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Original Research Article

The Relationship between Oxidative Stress and Osteopontin Levelsin Diabetic Patient with Acute Coronary Syndrome

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Abstract

This study was performed to determine osteopontin levels in sera of diabetic patients having acute coronary syndrome and finding the relationship betweenosteopontin and oxidative stress in the corresponding patients .A case –controlled study for 60Iraqi diabetic patients (33male and 27 female)with acute coronary syndrome (STEMI and NSTEMI) and 20 diabetic patients without myocardial infraction were selectedforthisstudy. Besides,Forty healthy subjects served as control group.The serum levels of Osteopontin (OPN),HbA1c,SOD,CAT and Malondialdehyde (MDA) were determined for the corresponding patients.The result obtained were compared with those 40 subject(20male and 20 female) who are apparently healthy (control group).All the selected patients were non -smokers,non –hypertensive and have no inflammatory disease which may affected the results of the measured parameters. Osteopontin levels were significantly increasedin patientswith DM,STEMI and NSTEMIcompared to the controls. The levels of MDA and HbA1Cin the patients were significantlyhigher compared to the controls whileSOD ,CATwere significantly lower compared to the controls, and a significant positive was found between osteopontin,HbA1C and MDA and negative correlation was found with SOD and CAT levels.The increase Osteopentin levels inrelevance to oxidative stress and HbA1C level may indicate the role of hyperglycemia in the induction of this acute inflammatory marker which can be considered as a prognostic indicator of diabetic complications especially diabetic patients with MI.

Key words: OPN,MDA,DM,STEMI,NSTEMI,SOD, CAT and HbA1C,MI.

العلاقة بين الجهد التأكسدي وبروتين الاوستيوبونوتين في مرضى السكري الذين يعانون من مرض متلازم الانسداد الحاد

الخلاصة

اجريت الدراسة على ستون مريض عراقي الذين يعانون من السكري اربعين منهم يعانون من متلازمة الشريان التاجي الحاد (STEMI و NSTEMI) وعشرون مريض يعانون السكري فقط. الى جانب ذلك عملت أربعون أصحاء كمجموعة سيطرة(كونترول). تم قياس مستويات المصل من اوستيوبونوتين OPN، HbA1c، SOD، CAT,MDA, للمرضى وتمت مقارنة النتائج التي تم الحصول عليها مع مجموعه السيطرة اربعون (عشرون ذكر وعشرون إناث) الذين يتمتعون بصحة جيدة (كونترول). وكان جميع المرضى المختارين لهذه الدراسة غير مدخنين ولا يعانون من مرض ارتفاع ضغط الدم وكذلك أي حاله التهاب والتي قد تؤثر على نتائج القياس.

