International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 08, 2020
ISSN: 1475-7192

# The relationship between oxidative stress marker and atherosclerosis in diabetes hyperlipidemic patient

[1]Sura Abdul Kareem Esmat, [2]Hiba Ghassan Rijab, [3]Najeeb Butrus Al-sabea

***Abstract***

*Diabetes mellitus can defined as disease characterized by many metabolic abnormalities like increased in blood glucose level (hyperglycemia) and failure in either synthesis or action of insulin that synthesized in the body by β-cell of pancreas, this disease accompanied by high mortality and morbidity due to the high percentage of exposure to cardiovascular complications (CVD)***.** *Seventy five people included in this study, all from Al-Kadhmiya Teaching Hospital during period from December 2019 to February 2020, study design (case –control study). Subjects were divided into two groups: 1- Control group (non- patient group): involve 50 healthy subjects 2-Patient study group: involve 25 diabetic hyperlipidemic subjects. All patients were diagnosed with hyperglycemia have Fasting blood sugar (FBS 125 – 300 mg/dl) with age between ( 40 – 65 years) and BMI (29.8% – 33%) and suffering of dyslipidemia; while control group have (FBS 74 – 120 mg/dl) and with age between ( 40 – 65 years) and BMI ( 28% - 31%)with normal lipid profile. There was significantly increasing in mean value of HbA1c, lipid profile, CRP and glutathione peroxidase in diabetic patient group than healthy control people ($p<0.001$) We can conclude that accelerated factors of atherosclerosis in diabetic patients is hyperglycemia in addition to dyslipidemia and increasing oxidative stress that lead to production of inflammatory factors and promote inflammatory reaction with blood vessels wall.*

*Diabetic hyperlipidemic vasculature is a key mediator for atherosclerosis*

***Keywords:*** *Hyperglycemia, Dyslipidemia, Glutathione peroxidase, HbA1c, CRP*

## I. Introduction

Diabetes mellitus can defined as disease characterized by many metabolic abnormalities like increased in blood glucose level (hyperglycemia) and failure in either synthesis or action of insulin that synthesized in the body by β-cell of pancreas, this disease accompanied by high mortality and morbidity due to the high percentage of exposure to cardiovascular complications (CVD) [1].

[1] *College of pharmacy, Institution affiliation, Uruk University*
[2] *College of pharmacy, Institution affiliation, Uruk University*
[3] *College of pharmacy, Institution affiliation, Uruk University*

Mortality of diabetes link to CVD and this percentage directly increased according to the extent of glycemic control. Increasing in the blood glucose level for long time lead to macro(stroke, coronary heart disease) and micro –vascular complications (retino, neuro and nephropathy), in addition there were many factors play an important role in the morbidity of diabetes like hyperlipidemia and oxidative stress.[2]

Because of the presence of the two causative factors which are insulin resistance and hyperglycemia which considered the major cause to develop atherosclerosis. [3, 4, 5]

Reactive Oxygen Species ROS can define as very reactive chemical species with one or more unpaired electron that formed inside mitochondria as a result of many biochemical reactions. [6]Have the ability to attack and destroy cells in order to be more stable, its action can be neutralized by substances have the ability to inhibit or reduce oxidation of substances by antioxidant mechanism that act to protect body from damaging effect of ROS; any imbalance between oxidant and antioxidant mechanism lead to different diseases, thus we can use antioxidant as biomarker for oxidative stress. [7, 8, 9]

Elevation in the level of ROS in diabetes patient lead to increase production of antioxidant like Catalase, Glutathione peroxidase and Superoxide dismutase. The mechanism of formation ROS in diabetic patient because of increasing glucose level in their blood lead to glycation of protein (protein glycation) and this process occur non-enzymatically and the major product of this process is HbA1c (glycoalbumin) and this will lead to damaging vascular hemostasis, many studies observed increasing in the inflammatory mediator like interlukin-6 (IL-6) and C-reactive protein CRP; this considered major vital association between diabetes and atherosclerosis. [10, 11, 12]

