

Study of Osteopontin in Diabetic Patients as Indicator for Myocardial Infarction

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Abstract

This study included (180) human samplest (89 males, 91 females), classified into 4 groups: (49) diabetic patients (21 males, 28females) 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)vas (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 Osteopontin (OPN).

The results showed a significant increase ($P<0.05$) in FBS of 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.

Levels of serum OPN increased in all patients significantly in comparing with the control group. MI group was higher significantly than DM group. No significant difference in serum OPN found between males and females. According to age range, there is a significant increase in OPN levels in all patients in the age range (40-50) when compared with control subjects group in this age range, but OPN levels didn't differ significantly among study groups aged 51-60 years. In elderly (>60 years) MI patients showed significant high levels of OPN in comparison with elderly DM patients.

In conclusion, serum OPN levels were higher in MI patients and in diabetic patients suffering from myocardial infarction, so this parameter could be a risk marker for MI in diabetic patients.

keywords: osteopontine, Diabetic M., Myocardial Infarction, lipid profile

الخلاصة

تضمنت هذه الدراسة 180 عينة (89 ذكراً و 91 انثى) صنفوا الى اربع مجاميع: (49) مرضى بالسكري (21 ذكراً و 28 انثى) كانوا يراجعون مركز امراض السكري والغدد الصماء في مدينة الصدر الطبية في النجف الاشرف وكانوا مسجلين في المركز مرضى بالسكري من النوع الثاني (مجموعة السكري DM). (40) مريضاً (23 ذكراً و 17 انثى) ادخلوا الى مركز العناية القلبية (CCU) في مدينة الصدر الطبية في النجف الاشرف، و شخّصوا على انهم مصابين باحتشاء القلب من النوع (STMI) (مجموعة MI). (46) مرضى بالسكري (21 ذكراً و 25 انثى) ادخلوا الى مركز العناية القلبية (CCU) في مدينة الصدر الطبية في النجف الاشرف، و شخّصوا على انهم مصابين باحتشاء القلب من النوع (STMI) وهم مشخصين مسبقاً بأنهم مرضى بالسكري من النوع الثاني (مجموعة DMMI). (45) من الاصحاء (24 ذكراً و 21 انثى) (كمجموعة سيطرة control) كل المرضى والاصحاء تراوحت اعمارهم 40-75 سنة، جميعهم كانوا غير بدنيين (دليل كتلة الجسم > 30). جمعت عينات الدم خلال الفترة من ايار 2011 الى شباط 2012. شملت المعايير المقطرة في الدم: سكر الدم الصائم، نسبة الهيموكلوبين السكري في الدم، نسبة الشحوم و مستوى الاوستيوبونتين في مصل الدم. اظهرت النتائج ما يلي:

زيادة معنوية ($P<0.05$) في مستوى سكر الدم الصائم في مجموعتي DM و DMMI. زيادة معنوية ($P<0.05$) في نسبة الهيموكلوبين السكري في كل المرضى (DM و MI و DMMI) مقارنة بمجموعة السيطرة. نسبة الشحوم كانت مرتفعة معنوياً ($P<0.05$) في كل المرضى مقارنة بالسيطرة.

ازدادت مستويات اوستيوبونتين المصل OPN معنوياً لدى جميع المرضى مقارنة بالسيطرة. ايضاً مجموعة MI كانت لأعلى معنوياً من مجموعة DM ولا توجد اختلافات بين الذكور والإناث. وبالمقارنة بين المستويات العمرية، هناك زيادة معنوية في مستويات الاوستيوبونتين لجميع

المرضى الذين تراوحت أعمارهم 40-50 سنة عندما قورنوا بالأصحاء بنفس مستوى العمر، لكن مستويات الأوستيوبوننتين لم تختلف معنوياً بين أفراد المجاميع المدروسة الذين تراوحت أعمارهم (51-60) سنة. في المسنين (< 60 سنة) مرضى MI كانت لديهم مستويات OPN أعلى معنوياً بالمقارنة مع مرضى DM المسنين.