Introduction

Diabetes is a metabolic disease associated with hyperglycemia which is resulting from defects in insulin function , insulin level, or both[1]. Chronic hyperglycemia may associated with long-term damage, dysfunction, and failure of different organs, such as; eyes, kidneys, nerves, heart, and blood vessels[2]. There are two major forms of diabetes, type 1diabetes mellitus (decreased of insulin production) and type 2diabetes (impaired natural response to insulin action and Beta-cell dysfunction). Both types lead to irregular hyperglycemia, massive urine production, thirst, fluid intake, blurred vision, weight loss, lethargy, and changes inmetabolism. T2DM were a complex heterogeneous groups of metabolic conditions lead to increase of blood glucose levels due to impaired in insulin action and/or insulin secretion[3].UsingHbA1Cpercentage to predict the progression ofdiabetes and demonstrationthat a strong, continuousassociation between HbA1C and subsequentcomplications[4].HbA1cisa molecule that isformed via a non-enzymatic glycationprocess, and is considered a reliable indicator ofthe glycemic status of the previous 3 months[5].Diabetes wasshown to contribute to the higher incidence and worse prognosis after myocardial infarction[6].Osteopontin (OPN) was secreted as calcium-binding molecule that has been implicated in both physiological and pathological events including cellular immunity, inflammation, tumor progression, and cellsurvival[7,8]. Recently OPN may exert important cardiovascular effects mayconsistent with its role in other ogenesis, OPN has been also identified as a component of human atherosclerotic lesions where it is produced by cells of the immunological cells, endothelial, and vascular smooth muscle cells[9,10]. In a specific way, OPN wasfound to be highly up regulation in symptomatic human carotid atherosclerosis in diabetic patients,and in calcified plaques[11].This protein plays an important role during both acute and chronic inflammation.Osteopontin(OPN)is upregulated in tissues during several pathological process including athero-sclerosis, valve stenosis, myocardial infarction and rheumatic arthritis[12]. The putative functions of Osteopontin are bone mineralization, regulation of immune cell function, inhibition of calcification, control of tumor cell phenotype and cell activation [13]. It is found to be expressed in smooth muscle cells (SMC)in the atherosclerotic lesion in angiogenic endothelial cells and macrophages [14].Antioxidants, are produced either endogenously or are derived from dietary sources. Oxidative stress is defined as the increased generationof free radicals and/or the impaired compensatory response of endogenous antioxidant defenses, both observed in type2 diabetes [15,16].Malondialdehydeis considered as an index of oxidative stress,it is end product of peroxidation ofpoly unsaturated fatty acid (PUFA) and related esters [17]. Malondialdehyde is a biological marker of lipid peroxidation caused by oxidative stress [18].It reacts with amino groups and with any ketones or aldehydes from other sources, for example, attached sugars or glycation products [19].Some studies indicated the role of osteopontin in diabetic patients and its complications but there is no previous study indicated the relationship between oxidative stress in diabetic in this study we tried to the relationship of this inflammatory marker with oxidative stress and chronic hyperglycemia .In this study ,we tried to clarify such relationship and elucidate those changes in diabetic patients with acute coronary syndrome .

Materials and Methods

Sixty diabetic patients who were admitted to coronary care units (CCU) in Merjan teaching hospital in Hilla city were selected for this study .Those patient

were subdivided into three groups as following

1-Twenty diabetic patients with no complications.

2-Twenty diabetic patients with ST segment elevation acute myocardial infraction and ST segment elevation (STEMI).

3-Twenty diabetic patients withoutST segment elevation acute myocardial infraction and non ST segment elevation (NSTEMI).

Forty subjects who are apparently healthy were chosen as control group in this study.

Venous blood samples (5ml)were withdrawn form patients and control group using disposable syringe in the sitting position.Another blood samples were obtained from the patients for determination of HbA1Cusing Fast Ions – Exchange Resin Separation Method. Serum level Osteopontin and catalasewere determined in different subgroups using sandwich ELISA technique.While serum Superoxide dismutase (SOD)was determined using Competitive ELISA technique., Serum Malondialdehyde (MDA) concentration was determined according to modified procedure described by Guidet. and Shah[38].The results were expressed as a (Mean± SD) by using descriptive analysis. Student t-test and linear regression analysis wereused for the evaluation of the data. Statistical analyses were performed with SPSSversion 20software.A p-value($P<0.05$) was considered to be statistically significant.

Results

1.Changes in serum Osteopontin(OPN)levels in different groups

The results in table (1)-a showed that serum osteopontin was significantly higher in patients group compared with control group as shown in table .Also there were significant differences between STEMI and NSTEMI patients in comparison with diabetic patients with no myocardial complication.

Table 1 a: Serum level of OPN in all groups of patient and comparison with control group

Parameter	Groups		NO.	M±SD	Range
OPN(ng/ml)	control		40	5.18±1.26	3.1-6.3
	DM		20	8.3±3.73	5.9-11.5
	ACS	STEMI+DM	20	12.79±5.25	7.2-19.2
		NSTEMI+DM	20	9.42 ± 3.93	6.5-18.4

Table 1 b: Statistical significance of OPN change among different diabetic groups and control

OPN	Control	DM	STEMI	NSTEMI
Control				
DM	0.008			
STEMI	0.001	0.001		
NSTEMI	0.001	0.03	0.005	

2.Changes in serum Malondialdehyde(MDA) levels in different groups.

The results in tables (2)-a showed that serum Malondialdehyde levels was significantly higher in patients group compared with control group as shown in table. The result also revealeda significant changes among different subgroups .