Hyperlipidemia (increasing in the cholesterol and LDL which considered as atherogenic agent) and hyperglycemia with increasing oxidative stress can collectively lead to develop atherosclerosis in diabetic patient. Atherosclerosis may define as lesion formed by action local inflammatory reaction occurs in the blood vessel wall and highly induced by hyperlipidemia. [13, 14]Hyperlipidemia and hyperglycemia together make cell more susceptible to lipid peroxidation by oxidative stress, ROS will attack the vascular wall and forming high level of oxidized LDL that will accumulate and adhere to injured vessels wall forming foam cell that lead to secrete many inflammatory mediator like CRP in order to attract monocyte to the sub endothelial space to uptake the oxidized lipid; this accumulation of oxidized LDL , inflammatory cell and fibrous element will decrease the radius of arteries by deposition plaque and this will lead to alteration in the blood flow and atherosclerotic process will develop. [15, 16, 17]

## II. Subject & Method

Seventy five people included in this study, all from Al-Kadhmiya Teaching Hospital during period from December 2019 to February 2020, study design (case –control study).

Subjects were divided into two groups:

1- Control group (non- patient group): involve 50 healthy subjects

2-Patient study group: involve 25 diabetic hyperlipidemic subjects

All patients were diagnosed with hyperglycemia have Fasting blood sugar (FBS 125 – 300 mg/dl) with age between ( 40 – 65 years) and BMI (29.8% – 33%) and suffering of dyslipidemia; while control group have (FBS 74 – 120 mg/dl) and with age between ( 40 – 65 years) and BMI ( 28% - 31%)with normal lipid profile. Five milliliters of venous blood was obtained from all groups (patient and control group) then blood samples were collected in plain tubes let them at room temperature for 10-20 minutes for clotting then centrifuged at 2000-3000 rpm for approximately 20 minutes after that the supernatant collected carefully, transferred to another tube and frozen at -20°C to be analyzed, any hemolyzed samples were rejected.

All blood samples were analyzed for FBS, Glycosylated hemoglobin (HbA1c), Lipid profile, C-reactive protein (CRP as inflammatory marker), glutathione peroxidase (as oxidative stress marker).

Enzyme-linked Immunosorbent assay ELISA kit was used that involve antigen antibody reaction , the principle of this kit is double antibody sandwich one step ELISA this process was used to assay the levels of glutathione peroxidase in all samples collected. All serum samples were analyzed for quantitative in-vitro measurement of GSH-PX by using Human Glutathione Peroxidase (GSH-PX) ELISA kit manufactured by (SHANGHAI YEHUA Biological technology Co, Ltd, China).

Principle of this kit used enzyme – linked immune sorbent assay ELISA depend on biotin double antibody sandwich technology. Wells were pre-coated with Glutathione Peroxidase (GSH-PX) monoclonal antibody.

We added serum that containing GSH-PX to pre-coated wells and incubated them, after incubation add anti GSH-PX antibodies labeled with biotin to unite with streptavidin-HRP, the immune complex was formed. Any amount of unbound enzymes after incubation was removed by washing. Then chromogenic agent (substrate A and substrate B) added to change color to blue then to yellow by the action of acid.

The Procedure involves the following steps:

1-Serum samples were allowed to dissolve at room temperature, reagents and standard solution prepared (standard solution prepared by dilution from original concentration).

2-Samples, blank and standard were prepared by addition ELISA solutions, GSH-PX antibodies labeled with biotin, streptavidin- HPR , incubate for one hour to complete reaction at 37°C with gently mixing.

3-Washing solution had been prepared by dilute it 30 times with distilled water.

4-By removing the seal membrane of plate and removed liquid then shaking the rest amount, the well filled with washing solution stay for 30 second and drain, this process repeated five times.

