A Study of Osteoprotegerin in Diabetic Patients as Indicator for Myocardial Infarction

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Abstract

The study included (180) subject (89 males, 91 females), classified into 4 study groups: (49) diabetic patients (21 males, 28 females) were attended to the diabetic and endocrinology center at Al-Sader medical city in Al-Najaf Al-Ashraf, and registered in the center as type 2 diabetic mellitus patients (DM) group. (40) patients (23 males, 17 females) attended to the coronary care unit (CCU) at Al-Sader medical city in Al-Najaf Al-Ashraf and diagnosed as having myocardial infarction (they all showed S-T elevation) (MI) group. (46) patients (21 males, 25 females) attended to the coronary care unit (CCU) at Al-Sader medical city in Al-Najaf Al-Ashraf and diagnosed as having myocardial infarction (they all showed S-T elevation), and previously diagnosed as type 2 diabetic patients (DMMI) group. (45) healthy subjects (24 males, 21 females) vás (control) group. all patients and control subjects were aged between 40-75 years. All of them were non obese (Body Mass Index < 30). Samples collected during the period from May 2011 till February 2012. The measured parameters include: fasting blood sugar (FBS), glycosylated haemoglobin (HbA1c %), lipid profile (LDL, TG, HDL, vLDL, atherogenic Index), serum Osteoprotegerin (OPG). The results obtained were:

There was a significant increase (P<0.05) in FBS in DM and DMMI groups. A significant increase (P<0.05) in the percentage of HbA1c in all patients (DM, MI, and DMMI groups) compared with the control. Lipid profile parameters and atherogenic index were significantly (P<0.05) higher in all patients than healthy individual. Serum Osteoprotegerin (OPG) levels elevates significantly (P<0.05) in all patients compared with the control group, the MI patients showed the highest OPG levels in all patients. Also there were no significant difference between males and females in serum OPG levels. In DM and DMMI groups serum OPG levels were significantly higher in elderly patients (aged more than 60 years) than patients aged 40-50 years. In conclusion, serum OPG levels were higher in diabetic patients suffering from myocardial infarction, so this parameter could be a risk marker for MI in diabetic patients.

Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism(1). Data compiled by the centers of disease control and prevention indicate that 26 million people in the united states have diabetes mellitus (DM) with the vast majority (90-95%) having type 2 DM.(2)

Diabetes mellitus is also a considerable public health concern and more aggressive management of the condition and its complications, particularly cardiovascular disease is warranted (3), this situation requires a more profound understanding of pathophysiological conditions preceding vascular damage in diabetes, the identification and adaptation of new and innovative diagnostic strategies in selecting diabetic patients at high cardiovascular risk,
the development and availability of effective and safe drugs for blood glucose control and for the reduction of vascular risk. (4). Poor long-term glycemic control in patients with DM can lead to a wide range of microvascular (e.g. renal, retinal) and macrovascular (primarily cardiovascular) complications. (5)

Macrovascular disease characterized by the atherosclerotic changes in large blood vessels, is the major cause of morbidity and mortality 80% in type 2 DM (6), the major clinical effects are seen in the coronary arteries (angina, myocardial infarction), lower extremities (gangrene), and carotid arteries (shoke). (6) Since cardiovascular disease is asymptomatic in diabetic patients, and is therefore at an advanced stage when it becomes clinically manifest (7) consequently, detecting cardiovascular marker, atherosclerotic marker in type 2 DM patients at early stage before the clinical complication incidence will be beneficial management, treatment and prevention of cardiovascular disease. Osteoprotegerin (OPG) is a promising predictor of cardiovascular disease in high risk diabetic populations as well as in other populations (8; 9; 10; 11). (OPG) is a member of the tumor necrosis factor (TNF) receptor superfamily acting as a soluble decoy receptor for the receptor activator of nuclear factor-β ligand (RANKL) and TNF-related apoptosis-inducing ligand (TRAIL) preventing osteoclast activation and bone resorption, and participating in immune regulation and cell survival (12) It also plays a role in the homeostasis of the immune system, e.g. the differentiation of dendritic cells, lymphocyte development, lymph node organization (13) and in some aspects of tumor biology such as bone metastasis (14). It has been shown that OPG is produced by wide range of tissues, including the cardiovascular system (15; 16). In the vascular system, OPG seems to protect against vascular calcification, since OPG/-/- mice have a combined phenotype of osteoporosis and calcification of large arteries (17). Recently, circulating OPG levels were reported as independent predictor of cardiovascular mortality in patients with stable coronary artery disease (18). In a symptomatic type 2 DM patients, OPG levels predicted subclinical atherosclerosis and cardiovascular event (10).he aim of this study was to investigate the levels of OPG in diabetic patients, myocardial infarction patients and diabetic patients with myocardial infarction.