Table 2 a: Serum level of in MDA all groups of patient and comparison with control group

Parameter	Groups		NO.	M±SD	Range
MDA(μM)	control		40	2.75±0.21	2.1-3.5
	DM		20	4.41± 0.65	3.2-5.1
	ACS	STEMI+DM	20	6.42±0.95	5.3-7.0
		NSTEMI+DM	20	٥.٧٦ ±0.74	4.5-6.2

Table 2 b: Statistical significance of MDA change among different diabetic groups and control

MDA	Control	DM	STEMI	NSTEMI
Control				
DM	0.001			
STEMI	0.001	0.0001		
NSTEMI	0.001	0.0001	0.001	

3. Changes in serum SOD levels in different groups

The results in (3)-a showed that serum Superoxide dismutase(SOD) levels was significantly lower in patients group compared with control group as shown in table.Also there were significant differences between STEMI and NSTEMI patients in comparison with diabetic patients with no myocardial complication.

Table 3 a: Serum level of in SOD all groups of patient and comparison with control group

Parameter	Groups		NO.	M±SD	Range
SOD(pg/ml)	control		40	2241.65±438.08	2078.2-3763.5
	DM		20	1316.0±154.2	1033.4-1652.9
	ACS	STEMI+DM	20	1054.95±215.7	789.6-1098.7
		NSTEMI+DM	20	1193.05±206.59	889.1-1100.4

Table 3 b: Statistical significance of SOD change among different diabetic groups and control

SOD	Control	DM	STEMI	NSTEMI
Control				
DM	0.001			
STEMI	0.001	0.002		
NSTEMI	0.001	0.01	0.05	

4. Changes in serum Catalase(CAT) levels in different groups

The results in table (4)-a: showed that serum Catalase(CAT) levels was significantly lower in patients group compared with control group as shown in table.The result also revealeda significant changes among different subgroups .

Table 4 a: Serum level of in CAT all groups of patient and comparison with control group

Parameter	Groups		NO.	M±SD	Range
CAT(pg/ml)	control		40	121.02±37.01	72.5-189.3
	DM		20	67.43±18.98	55.3-99.9
	ACS	STEMI+DM	20	52.71±14.79	32.1-75.3
		NSTEMI+DM	20	66.46±29.63	44.4-85.7

Table 4 b: Statistical significance of CAT change among different diabetic groups and control

CAT	Control	DM	STEMI	NSTEMI
Control				
DM	**0.001**			
STEMI	**0.001**	**0.02**		
NSTEMI	**0.001**	**0.05**	**0.02**	

5.Changes in serum HbA1C levels in different groups.

The results in table (5)-a showed that HbA1C levels significantly higher in patients group compared with control group as shown in table The result also revealeda significant changes among different subgroups .

Table 5 a: Serum level of in HbA1C all groups of patient and comparison with control group

parameter	Groups		NO.	M±SD	Range
HbA1C%	control		40	4.570±0.25	4.3-5.1
	DM		20	٧.٣٤±١.٠٣	6.1-9.2
	ACS	STEMI+DM	20	10.16±3.93	8.0-12.2
		NSTEMI+DM	20	8.6± 2.39	7.1-10.5

Table 5 b: Statistical significant of HbA1C change among different diabetic groups and control

HbA1C	Control	DM	STEMI	NSTEMI
Control				
DM	0.001			
STEMI	0.001	0.05		
NSTEMI	0.001	0.05	0.01	

6. Correlations between the measured parameters in patients with MI

6.1. Correlation among studied parameter

Table (6) showed the correlation among the measured parameters in diabetic patient with acute coronary syndromes(both STEMI and NSTEMI).

Table 6 : Correlation between all measured parameter study in MI patients (STEMI and NSTEMI)

Correlations							
		OPN	**MDA**	**SOD**	**CAT**	**HbA1C**	**BMI**
OPN ng/ml	**Pearson Correlation**	**1**					
MDA μM	Pearson Correlation	.414**	1				
		P =0.008					
SOD pg/ml	Pearson Correlation	-.461**	-.362*	1			
		p=0.003	P=0.02				
CAT pg/ml	Pearson Correlation	-.444**	-.512**	.347*	1		
			P=0.01				
HbA1C%	Pearson Correlation	.336*	.364*	-.339*	-.324*	1	
		p.=0.03	P=0.03				
BMI Kg/m^2	Pearson Correlation	.217	.270	-.169	-.278	.211	1
**. Correlation is significant at the 0.01 level							
*. Correlation is significant at the 0.05 level							

7. Correlation between osteopontin and measured parameters in diabetic patients with MI.

7.1. Correlation between levels of osteopontin and MDA, SOD and HbA1C indiabetic MI patient.

The correlation between the levels of osteopontin HbA1C,SOD and MDA in diabetic MI patients is represented in the table(6).