5-For color development we added chromogenic reagent A then added chromogenic reagent B and mixed gently then allowed 10 minute for incubation at 37°C.

6-Stop solution had been added to stop the reaction, at the same time the color changed from blue to yellow.

7- Optical densities had been read within 10 minute and calculate samples concentration from standard curve.

Equipment, instruments and glass wares used in this study

1- Centrifuge (Hermile LabTechnik Co., Germany).

2- Micro titer plate reader (Stat Fax 4200, Awareness Technology, USA).

3- Micro titer plate shaker (Biotek Co., USA).

4- Micro titer plate washer (Biotek Co., USA).

5- Glass wares (test tubes, glass khan tubes, plain tubes).

6- Disposable syringe of G21 needles.

7- Disposable containers.

8- Different size micro pipette and tips.

Statistical package for social sciences version 24 (SPSS v24) used to analyze data. Continuous variables presented as means with standard deviation and discrete variables presented as numbers and percentages.

ANOVA and Kruskal Wallis tests were used as appropriate to test the significance of observed difference in means of more than two independent samples.

T test for two independent samples and Mann-Whitney test were used as appropriate to test the significance of observed difference in means of two independent samples.

Findings with P value less than 0.05 were considered significant.

## III. Result

Result found in table (1) showed that the mean value of HbA1c in diabetic patient group (9.5 ± 1.3) was higher than the mean value in non-diabetic healthy control group (5.9 ± 0.6).

Mean value of HbA1c was significantly higher in diabetic patient group than non-diabetic healthy control group ($P<0.001$) as showed in figure (1).

**Table (1) HbA1c levels in study groups**

| Variable | Study Group | Mean ± SD | P value* |
|---|---|---|---|
| HbA1C % | Control Group | 5.9 ± 0.6 | < 0.001 |
| | Diabetic patient | 9.5 ± 1.3 | |

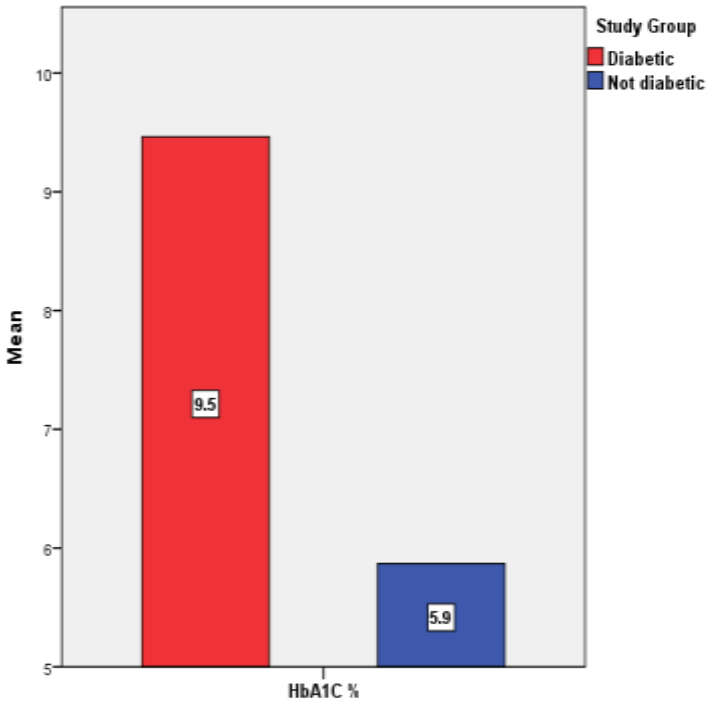


**Figure (1): HbA1c levels in study group**

Result found in table (2) showed that the mean value of lipid profile in diabetic patient group (cholesterol 226 ± 58.3 mg/dl, Triglyceride 244.6 ± 107.4 mg/dl, LDL 145.3±17.1 mg/dl) was higher than the mean value of healthy non-diabetic control group (cholesterol 170.9 ± 34.8 mg/dl, Triglyceride 136.4 ± 35.7 mg/dl, LDL 107.1 ± 24.2 mg/dl), while the mean value of HDL in diabetic patient group (36.8±9.9mg/dl )was lower than the mean value of healthy non-diabetic control group (HDL 47.9 ± 16.7 mg/dl)