يمكن الاستنتاج ان مستويات OPN في المصل كانت اعلى لدى مرضى الاحتشاء القلبي ولدى مرضى السكري الذين يعانون من احتشاء القلب، لذا يمكن ان يكون هذا القياس مؤشراً لأحتشاء القلب لدى مرضى السكري.

الكلمات المفتاحية: اوستيوبوننتين ، مرض السكري . احتشاء العظلة القلبية ، ملف الدهون

Introduction

Diabetes mellitus is one of the most prevalent diseases in the world, The total number of people with diabetes worldwide was estimated to be between 151 million and 171 million at the turn of the century, and is expected to rise to 366 million by 2030. Shaw *et al.* (2010) estimated the world prevalence of diabetes among adults will be 6.4%, affecting 285 million adults, in year 2010, and will increase to 7.7%, and 439 million adults by year 2030.

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. (Dailey 2011)

Macrovascular disease characterized by the atherosclerotic changes in large blood vessels, is the major cause of morbidity and mortality 80% in type 2 DM (He *et al.*, 2010), the major clinical effects are seen in the coronary arteries (angina, myocardial infarction), lower extremities (gangrene), and carotid arteries (stroke). (He *et al.*, 2010)

Since cardiovascular disease is asymptomatic in diabetic patients, and is therefore at an advanced stage when it becomes clinically manifest 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.

Osteopontin (OPN) is a phosphorylated acidic glycoprotein with a diverse range of biologic activities including cell adhesion, proliferation and migration. OPN is now considered as a strong chemoattractive and pro-inflammatory molecule, which is involved in a number of physiologic and pathologic events, e.g., angiogenesis, apoptosis, inflammation, wound healing and tumor metastasis (Lorenzen *et al.*, 2010).

OPN has been implicated as a key factor in the development of atherosclerosis (the main cause of coronary artery diseases). Plasma levels of OPN are elevated in essential hypertension, in patients with coronary artery disease (CAD), and restenosis. (Lorenzen *et al.*, 2010).

The aim of this study was to investigate the levels of OPN in diabetic patients, myocardial infarction patients and diabetic patients with myocardial infarction.

Materials and Methods

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

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.
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.

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.

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.

Five 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 ependroff tubes and stored at -20°C until the measurement of the study parameters.

Measurements of glycohemoglobin (HbA1c) (Trivelli *et al.*, 1971), Glycohemoglobin kit for quantitative colorimetric determination of glycohemoglobin in whole blood was supplied by StanBio laboratory (USA)

Measurements of glucose in serum (Trinder, 1969), Serum glucose level was measured by glucose (Glucose-PAP) kit (AUDIT DIAGNOSTICS, Ireland)

Measurements of total cholesterol (Tietz, 1999)

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

Measurement of Triglycerides (Fossati and Principe, 1982), 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 (Friedewald *et al.*, 1972), This was done by using the following equation:

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

Calculation of VLDL-Cholesterol (Friedewald *et al.*, 1972), This was done by using the following equation:
$$\text{VLDL in mg/ dl} = \text{Triglyceride} / 5$$

Calculation of Atherogenic index (Wilson *et al.*, 1998), Atherogenic index is the ratio between total cholesterol / HDL- cholesterol.

To calculate Atherogenic index the following equation was used:

$$\text{Atherogenic Index} = \text{Total Cholesterol} / \text{HDL- cholesterol}$$

Measurement of serum OPN

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

The data expressed as mean \pm 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 over weight (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 21.735 administered insulin, 45.65% glibin clamide, 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 $10.21\% \pm 0.34\%$ in DM patients, $8.33\% \pm 0.41\%$ in MI patients, $10.32\% \pm 0.34\%$ in DMMI patients, compared with $6.12\% \pm 0.19\%$ in control group. Atherogenic index and lipid profile increased significantly in all patient in comparison to control group (table 2).

Serum OPN

Levels of serum OPN increased in all patients significantly in comparing with the control group, figure (3), it was 20.856 ± 3.08 , 29.52 ± 2.46 , 23.33 ± 2.62 ng/ml in DM, MI, DMMI groups respectively compared with 9.02 ± 1.15 ng/ml in the control group. Also MI group was higher significantly than DM group. No significant difference in serum OPN found between males and females (figure 4).