According to this statistical analysis there is a positive significant correlation between the levels of OPN versus HbA1C and levels of OPN versus MDA in diabetic MI patients is represented in figure (1) and (2) respectively and a negative significant correlation between the levels of OPN versusSODisrepresented in figure (3)

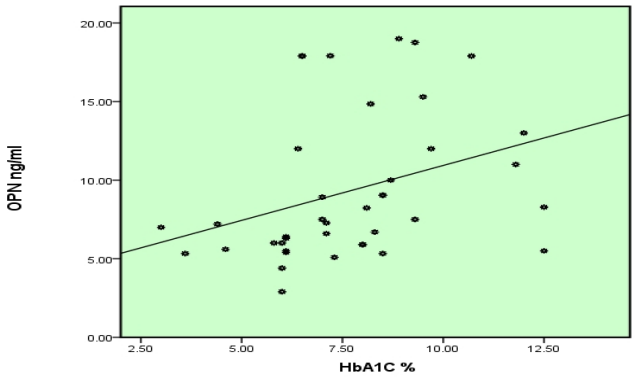

Figure 1 : Relationship the level of OPN and HbA1C in diabetic MI patients

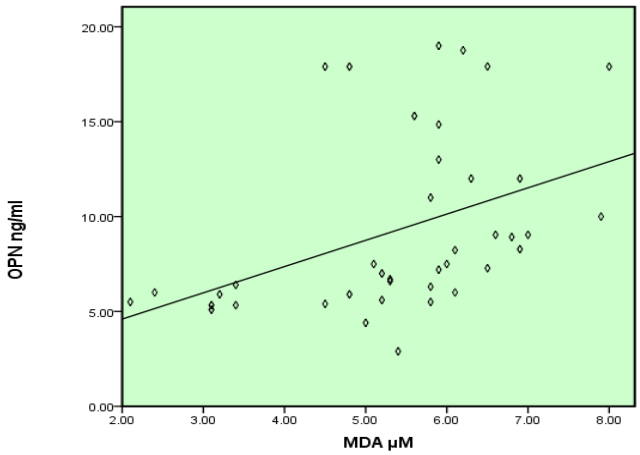

Figure 2 : Relationship the levels of OPN and MDA in diabetic MI patients

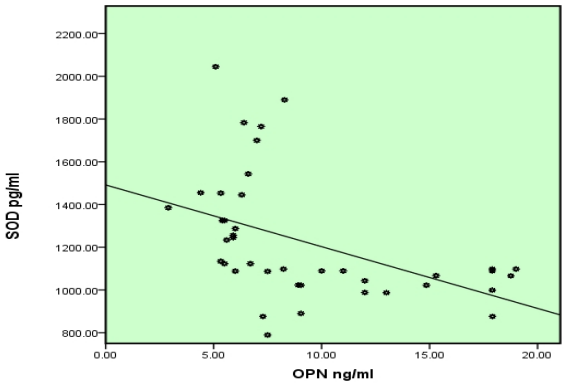

Figure 3 : Relationship the levels of OPN and SOD in diabetic MI patients

8. Correlation between levels of Malondialdehyde MDA and SOD ,CAT and HbA1Cindiabetic MI patient.

The correlation between the level of MDA versus SOD,CAT and HbA1C in diabetic MI patients is represented in the table(6).

According to this statistical analysis there is a negative significant correlation between the levels of MDA versus SOD and levels of MDA versus CAT in diabetic MI patients is represented in figure (4) and (5) respectively and a positive significant correlation between the levels of MDA versus HbA1Cis represented in figure (6).