Mean value of lipid profile (cholesterol, Triglyceride, LDL) was significantly higher in diabetic patient group than healthy non-diabetic control group (P<0.001) as showed in figure (2).

**Table (2) lipid profile levels in study groups**

| | Study Group | | | | |
|---|---|---|---|---|---|
| | Diabetic | | Not diabetic | | |
| Variables | Mean | SD | Mean | SD | P value |
| Cholesterol(mg/dl) | 226.0 | 58.3 | 170.9 | 34.8 | <0.001 |
| TG(mg/dl) | 244.6 | 107.4 | 136.4 | 35.7 | <0.001 |
| LDL(mg/dl) | 145.3 | 17.1 | 107.1 | 24.2 | <0.001 |
| HDL(mg/dl) | 36.8 | 9.9 | 47.9 | 16.7 | 0.003 |

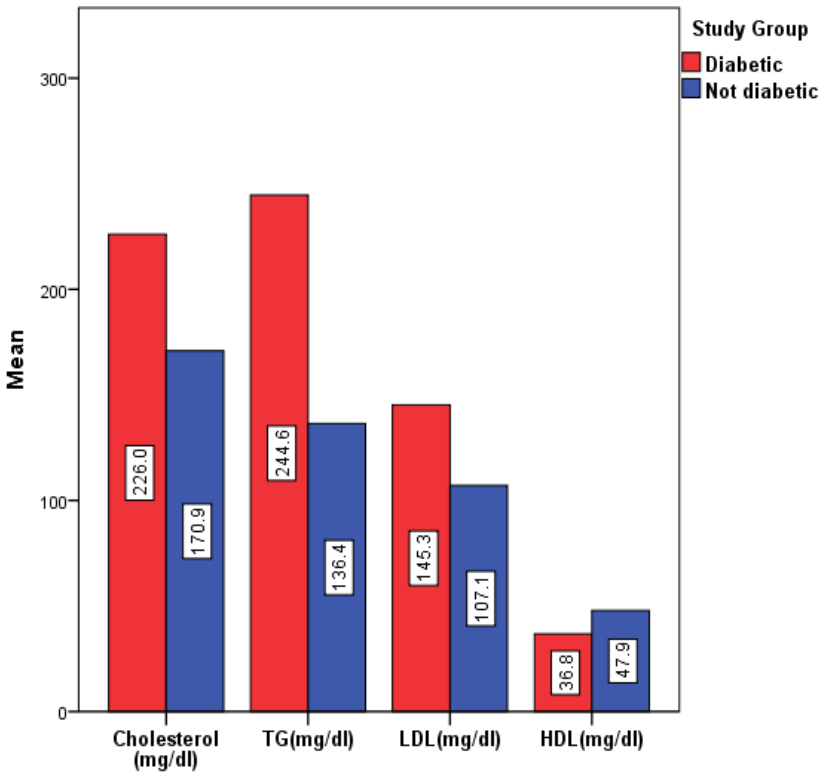


**Figure (2): lipid profile levels in study groups**

Result found in table (3) showed that the mean value of CRP in diabetic patient group (32.2 ± 12.2 mml/l) was higher than the mean value in non-diabetic healthy control group (20.4 ± 4.1mmol/l).

Mean value of CRP was significantly higher in diabetic patient group than non-diabetic healthy control group (P<0.001) as showed in figure (3).

