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

Ayman S. Alharbi1*, Zainy M. Banjar2, Osama M. Shenawy3

Mohammad Al hasan Al falgi4, Saad S. Al Zahran5, Yousri M. Husseyn6

Faculty of Applied medical science, Taif university1

Faculty of medicine, King Abdul-Aziz university2

Faculty of medicine, King Abdul-Aziz university3

Internal Medicine, King Faisal Hospital, Taif, Saudi Arabia 4

Family Medicine Department, Faculty of Applied Medical Science, Taif University, Saudi Arabia 5.

Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt, Medical Laboratories Department, Faculty of Applied Medical Science, Taif University, Saudi Arabia 6.

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, 35 obese 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 microalbuminuria 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 resistance.

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 cardiovascular 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 survival3,4). 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 vasculature5,6,7). Recently, an elevated plasma OPG level was shown to predict increased mortality in patients with type 1 diabetes and diabetic nephropathy8,9). 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/m², or HOMA-IR >3.60 and BMI >27.5 kg/m²)(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.

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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, ura and OPG, while EDTA plasma sample used to determined HbA1c, and ura sample for microalbuminuria determination.

The serum insulin level was determined by sandwich electrochemiluminescence immunoassay (ELYSIS; 2020) (11), while serum glucose, urea, and creatinine was measured by spectrophotometry (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 variance). 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, ura, 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 ura 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 ura 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 microalbuminuria compared with type 2 diabetic patient without microalbuminuria (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)**

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

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.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control n = 15</th>
<th>Obese with insulin resistance n = 35</th>
<th>Type 2 diabetic(s) n = 40</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>22.2 ± 1.6</td>
<td>36.1 ± 7.2</td>
<td>29.9 ± 4.4</td>
<td>3.022E − 09 **</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>103 ± 6</td>
<td>119 ± 24</td>
<td>196 ± 73</td>
<td>3.840E − 08 **</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>3.4 ± 0.3</td>
<td>4 ± 0.7</td>
<td>7.7 ± 0.9</td>
<td>0.00E + 00 **</td>
</tr>
<tr>
<td>OPG (pmol/l)</td>
<td>3.74 ± 0.70</td>
<td>4.47 ± 0.97</td>
<td>6.00 ± 0.99</td>
<td>1.187E − 10 **</td>
</tr>
<tr>
<td>Urea level (mg/dl)</td>
<td>24.8 ± 4.6</td>
<td>29.2 ± 8.1</td>
<td>35.1 ± 10.7</td>
<td>3.00E − 03 **</td>
</tr>
<tr>
<td>Creatinine level (mg/dl)</td>
<td>0.92 ± 0.19</td>
<td>0.99 ± 0.28</td>
<td>1.10 ± 0.24</td>
<td>0.0796</td>
</tr>
</tbody>
</table>

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

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

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

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

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(28), 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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Osteoprotegerin Level In Saudi Obese

By

Dr. Aiman Saeed Al Harbi 1, Dr. Zinzi B. Al Abashr 2, Dr. Mohammed Al Sattar Al Falah 3

1 Assistant Professor, Department of Chemical Pathology, College of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
2 Assistant Professor, Department of Chemical Pathology, College of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
3 Assistant Professor, Department of Pathology, College of Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

Abstract

Osteoprotegerin (OPG) is a membrane glycoprotein that belongs to the tumor necrosis factor (TNF) family. It is a natural inhibitor of osteoclast formation and function. The understanding of the role of OPG in the maintenance of vascular health is important.

Previous studies have shown an increased level of OPG in patients with type 2 diabetes and patients with cardiovascular disease who do not have diabetes.

The aim of this study was to determine the level of OPG in the blood of obese Saudi individuals and individuals with resistance to insulin, as well as patients with type 2 diabetes (not dependent on insulin) and others with impaired renal function, and to compare it with the level of OPG in a healthy group.

This study was conducted on 90 Saudi men aged 33 to 53 years. They were divided into 15 healthy men, 35 men with obesity and insulin resistance, and 43 men with type 2 diabetes.

The OPG level was measured in all study participants in addition to creatinine and uric acid in the blood of the patients with obesity and type 2 diabetes, and albumin in the urine of diabetic patients.

It was found that there was a significant increase in the level of OPG in the blood of obese individuals compared to healthy individuals.

Similarly, there was a significant increase in the level of OPG in the blood of patients with type 2 diabetes compared to healthy individuals.

It was also found that there was a positive correlation between the level of OPG and the level of creatinine in the blood of obese individuals.

It can be concluded that there is a relationship between the increased level of osteoprotegerin and the complications that occur in type 2 diabetes, but it is not possible to determine whether this increase is a causative factor or a result of the disease.

Therefore, it is recommended to conduct a larger study to determine the source of OPG and its relationship with type 2 diabetes on a large scale.

References

- Al Harbi, A. S. (2013). Osteoprotegerin Level In Saudi Obese. Z.U.M.J. Vol. 19; N. 2; March; 2013

Note: The reference list is not provided in the text.