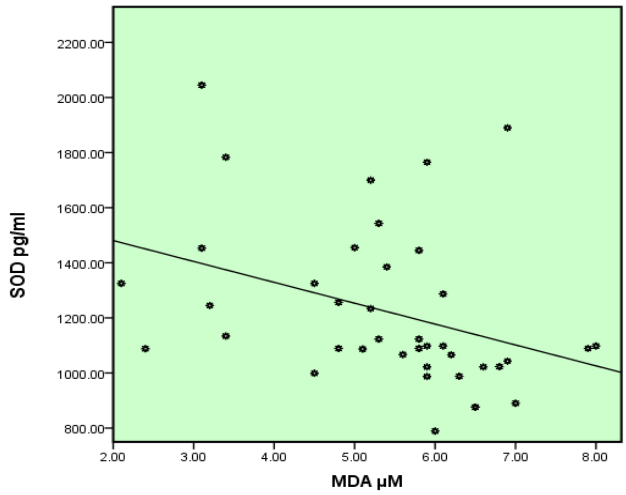

Figure 4 : Relationship of levels of MDA and SOD in diabetic MI patients

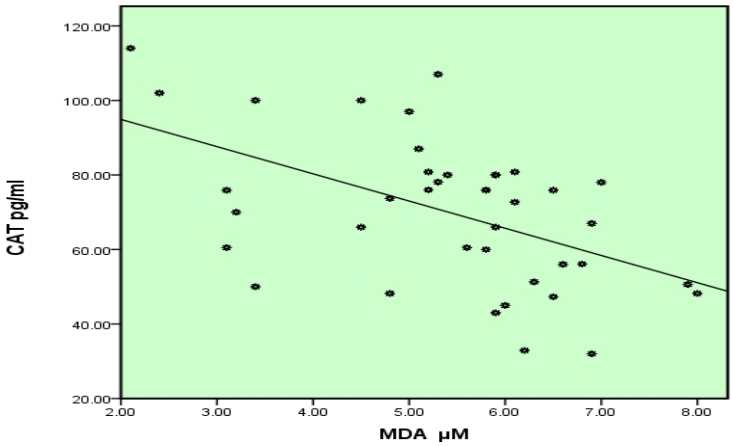

Figure 5 : Relationship of levels of MDA versus CAT in diabetic MI patients

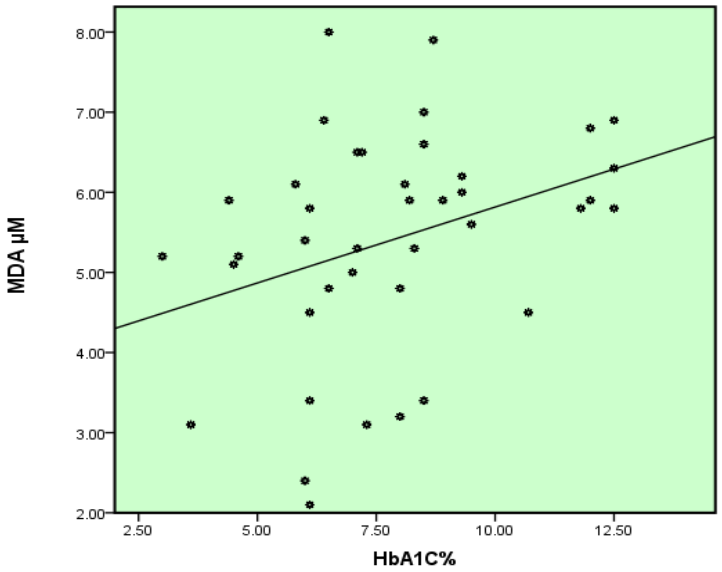

Figure 6 : Relationship levels of MDA versus HbA1C in diabetic MI patients

Discussion

It is well known that the poor long term control of hyperglycemia can lead to a wide range of microvascular (e.g. renal, retinal) and macrovascular(primarily cardio-vascular)complications [20].Atherosclerotic changes in large blood vessels is the major causes of morbidity and mortality of 80 patients with type 2 DM[21]. Since cardiovascular disease is a symptomatic in diabetic patients and its clinical manifestation occurs in advanced stage it becomes necessary to detect some cardiovascular or atherosclerotic markers in diabetic patients for the beneficial diagnosis ,better management and prevention of cardiovascular disease.

Cardiovascular markers must be related to other different biochemical parameters to clarify the association between them in order to get some confirmatory evidence for the a etiology and the prognosis of cardiovascular disease[22].