According to age range, figure (5) shows a significant increase in OPN levels in all patients in the age range 40-50 when compared with control subjects in this age range, but OPN levels didn't differ significantly among study groups aged 51-60 years, (figure 6). In elderly (>60 years) MI patients show significant high levels of OPN in comparison with elderly DM patients (figure 7). Table 3 represent a comparison of OPN levels between age ranges in each study group, there was only a one significant difference in DM patients between (40-50)year and (>60) year, the former was the higher.

Discussion

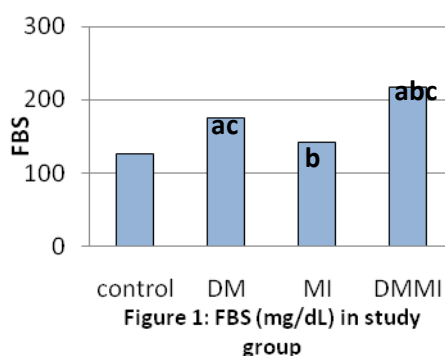
In this study, fasting blood sugar was higher in DM and DMMI groups comparing with the control and MI groups. The percentage of HbA1c that represent the control of blood sugar during a period about several months, was elevated in all patient groups (DM, MI, DMMI) compared with the control. Also this percentage was lower in MI patients than diabetic patients (figure 2). This result was agreed with Gerstein *et al.* (2010), who study the dysglycaemia in multiple 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.⁴ 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. (Betteridge, 2011).

Table 1: characteristic features of control and patients

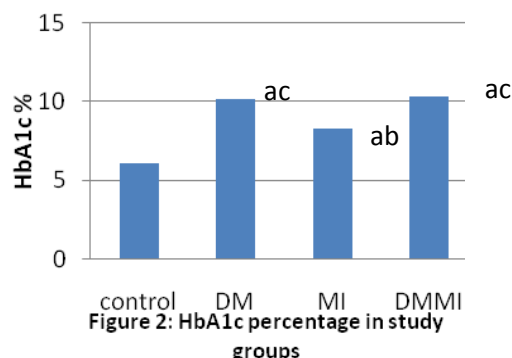
Characteristics	Control	DM	MI	DMMI
No.	45	49	40	46
Gender M/F	24/21	21/28	23/17	21/25
Age (average)	49.06	52.75	55.55	54.19
40-50y	29	20	13	12
50-60y	10	17	8	19
>60y	6	12	19	15
Family history of DM	-	55%	-	50%
Family history of MI	-	-	25%	19.5%
Duration of DM (y)	-	7.58 ±0.93	-	7.26±1.05
Hypertention %	-	1%	32.5%	45.5%
Diastolic BP	80.4 ± 0.59	78.8 ± 0.69	78.0 ± 1.84	76.5 ± 1.7
Systolic BP	118.9 ± 0.57	125.9 ± 1.9	120.1 ±2.97	121.5 ± 3.4
BMI	27.85 ±0.23	28.48 ±0.48	25.96 ±0.42	27.45 ±0.41
Medications				
insulin	-	16.32%	-	21.73%
Glibinclamide	-	69.38%	-	45.65%
Metformin	-	42.85	-	13.04%
Capotin	-	-	27.5%	8.69%



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.



a significant difference ($P < 0.05$) with control group.

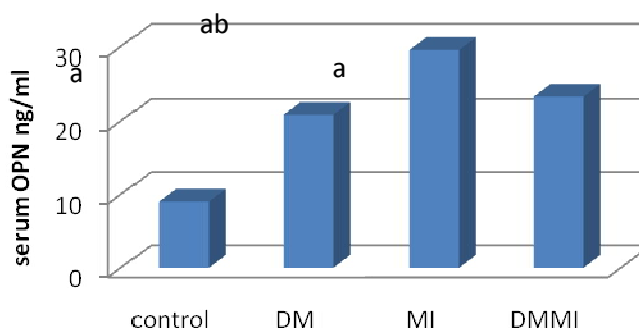
b significant difference ($P < 0.05$) with DM group.

c significant difference ($P < 0.05$) with MI group.