**Table (3) CRP levels in study groups**

| Variable | Study Group | Mean ± SD | P value* |
|---|---|---|---|
| CRP mmol/l | Control Group | 20.4 ± 4.1 | < 0.001 |
| | Diabetic patient | 32.2 ±12.2 | |

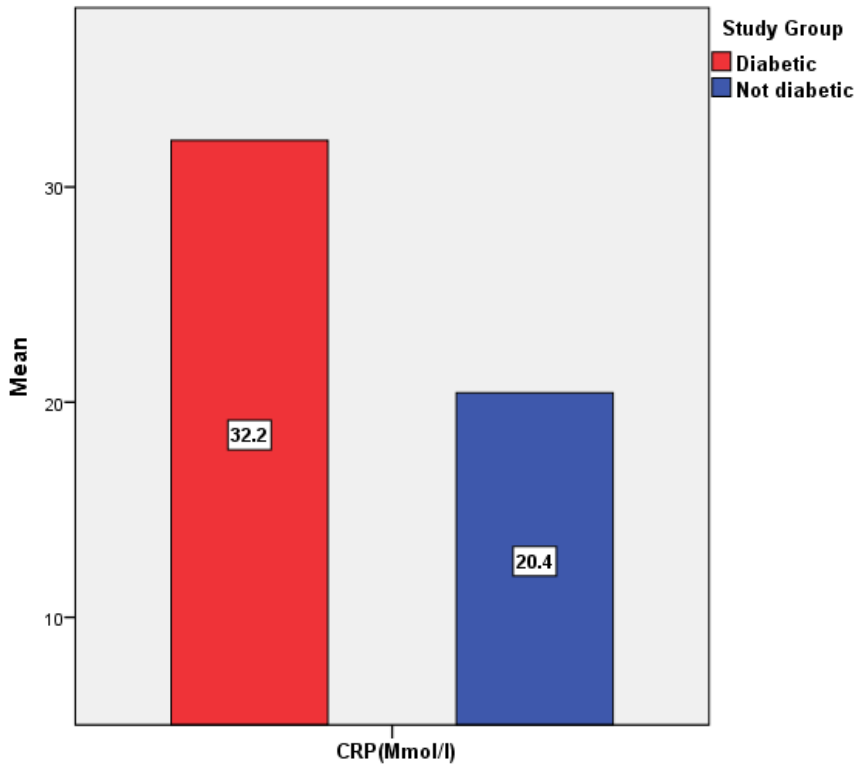


**Figure (3): CRP levels in study groups**

Result found in table (4) showed that the mean value of glutathione peroxidase in diabetic patient group (253.8 ± 56.3 U/ml) was higher than the mean value in non-diabetic healthy control group (97.6 ± 18.2 U/ml).

Mean value of glutathione peroxidase was significantly higher in diabetic patient group than non-diabetic healthy control group (P<0.001) as showed in figure (4).

**Table (4) Glutathione peroxidase levels in study groups**

| Variable | Study Group | Mean ± SD | P value* |
|---|---|---|---|
| Glutathione peroxidase U/ml | Control Group | 97.6 ± 18.2 | < 0.001 |
| | Diabetic patient | 253.8± 56.3 | |