The results in table (1)-a show that a significant increase in OPN levels in diabetic patients complications.This assists in prognosis of the disease and monitoring the staging the clinical changes in acute myocardial in fraction (both STEMI and NSTEMI). This result is a good agreement with the study done by Xie*et al.,*2001 who demonstrated that the increased in the OPN expression in cardiac myositis and cardiac fibroblasts appears to be due to the activation of extracellular related kinases and production of reactive oxygen species (ROS).

Another study revealed that the increase in OPN can be attributed to its increased expression by many cell types especially macrophages in response to biological stressors,reported that OPN expression play are important role in macrophage and vascular smooth muscle cell migration besides its relationship to vascular remodelling and the development of atherosclerosis[24].

Some investigators by demonstrated that the OPN has been identified as a prominent component of human atherosclerotic lesions where it is synthesized by cells of the monocyte/ macrophage lineage and to a lesser extent by endothelial and vascular smooth muscle cells. Specifically, OPN was found to be highly up- regulated in symptomatic human atherosclerosis in diabetic patient[25,26].

It is noteworthy to mention that the significant increase in OPN levels in STEMI and NSTEMI diabetic patients in

comparison with its mean value in diabetic patients with no complications gives a special consideration for this inflammatory factor in the prognosis of the disease ,The results are in good with those reported demonstrate a significant elevation in osteopontin levels in patients with angina pectoris and STEMI patients and NSTEMI patients[27]. Also,the significant changes between STEMI and NSTEMI subgroups gives a good indications for the use of OPN as a diagnosis marker besides ECG investigation.

Table (6) show significant positive correlation between MDA and HbA1CThis indicates that oxidative stress (is induced by chronic hyperglycemia) which lead consequently to increase in acute inflammatory marker [28].

Oxidative stress is produced due to imbalance between antioxidant status and different types of oxidative.MDA is considered as a good indicator of lipid peroxidation that may result from increased free radical generation and suppressed scavenging mechanism. Our results are in good agreement with those obtained by Morrow *et al*., who found a direct relationship between MDA and the severity of acute coronary syndromes.Also, there was a good extent of similarity with those obtained by AL-Rubaye who reported the association between oxidative stress and some biomarkers of cardiovascular disease.

There was a highly significant negative correlation between SOD and HbA1C .This gives a confirmatory evidence for the role of this antioxidant enzyme in prevention of cardiovascular disease. the oxidative stress is defined as the increased generation of free radicals and/or the impaired compensatory response of endogenous antioxidant defenses, both observed in type2 diabetes. Oxidative stress is a pathologic condition resulting from either increased production of free radicals or decreased levels of antioxidants.The same trend was observed with catalase but to a lesser extent. This indicates that SOD play the major role as an antioxidant enzyme in diabetic patients .Previous studies indicted to the role of those mentioned antioxidant enzymes in the a etiology of acute coronary syndrome in patients with diabetic mellitus[32,33].

Many previous studies considered these enzymes as a good markers for cardiovascular injury in both diabetic and non–diabetic patients.Both enzymes participated in the destruction of free radicals and elimination of its harmful effects SOD convert superoxide anion into hydrogen peroxide , then catalase reduced hydrogen peroxide to water.Catalase was shown to play the predominate role in controlling the concentration of H_2O_2 and consequently protects pancreatic β-cell from damage by H_2O[34- 37].

Also,those investigators concluded thatlowering of SOD and catalase levels lead to increased risk for diabetes mellitus and its complications.

The results of this study insist on the role of hyperglycemia in the induction of oxidative stress which consequently lead to elevation of OPN levels.Therefore continuous monitoring of HbA1C and current determination of OPN in diabetic patients is necessary to assess the extent of oxidative stress which can be relieved by the intake of nutritional antioxidant preparations.

The results also indicated the importance of determination of serum OPN levels in diabetic patients with cardiovascular complications as a confirmatory test beside electro cardio-graphic results.