Table 2: lipid profile in the study groups (Mean \pm Std.Error)

Group	n.	Chol.	T.G.	HDL	vLDL	LDL	Athero. index
Control	45	143.78 \pm 2.87	140.68 \pm 8.6	39.5 \pm 0.6	28.13 \pm 1.72	76.15 \pm 3.66	3.67 \pm 0.09
DM	49	182.95 a \pm 5.59	189.42 a \pm 10.9	38.45 \pm 1.4	37.88 a \pm 2.18	106.59 a \pm 5.05	5.02 a \pm 0.26
MI	40	177.72 a \pm 5.44	205.65 a \pm 20.46	38.54 \pm 1.41	41.13 a \pm 4.09	98.02 a \pm 4.29	4.87 a 0.24
DMMI	46	164.35 ab \pm 5.14	174.6 \pm 8.16	35.21 a \pm 1.43	34.05 \pm 1.8	95.06 a \pm 4.47	4.95 a 0.24

a significant difference ($P < 0.05$) with control group.
b significant difference ($P < 0.05$) with DM group.



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.

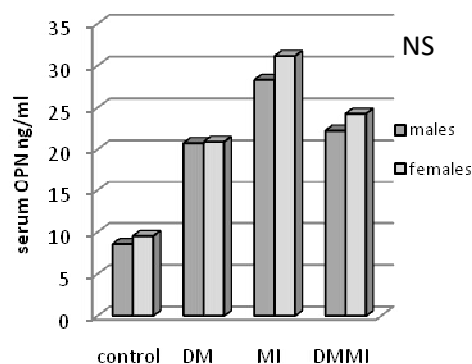


Figure 4: Serum OPN (ng/ml) between males and females.

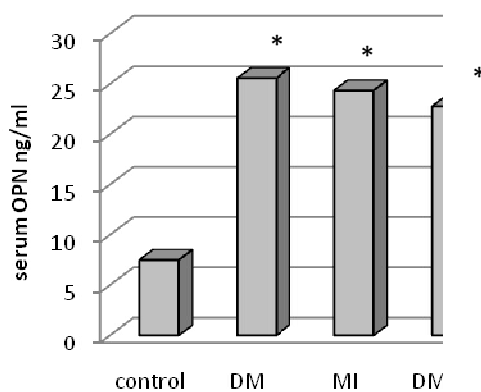


Figure 5: Serum OPN (ng/ml) in sub (40-50y).

NS: no significant differences.

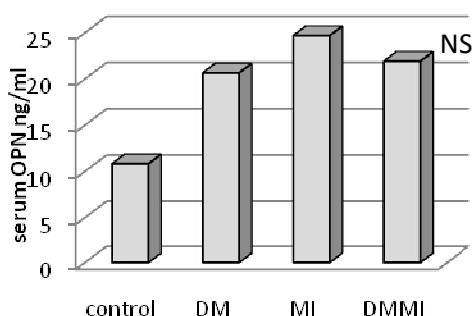


Figure 6 : Serum OPN (ng/ml) in the age range (50-60)y.

NS: no significant differences.

* significant difference ($P < 0.05$) with control

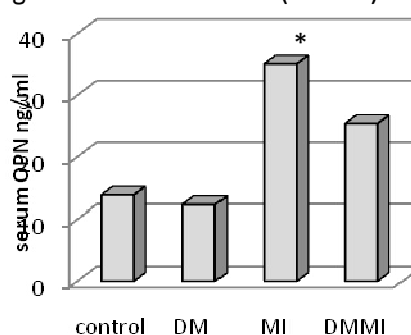


Figure 7: Serum OPN (ng/ml) in the age range (>60)y.

* significant difference ($P < 0.05$) with DM group.

Table 3: Serum OPN, a comparison in age ranges in each study group.