Methods

1. Subjects: The study included (180) subject (89 males, 91 females), classified into 4 study groups as following:

Group 1: (49) diabetic patients (21 males, 28 females) were attended to the diabetic and endocrinology center at Al-Sader medical city in Al-Najaf Al-Ashraf, and registered in the center as type 2 diabetic mellitus patients (DM) group. Group 2: (40) patients (23 males, 17 females) attended to the coronary care unit (CCU) at Al-Sader medical city in Al-Najaf Al-Ashraf and diagnosed as having myocardial infarction (MI) group. They were diagnosed by physicians based on history, clinical presentation, electrocardiogram, angiography (they all showed S-T elevation), and its their first exposure to an ischemic heart disease. Group 3: (46) patients (21 males, 25 females) attended to the coronary care unit (CCU) at Al-Sader medical city in Al-Najaf Al-Ashraf and diagnosed as having myocardial infarction, and previously diagnosed as type 2 diabetic patients (DMMI) group. They were diagnosed by physicians based on history, clinical presentation, electrocardiogram, angiography (they all showed S-T elevation), and its their first exposure to an ischemic heart disease. Group 4: (45) healthy subjects (24 males, 21 females) was (control) group. All patients and control subjects were aged between 40-75 years, non smokers, have no history of renal dysfunction, liver disease, or chronic inflammatory disease. All of them were non obese (Body Mass Index < 30). Samples collected during the period from May 2011 till February 2012.
2. Blood Samples

5 milliliters of venous blood was obtained by antecubital venipuncture using G23 needle were drawn between 8:30-10:00 A.M. after 12 hour fasting. 1.5 mL were put in ethylin diadine tetra acetic acid (EDTA) containing tube for HbA1c measurement. The remaining blood was allowed to clot in plain test tube at room temperature, the serum was aspirated after centrifugation at 3000 rpm for 10 min., divided into aliquots in epindroff tubes and stored at -20°C until the measurement of the study parameters. Some measurements were performed on the collection day: HbA1c, fasting blood sugar (FBS), total cholesterol, and triglyceride (TG), HDL-cholesterol,FBS, total chol., TG, and HDL-chol. Levels were measured in serum by routin enzymatic methods.

**Measurements of glycohemoglobin (HbA1c) (19)**
Glycohemoglobin kit for quantitative colorimetric determination of glycohemoglobin in whole blood was supplied by StanBio laboratory (USA).

**Measurements of glucose in serum (20)**
Serum glucose level was measured by glucose (Glucose-PAP) kit (AUDIT DIAGNOSTICS, Ireland)

**Measurements of total cholesterol (21)**
Quantitative-enzymatic1-colorimetric determination of total cholesterol in serum (from Stanbio cholesterol Liquid color)

**Measurement of Triglycerides (22)**
Stanbio triglyceride liquid color ® a quantitative enzymatic-colorimetric determination of triglyceride in serum.

**Measurements of HDL-cholesterol**
Quantitative-enzymatic1-colorimetric determination of HDL-cholesterol in serum (from Stanbio cholesterol Liquid color)

**Calculation of LDL-Cholesterol (23)**
This was done by using the following equation:

\[
LDL \text{ in mg/ dl} = \text{Total cholesterol} - (VLDL + \text{HDL-cholesterol})
\]

**Calculation of VLDL-Cholesterol (23)**
This was done by using the following equation:

\[
VLDL \text{ in mg/ dl} = \frac{\text{Triglyceride}}{5}
\]

**Calculation of Atherogenic index (24)**
Atherogenic index is the ratio between total cholesterol / HDL-cholesterol.
To calculate Atherogenic index the following equation was used:
Atherogenic Index = Total Cholesterol / HDL-cholesterol.

**Measurement of serum OPG**
OPG was measured by the RayBio Human osteoprotegerin ELISA kit. The RayBio Human osteoprotegerin ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human osteoprotegerin in serum, plasma, cell culture supernatants and urine.

**Statistical Analysis**
The data expressed as mean ± S.E. SPSS version 14 for window was used for all statistical analyses. Statistical significance was assessed by ANOVA, P values of less (0.05) was considered significant.