Conclusion

1. Myocardial infraction (both STEMI and NSTEMI)is accompanied by increase in osteopontin level in diabetic patients which can be also used as a diagnostic marker in those patients.
2. The increase in osteopontin is caused by oxidative stress which is induced by chronic hyperglycemia.
3. The direct correlation among HbA1C oxidative stress,osteopontin and antioxidant enzymes gives good indicator for interrelationship of those parameters in

both pathogenesis and prognosis of the disease

References

1. Cefalu WT., George Bakris G., Blonde L., Andrew F., Boulton J.M., *et al*.Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2014; 37(1).
2. Maritim A. C,. Sanders R. A, and Watkins J. B III2. Diabetes, Oxidative Stress, and Antioxidants. A Review JBiochem Molecular Toxicology.2003; 17(1).
3. Lin Y. and Sun Z. Current views on type 2 diabetes Journal of Endocrinology .2010; 204: 1–11.
4. American Diabetes Association. Standards of Medical Care in Diabetesd 2013.Diabetes Care. 2013; 36(1).
5. Giniş Z., Öztürk g. and Sirmali R. The role of HbA1c as a screening and diagnostic test for diabetes mellitus in Ankara. Turk J Med Sci.2012; 42 (2): 1430-1436.
6. Thiele H., Wohrle J.,Hambrecht R.,Rittger H.,Birkemeyer R., Lauer B.,*etal* .Intracoronary versus intravenous bolus abciximab during primary percutaneous coronary intervention in patients with acute ST- elevation myocardial infarction :a randomised trial. Lancet 2012;379:923–31.
7. Denhardt DT., Noda M ., O'Regan AW., Pavlin D., Berman JS. Osteopontin as ameans to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Invest 2001;107:1055–1061.
8. Kipari T., Ophascharoensuk V., Wesson JA., Johnson R., Hughes J.Osteopontin-a molecule for all seasons. QJM 2002;95:3–13.
9. Golledge J., McCann M., Mangan S., Lam A., Karan M etal.,Osteoprotegerin and osteopontin are expressed at high concentrations within symptomatic carotid atherosclerosis. Stroke 2004;35:1636–1641.
10. Chen NX.and Moe SM. Arterial calcification in diabetes. CurrDiab Rep.2003;3:28-32.
11. Strom A., Franzen A., Wangnerud C., Knutsson AK., Heinegard D., Hultgardh-Nilsson A.etal., Altered vascular remodeling in osteopontin-deficient atherosclerotic mice. J Vasc Res.2004;41:314–322.
12. GursoyYA. andAlagoz S. Ankara E.—itimVeAratirma Hastanesi III. Dahiliye Klinij, Ankara. Osteopontin: A multifunctional molecule, J Med Medical Sci. 2010; 1(3):055-060.
13. MazzaliM., Kipari T.and Ophascharoensuk V. Osteopontin- a molecule for all seasons. Q.J. Med. 2002; 95:3-13.
14. Scetana M. And Guachelli C.M.Osteopontin: A multifactorial molecule regulating chronic inflammation and vascular disease. Arteroscler.ThrombVasc Biol. 2007; 27:2302-2309.
15. Cusi K. Role of insulin resistance and lipotoxicity in non-alcoholic steatohepatitis. Clin Liver Dis 2009, 13(4):545–563.
16. Wild SH., Roglic G., Green A., Sicree R, King H.,*etal*. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27(10):2569.
17. Esteghamati A., Gouya MM., Abbasi M., Delavari A., Alikhani S., Alaedini F., *et al.* Prevalence of diabetes and impaired fasting Glucose in the adultpopulation of Iran. Diabetes Care. 2008; 31(1):96.
18. Abha C. andVed C. Oxidative stress in autism. Pathophysiology. 2006; 13: 171.
19. Hung H., Tung S.and Chen I. Lipid peroxidation and oxidative status compared in workers at a bottom ash recovery plant and fly ash treatment plants. Journal of occupational Health. 2008; 50: 492 497.
20. Dailey G. Early and Intensive Therapy for Management of Hyper-glycemia and Cardiovascular Risk Factors in Patients With Type 2 Diabetes. Clinical Therapeutics.2011; 33(6): 666-678.
21. He C., Yang Z., Chu Z., Dong Z., Shao H., Deng W., Chen J., Peng L., Tang S., Xiao J., *et al.* Carotid and cerebro-