Group	Age range	n.	Mean \pm Std. Error
Control	40-50	29	7.49 \pm 1.4
	50-60	10	10.82 \pm 2.7
	>60	6	13.83 \pm 1.8
DM	40-50	20	25.57 \pm 5.87
	50-60	17	20.65 \pm 4.4
	>60	12	12.25 \pm 3.8 *
MI	40-50	13	24.34 \pm 3.2
	50-60	8	24.69 \pm 5.7
	>60	19	35.09 \pm 3.8
DMMI	40-50	12	22.78 \pm 3.6
	50-60	19	21.92 \pm 3.9
	>60	15	25.38 \pm 5.8

* significant difference between (40-50) group and (>60)

Levels of serum OPN increased in all patients significantly in comparing with the control group, MI group was higher significantly than DM group. The expression of OPN is highly induced by glucose, higher serum OPN is found in diabetic patients, suggesting that it may be involved in the accelerated atherosclerosis seen in diabetes. (OPN) is a pleiotropic cytokine that is a common and relevant component of many acute and chronic vascular or endothelial responses to injury characterized by inflammation and or fibrosis, including arterial neointimal hyperplasia, atherosclerosis, aortic stenosis (Lorenzen *et al.*, 2010; Rangaswami *et al.*, 2006; Rangaswami *et al.*, 2005; Rangaswami *et al.*, 2004). as well as the vascular damage which accompanies diabetes [Riedl *et al.*, 2008]. OPN, together with other inflammatory cytokines such as IL-6, has been implicated in mediating the vascular effects of angiotensin II (Ang II) and aldosterone in hypertension (Marchesi *et al.*, 2008).

OPN has been implicated as a key factor in the development of atherosclerosis (Ikeda *et al.*, 1993; Giachelli *et al.*, 1998; Isoda *et al.*, 2003). Plasma levels of OPN are elevated in essential hypertension, in patients with coronary artery disease (CAD), and restenosis (Kurata *et al.*, 2006; Ohmori *et al.*, 2003; Kato *et al.*, 2006).

Experimental studies have shown a reduction of atherosclerotic plaques through angiotensin receptor blockage mediated by osteopontin (Arishiro *et al.*, 2007). Lorenzen *et al.* (2010) show that elevated OPN levels in hypertensive patients correlate with adhesion molecules and inflammation markers. It was further demonstrated that OPN is involved in Th1-mediated cellular immunity. Other cell types of the immune response are also under the control of OPN. In this regard, OPN is a mast cell mediator and enhances mast cell response to antigens (Nagasaka *et al.*, 2008). A role for OPN as a critical mediator of inflammation was also described, especially in macrophage tissue infiltration (Giachelli *et al.*, 1998), migration, and activation and in neutrophil recruitment and migration (Koh *et al.*, 2007). OPN regulates the production of inflammatory cytokines and nitric oxide in macrophages (reviewed in (Singh *et al.*, 2007). In addition, OPN-deficient mice exhibited decreased markers of inflammation, definitively demonstrating the role of this cytokine (Nomiyama *et al.*, 2007; Mazière *et al.*, 2010).

In the cardiovascular system, OPN is one of the major regulators of chronic inflammation and vascular disease (Scatena *et al.*, 2007). It was reported that OPN expression increases in the heart during hypertrophy (Xie *et al.*, 2004) and heart failure (Soejima *et al.*, 2007). Its role in cardiac fibrosis and remodeling was also described (Matsui *et al.*, 2004). OPN is expressed in smooth muscle-derived foam cells in human atherosclerotic lesions of the aorta, and the OPN plasma level has been correlated with carotid atherosclerosis (Kurata *et al.*, 2006). It was demonstrated in an OPN-deficient mouse model that OPN has a promoting effect in atherosclerosis (Matsui *et al.*, 2003). Conversely, over expression of OPN in lymphoid tissues of transgenic mice is associated with an increase in aortic lesion size. Consistently, tissue-wide over expression of OPN in fat-fed mice resulted in fatty and mononuclear cell-rich lesion formation (Isoda *et al.*, 2003).