**Results**

Table (1) shows the characteristics of the study subjects. The study performed on 180 subjects (89 males and 91 females), aged 40-75 years, 55% of DM patients have family history of the disease, and 50% of DMMI group patients have a family history of DM. Also 25% of MI group patients have a family history of MI, and 19.5% of DMMI group patients have a family
history of MI. Although all subjects were not obese (their BMI less than 30), all subjects could be considered overweight (their BMI were 27.85, 28.48, 25.96, and 27.45 in control, DM, MI, and DMMI groups respectively). 1% of DM patients suffered from hypertension, and 32.5% of MI patients, 45.5% of DMMI patients also have hypertension. DM patients administered medications: insulin 16.32%, glibiclamide 69.38%, metformin 42.85%, most of them may have a combination of two type of these drugs. In DMMI group patients 21.73% administered insulin, 45.65% glibiclamide, and 13.04% metformin, also some combined more than one type. In MI group, 27.5% administered capotin, also 8.69% of DMMI group administered capotin. FBS increased significantly (P<0.05) in DM and DMMI group, (figure 1), it was 174.97 ± 7.39 and 218.158 ± 14.14 mg/dl in DM and DMMI groups respectively when compared with 117.17± 4.22 mg/dl in control group. HbA1c percentage increased significantly (P<0.05) in all patients when compared with control group (figure 2), it was 10.21% ± 0.34 % in DM patients, 8.33% ± 0.41% in MI patients, 10.32% ± 0.34% in DMMI patients, compared with 6.12% ± 0.19% in control group. Atherogenic index and lipid profile increased significantly in all patient in comparison to control group (table 2).

### Serum OPG

The results showed a significant increase (p<0.05) in serum OPG in DM group (1.189 ± 0.1) ng/ml, and MI group (1.619 ± 0.12) ng/ml, and in DMMI group (1.244 ± 0.12) ng/ml when compared with control group (0.753 ± 0.05) ng/ml. Serum OPG in MI group significantly different (p<0.05) from other groups (figure 3).

There was no significant difference (p<0.05)in serum OPG between males and females(figure 4). Figure (5) shows a significant difference (p<0.05) in serum OPG between DM patients aged 40-50 years and DM patients >60 years, and a significant difference (p<0.05) in DMMI patients between age ranges 40-50 years and >60 years.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>DM</th>
<th>MI</th>
<th>DMMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>45</td>
<td>49</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>24/21</td>
<td>21/28</td>
<td>23/17</td>
<td>21/25</td>
</tr>
<tr>
<td>Age (average)</td>
<td>49.06</td>
<td>52.75</td>
<td>55.55</td>
<td>54.19</td>
</tr>
<tr>
<td>40-50y</td>
<td>29</td>
<td>20</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>50-60y</td>
<td>10</td>
<td>17</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>&gt;60y</td>
<td>6</td>
<td>12</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Family history of DM</td>
<td>-</td>
<td>55%</td>
<td>-</td>
<td>50%</td>
</tr>
<tr>
<td>Family history of MI</td>
<td>-</td>
<td>-</td>
<td>25%</td>
<td>19.5%</td>
</tr>
<tr>
<td>Duration of DM (y)</td>
<td>-</td>
<td>7.58 ±0.93</td>
<td>-</td>
<td>7.26±1.05</td>
</tr>
<tr>
<td>Hypertension %</td>
<td>-</td>
<td>1%</td>
<td>32.5%</td>
<td>45.5%</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>80.4 ± 0.59</td>
<td>78.8 ± 0.69</td>
<td>78.0 ± 1.84</td>
<td>76.5 ± 1.7</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>118.9 ± 0.57</td>
<td>125.9 ± 1.9</td>
<td>120.1 ±2.97</td>
<td>121.5 ± 3.4</td>
</tr>
<tr>
<td>BMI</td>
<td>27.85 ±0.23</td>
<td>28.48 ±0.48</td>
<td>25.96 ±0.42</td>
<td>27.45 ±0.41</td>
</tr>
<tr>
<td>Medications</td>
<td>insulin</td>
<td>-</td>
<td>16.32%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Glibiclamide</td>
<td>-</td>
<td>69.38%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Metformin</td>
<td>-</td>
<td>42.85%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Capotin</td>
<td>-</td>
<td>27.5%</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2: lipid profile in the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n.</th>
<th>Mean ± Std.Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chol.</td>
<td>T.G.</td>
</tr>
<tr>
<td>Control</td>
<td>45</td>
<td>143.78 ± 2.87</td>
</tr>
<tr>
<td>DM</td>
<td>49</td>
<td>182.95 a ± 5.59</td>
</tr>
<tr>
<td>MI</td>
<td>40</td>
<td>177.72 a ± 5.44</td>
</tr>
<tr>
<td>DMMI</td>
<td>46</td>
<td>164.35 ab ± 5.14</td>
</tr>
</tbody>
</table>