vascular disease in symptomatic patients with type 2 diabetes: assessment of prevalence and plaque morphology by dual-source computed tomography angiography. Cardiovascular Diabetology.2010; 9:91.
22. Lorenzen JM., Neunhoffer H., David S., Kielstein JT., Haller H., DaniloF.,etal. Angiotensin II receptor blocker and statins lower elevated levels of osteopontin inessential hypertension—Results from the Eutopia trial. Atherosclerosis. 2010; 209: 184–188.
23. Xie, Z., Pimental D. R., Lohan, S., Vasertriger, A., Pligavko, C., Colucci, W. S., *et al*. Regulation of angiotensin II-stimulated osteopontin expression in cardiac micro vascular endothelial cells: Role of p42/44 mitogen-activated protein kinase and reactive oxygen species. J Cell Physiol.2001; 188(1), 132-8
24. Rosenberg M., Christian Z., Manfred N., ClausJ.,*et al.*Osteopontin, a New Prognostic Biomarker in Patients With Chronic Heart Failure*, Circ Heart Fail* . 2008.
25. Sudhir P. S. , JayashreeV.G.and Nitin N., Osteopontin: A Novel Protein Molecule Indian Medical Gazette . 2012.
26. KonstantinosF., VasiliosM., Galyfos G., Fragiska S.,*etal.*Osteopontin and Osteoprotegerin as Potential Biomarkers in Abdominal Aortic Aneurysm before and after Treatment. International Scholarly Research Notices. 2014; 461239: 6.
27. Toni V.T., EminaB.T., Ksenija L., SanjaŠ.,*etal*. Plasma Levels of Osteopontin and Vascular Endothelial Growth Factor in Association with Clinical Features and Parameters of Tumor Burden in Patients with Multiple Myeloma,Hindawi Publishing Corporation Bio Med Research International Volume. 2014; 513170: 6.
28. Mawatar S., Kazuyuki S., Kaori M., and TakehikoF. Absence of Correlation Between Glycated Hemoglobin and Lipid Composition of Erythrocyte Membrane in Type 2 Diabetic Patients. 2004; 53(1): 123-127.
29. Morrow DA., Cannon CP., Jesse RL., Newby LK., Ravkilde J., Storrow AB., *et al*. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: clinical characteristics and utilization of biochemical markers in acute coronary syndromes. Clin Chem. 2007;53:552-74.
30. AL-Rubaye F.Gh. Correlation of serum Malondialdehyde Acetylaldehyde Adduct to serum Malondialdehyde as oxidative stress markers in Acute Coronary Syndrome patients. 2014 ; 4 (6):2249-555 .
31. Devaki M., Nirupama R., and Yajurvedi H.N., Reduced antioxidant status for prolonged period due to repeated stress exposure in rat,Journal of stress physiology and biochemistry.2011**;** 7 (2).
32. Huige Li., Sven Horke., and Ulrich F. Oxidative stress in vascular disease and its pharmacological prevention, Trends in Pharmacological Sciences. 2013; 34(6).
33. Maria E., JoanneM.D., Roland Stocker. Actions of ‘‘antioxidants’’ in the protection against atherosclerosis, Free Radical Biology and Medicine. 2012; 53: 863–884.
34. LeiliA., Bahareh S., RezvanB.And Hajar S. Designing a Hydrogen Peroxide Biosensor Using Catalase and Modified Electrode with Magnesium Oxide Nanoparticles. Int. J. Electro chem. Sci. 9,2014**;** 257- 271.
35. Brent L. N., Dan S., Johan H., Francis E. R., TamirG.J.,*etal*. Structure of catalase determined by Micro ED. Biochemistry, Biophysics and structural biology, e Life. 2014;3:03600.
36. Chamari M., Hassan M.,Javanbakht.and Siadat Z. Assessment of Range of Malondialdehyde in Serum and Some Anti-Oxidant Enzymes in Patients with Diabetes Type 2 with Controlled Blood Sugar In Comparison with the Diabetics with Uncontrolled Bs Advances in Environmental Biology. 2014; 8(9): 18-22.
37. Benedicta D.S., Vivian D.S., Sowmya S., Seema G.*et al* . A comparative study on oxidative stress and antioxidant status in ischemic stroke patients with and without diabetes . Indian Journal of

Clinical Biochemistry. 2008; 23 (3): 218-222.
38. Guidet B. and Shah S. Enhanced in vivo H2O2 generation by rat kidney in glycerol induced renal failure. American Journal of Physiology. 1989; 1257: 440-444.