In the current study results, serum OPN elevated in diabetic patients, this agreed with Kiefer *et al.*, 2010 who report an increase of OPN in diabetics and there is a specific local role of OPN in obese adipose tissue. Therefore, OPN could be a critical regulator in obesity induced adipose tissue inflammation and insulin resistance. Later the same group found that the neutralization of OPN inhibits obesity-induced inflammation and insulin resistance (Kiefer *et al.*, 2010).

Also the results showed increase OPN in MI patients. The acute coronary syndromes are typically initiated by an unpredictable and abrupt conversion of a stable atherosclerotic plaque to

an unstable and potentially life-threatening atherothrombotic lesion through rupture, superficial erosion, ulceration, fissuring, or deep hemorrhage. In most instances, the plaque change causes the formation of a superimposed thrombus that partially or completely occludes the affected artery (Fuster *et al.*, 2005). These acute events are often associated with intralesional inflammation, which mediates the initiation, progression, and acute complications of atherosclerosis (Ross, 1999)

Cho *et al.* (2009) concluded that OPN is highly expressed at sites with atherosclerotic plaques, especially those associated with macrophages and foam cells. However, the role of OPN in vascular calcification, which is closely related to chronic and active inflammation, is that of a negative regulator because it is an inhibitor of calcification and an active inducer of decalcification. (Cho *et al.*, 2009).

OPN may play a relevant role, its expression in the heart is potently regulated by Ang II and OPN is an important factor controlling cardiac fibroblast growth, adhesion to extracellular matrix, and collagen gel contraction. Several studies suggest that Ang II induces a dramatic increase in cardiac OPN expression (Murry *et al.*, 1994). OPN may modulate Ang II mediated fibrotic response and collagen accumulation in tissue injury, possibly as a result of alterations in cell proliferation and adhesion (Collins *et al.*, 2004).

OPN down- regulates plaque calcification and may promote plaque instability. Aside from direct effects, OPN may facilitate plaque destabilization indirectly. Available data suggest that OPN may induce matrix metalloproteinases release and angiogenesis within the atherosclerotic plaque leading to fibrous cap degradation and hemorrhage, respectively (Isoda *et al.*, 2003; Kadoglou *et al.*, 2005; Tureyen *et al.*, 2006).

Wang *et al.* (2009). Study the OPN and hsCRP levels before surgery in consecutive patients scheduled for mitral valve replacement. Cardioprotective effects were assessed by creatine kinase MB (CK-MB) and cardiac troponin T (cTnT) leakage postoperatively. The protective effects of OPN on neonatal cardiomyocytes against anoxia– reoxygenation-induced injury. They found that patients with higher plasma OPN levels had more activated extent of transcription factors, higher expression of effector proteins, and better cardioprotective effects, assessed by CK-MB and cTnT.

OPN plasma concentrations decreased after coronary artery bypass grafting CABG surgery in the early post-operative period. (Sbarouni *et al.*, 2012).

In another study Wang *et al.* (2012) reported that increased OPN mRNA expressions is coincident to the upregulated IL-6, monocyte chemoattractant protein 1 in vascular smooth muscle cells medium as preinflammatory properties at some cases like nicotine exposure.

In other aspects of vascular diseases, Yan *et al.* (2010) found that plasma OPN level, parallels with the severity of nephropathy and CAD in diabetes, suggesting that an increased plasma OPN level may be used as an indicator for screening diabetic vasculopathy. Also Rosenberg *et al.* (2012) believed that OPN could serve as a new biomarker in patients with pulmonary hypertension that may complement existing diagnostic tools in order to further improve risk stratification in this patient population.

In the current study, there was no significant difference in serum OPN found between males and females. There is no previous studies confirmed an gender effect on OPN. As proinflammatory marker, OPN may not affected with sex hormones.

According to age range, there was a significant increase in OPN levels in all patients in the age range 40-50 when compared with control subjects in this age range, but OPN levels didn't differ significantly among study groups aged 51-60 years. In elderly (>60 years) MI patients

show significant high levels of OPN in comparison with elderly DM patients. Kadoglou et al (2008) and Chen *et al.*(2009) found that Elevated plasma OPN levels were positively correlated with increasing age.

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