased OPG serum levels measured by this and other commercial assays may be due to an increase of free OPG, an increase of RANKL-OPG complexes, or both, and the second limitation is the smaller size of our subjects. In our study, we tried to eliminate potential confounding by age by closely matching these parameters in the individuals from each group. Also because OPG serum concentrations were increased in patients with renal failure[(20)], we excluded patients with creatinine serum concentrations above 2.0 mg/dl in our study.

In conclusion, our data show that OPG serum levels increase in type 2 diabetic patients and high level of OPG appear in type 2 diabetic patients with microalbuminuria, and because our study was a small sized, the effect of insulin resistance not clear, so a large populations is needed to determine the serum OPG levels in insulin resistant subjects and type 2 diabetic patients.

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مستوى الأوستيوبروتيجرين لدى الأشخاص السعوديين الذين يعانون من السمنة ومقاومة تأثير الأنسولين والأشخاص الذين يعانون من داء السكري من النوع الثاني

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الأستيوبروتيجرين أو الأو بي جي هو عبارة عن بروتين مثبط لعملية فقدان الكالسيوم من العظم، وقد تم اكتشافه حديثا. وقد دللت الدراسات الحديثة على دوره المنظم في عملية الحفاظ على سلامة الأوعية الدموية. وقد أظهرت تلك الدراسات ارتفاع مستوى الأو بي جي في الدم لدى مرضى السكري والمرضى الذين يعانون من أمراض القلب والشرايين ممن لا يعانون من مرض السكري. وقد أجريت هذه الدراسة لمعرفة العلاقة بين مستوى الأو بي جي في الدم لدى الأشخاص الذين يعانون من السمنة ومقاومة خلاياهم لعمل الأنسولين والأشخاص المصابين بداء السكري من النوع الثاني (الغير معتمد على نقص كمية الأنسولين) والذين ليس لديهم مضاعفات في وظائف الكلى، وآخرون مصابون بداء السكري ولديهم تلك المضاعفات ومقارنتها بمستوى الأو بي جي لدى أشخاص سليمين لا يعانون من أي مما سبق ذكره أو أي أمراض أخرى.
وقد أجريت هذه الدراسة على تسعين رجلا سعوديا تتراوح أعمارهم من ٣٠ إلى ٥٠ سنة وقد قسمو الى ١٥ رجلا سليما و٣٥ رجلا يعانون من السمنة ومقاومة خلاياهم لعمل الأنسولين و٤٠ رجلا مصابين بداء السكري من النوع الثاني. كما قسم الأشخاص الذين يعانون من داء السكري من النوع الثاني إلى ثلاثة مجموعتين حسب تواجد أو غياب نسبة الألبيومين في البول. وقد قمنا بتقدير مستوى السكر، الأو بي جي واليوريا، والكرياتينين في دم جميع الأشخاص المعنيون في البحث بالإضافة إلى قياس مستوى الأنسولين في دم الأشخاص الذين يعانون من السمنة ونسبة الألبيومين في بول مرضى السكري. وقد وجدنا ارتفاعا ملحوظا لمستوى الأو بي جي في دم الأشخاص الذين يعانون من السمنة مقارنة بالأشخاص السليمين، وكذلك وجدنا ارتفاعا ملحوظا في مستوى الأو بي جي في دم مرضى السكري من النوع الثاني مقارنة بالأشخاص السليمين أيضا. أما في الأشخاص الذين يعانون من داء السكري من النوع الثاني فقد وجدنا ارتفاعا ملحوظا في كمية الأو بي جي في الدم لدى مرضى السكري من النوع الثاني والذين يعانون من اختلال في وظائف الكلى مقارنة بمرضى السكري الذين لا يعانون من تلك الاعتلالات.
كما وجدت علاقة ايجابية بين مستوى الأو بي جي وكمية السكر في دم الأشخاص الذين يعانون من السمنة.ويستنتج من هذه النتائج على وجود علاقة بين ارتفاع مستوى الأوستيوبروتيجيرين والمضاعفات التي تحدث على مستوى الأوعية الدموية في مرضى السكري من النوع الثاني، ولكن لا يمكن تحديد ما إذا كان هذا الارتفاع كعامل حماية لتلك الأوعية الدموية أم هو المسبب لذلك العطب فيها.
لذا نوصي بإجراء دراسة أكبر لمعرفة مصدر الأو بي جي ودراسة علاقته مع مرض السكري من النوع الثاني على نطاق واسع.