- *a* significant difference (P<0.05) with control group.
- *b* significant difference (P<0.05) with DM group.
- *c* significant difference (P<0.05) with MI group.
Discussion

The risk of coronary artery disease (CAD) is markedly increased in diabetic patients, in whom CAD is the leading cause of death. Silent myocardial ischemia (SMI) occurs
frequently in numerous individuals and may result in more severe CAD upon initial presentation and worse outcomes in diabetic patients (25; 26). Once CAD is symptomatic, morbidity and mortality are higher and significantly worse in patients with than without diabetes.

In patients with diabetes, risk factors should be managed aggressively to prevent the development and progression of CAD (27; 28).

In this study, fasting blood sugar was higher in DM and DMMI groups comparing with the control and MI groups. The percentage of HbA1c that represent the control of blood sugar during a period about several months, was elevated in all patient groups (DM, MI, DMMI) compared with the control. Also this percentage was lower in MI patients than diabetic patients (figure 2). This result was agreed with (29), who study the dysglycaemia in multible ethnic groups, in their analysis of 15780 patients from 52 country, their findings clearly showed that dysglycaemia as measured by the HbA1c level in people with or without a history of diabetes is a strong, independent cardiovascular risk factor throughout different regions of the world and ethnicities. Overall, after accounting for the other major cardiovascular risk factors, for every 0.5% and 1% higher HbA1c there was a 9% and 19% higher odds of MI respectively. The findings suggest that dysglycaemia is closely linked to an unmeasured causal factor, and that therapeutic and/or population-based strategies that reduce the prevalence of dysglycaemia by preventing or reversing diabetes, or by slowing the rise of HbA1c with time may reduce the global burden of MI. Indeed, at least three large ongoing clinical trials are currently assessing the effect of preventing diabetes and/or of treating early diabetes on cardiovascular outcomes. These data presented here highlight the importance of the HbA1c as an important and robust independent risk factor for MI in the presence and absence of a history of diabetes.

Atherogenic index in this study showed in table 2 was significantly higher in all patients compared with the control, this result confirm dyslipidemia in all patients. In diabetic patients dyslipidemia is common, an important predictor of cardiovascular risk, and a feature open to therapeutic intervention. Dyslipidemia is strongly correlated with insulin resistance and hyperinsulinemia. Dyslipidemia is generally present at the time of diagnosis of type 2 diabetes and persists despite treatment of hyperglycemia. (30).

The implication of OPG in human cardiovascular disease has been increasingly recognized (31). High levels of OPG have documented in type 2 DM patients with asymptomatic coronary artery disease (32; 33) The over-expression of OPG by the vascular cell, may continue the process of calcification. In fact, OPG levels may rise further, as calcification progresses, concurred production of OPG by the newly differentiateed osteoclast-like cells from the calcifying vascular cells.

High OPG levels are reported to be positively correlated with inflammatory markers (CRP, IL-6, and fibrinogen, HbA1c levels and insulin resistance. (34; 35). Kadoglou et al. (2008) (36) found that the increased OPG concentrations in patients with carotid atherosclerosis and its independent association with carotid plaque. In diabetes mellitus, accumulation of OPG may be part of the generalized matrix change seen in the arterial wall (37; 38) which could relate to the fact that production of OPG from vascular smooth muscle cells is highly influenced by proinflammatory and hormonal factors in the diabetes melieu (16). an observational study found higher plasma OPG concentration in diabetes individuals compared with non diabetics, although the absolute concentration difference was limited (39) high levels of OPG have documented in type 2 DM patients with asymptomatic coronary artery disease(32; 33).
Our results also showed a significant increase in OPG levels in diabetic patients when compared with the control group. This result agreed with several studies on DM patients such (40).

Also the DM patients who were suffering from MI (DMMI group) showed high levels of OPG, also this result agreed with Chen et al., (40). Who found an association of plasma OPG and adiponectin with alteration function in type 2 DM they found that OPG level may be a marker of underlying mechanism linking the diabetic state to cardiac abnormalities, and documented that the use of OPG as marker might add to the risk stratification of (future) cardiac disease in asymptomatic men with less advanced type 2 DM prior the onset of coronary artery disease.

Several studies link the OPG levels with cardiovascular diseases in many aspects: Heymann et al. (2012) (41) found that OPG / RANK / RANKL molecular tried may play a role in differentiation calcification in carotid and femoral plaques. Breland et al. (2010)(42) found that inflammatory rheumatic disease patients with coronary artery disease have an inflammatory phenotype involving enhanced endothelial activation and increased OPG.Karatolios et al. (2012)(43) documented that pericardial and systemic OPG or TRAIL are potential diagnostic tools to discriminate between malignant or benign pericardial effusion.

In our study we didn’t found significant differences between males and females, but many studies linked OPG levels with atherosclerosis and cardiovascular diseases in postmenopausal women. In their study Shagrodsky et al (2009) found that OPG appears to represent the molecular link between bone resorption and vascular calcification and may help to explain the high prevalence of atherosclerosis and osteoporosis in postmenopausal women. Akinci et al. (2011)(44) demonstrated that serum OPG is related to cardiovascular risk factors and metabolic syndrome and might be involved in the development of cardiovascular disorders in women with previos gestational diabetes mellitus. The study of Nabipour et al. (2010)(45) showed that serum OPG levels are significant associated with type 2 DM in postmenoposal women independent of cardiovascular risk factors including hs-CRP and OPG levels in their study have no correlation with the metabolic syndrome.

Reinhard et al (2011)(12) linked the elevated plasma OPG with type 2 DM, they found that serum OPG is an independent predictor of the presence of coronary artery disease in asymptomatic type 2 diabetic patients. Singh et al. (2011)(46) reported that there is a high OPG levels in type 2 DM associated with foot vascular calcification. They suggest that high OPG may be a response to high 25 hydroxy vitamin D (25 OH D) levels and hyperlipideamia-induced calcification.

O'Sullivan et al. (2010)(47) found that peripheral artery disease is associated with high serum OPG, regardless of the coexistence of DM. This finding in addition to its correlation with severity of peripheral artery disease, suggests that OPG may be a novel marker for the presence of severity of peripheral artery disease, possibly by reflecting the degree of underlying vascular calcification.

Avegnon et al. (2007)(33) found that OPG is a novel independent marker for silent myocardial ischemia in asymptomatic diabetic patients.

Secchiero et al. (2006)(48) reported that serum OPG is significantly increased in diabetic patients, prompting expanded investigation of the correlation between OPG production / levels and glycemic levels and characterizes the early onset of diabetes mellitus and may contribut to endothelial cell dysfunction. A 17-year prospective observational study in type 2 DM patients showed a strong predictive value of OPG for all causes mortality, independent of conventional risk for cardiovascular disease including renal function (9). Singh et al. (2011)(46) suggest that OPG plays a major role in modulating the response of endothelial
cells and vascular smooth muscle cells to the action of calcification promoters from the stage of endothelial cell dysfunction through the development of medial arterial calcification. Chang et al. (2011)(49) demonstrated that serum OPG and TRAIL concentration are closely related to the severity of diabetic nephropathy which supports the hypothesis of close interplay between OPG and TRAIL. This study also suggested that OPG may be a marker of the severity of diabetic nephropathy. Chen et al. (2011)(40) documented that in asymptomatic type 2 DM, OPG and adiponectin may be marker of underlying mechanism linking the diabetic state to cardiac abnormalities. Poulsen et al. (2011)(50) linked the increased plasma OPG concentration is associated with carotid and peripheral arterial disease in patients with type 2 DM, whereas no relation is observed with respect to myocardial ischemia on myocardial perfusion scintigraphy.

We concluded that elevated serum OPG in DM patient could be a marker for myocardial infarction in DM patients, and early diagnosis could be beneficial in MI prevention.

References
25 BARI Investigators: Influence of diabetes on 5-year mortality and morbidity


osteoprotegerin and tumor necrosis factor related apoptosis inducing-ligand (TRAIL) are elevated in type 2 diabetic patients with albuminuria and serum osteoprotegerin is independently associated with the severity of diabetic nephropathy. Metabolism clinical and experimental 60 (2011) 1064-1069.