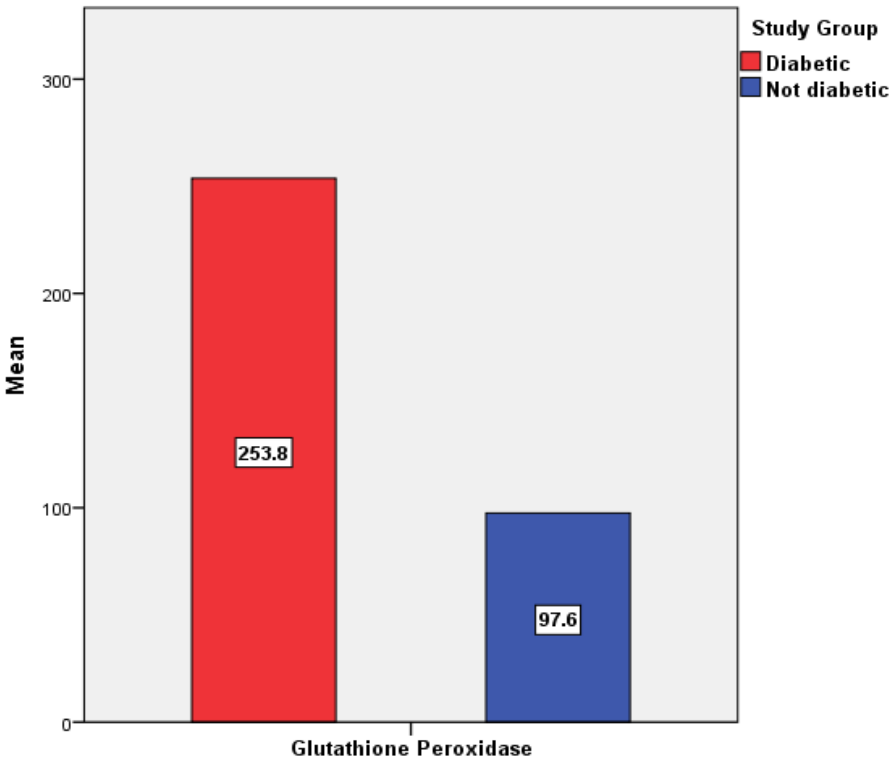


**Figure (4): Glutathione peroxidase levels in study groups**

## IV. Discussion

In present study it was found that mean level of HbA1c in diabetic patient group (9.5 ± 1.3) was higher than the mean value in non-diabetic healthy control group (5.9 ± 0.6) (P<0.001) This significant increasing in the level of HbA1c is in agreement with previous studies.

Many studies proves that hyperglycemia and poor glycemic control lead to spontaneous non enzymatic reaction which is protein glycation and lead to form of HbA1c which act as a record for glycemic control for at least 120 days. This high level of HbA1c in diabetic patient group considers the first step in beginning of complex reaction lead to formation of advanced glycation end product (AGEs) which act as causative factor of vascular complication in diabetic patient. [18, 19]

In present study it was found that mean level of lipid profile (cholesterol 226 ± 58.3 mg/dl, Triglyceride 244.6 ± 107.4 mg/dl, LDL 145.3±17.1 mg/dl) was higher than the mean value of healthy non-diabetic control group (cholesterol 170.9 ± 34.8 mg/dl, Triglyceride 136.4 ± 35.7 mg/dl, LDL 107.1 ± 24.2 mg/dl), while the mean value of HDL in diabetic patient group (36.8±9.9mg/dl )was lower than the mean value of healthy non-diabetic control group (HDL 47.9 ± 16.7 mg/dl)(P<0.001). This significant increasing in the levels of lipid profile is in agreement with previous studies.

Many studies showed that diabetes mellitus lead to disturbance in lipid profile and this will make cells very susceptible to lipid peroxidation. [20] In diabetic patients that have dyslipidemia with high level of LDL this will lead to glycation process of LDL that will promote its oxidation and lead to formation of oxidized-LDL, this high level of ox-LDL lead to attract macrophage in order to uptake ox-LDL and lead to formation of foam cell ;which considered as early step of atherosclerosis. [21, 22, 23, 24, 25, 26]

In present study it was found that mean level of CRP(32.2 ± 12.2 mml/l) was higher than the mean value in non-diabetic healthy control group (20.4 ± 4.1mmol/l) )(P<0.001). This significant increasing in the levels of CRP is in agreement with previous studies.

Many studies showed that increasing expression and production of inflammatory factors in order to increase monocyte and macrophage migration, also AGEs lead to stimulation of macrophage to release inflammatory factors and this play a major role in the cause of inflammatory reaction. [27, 28]

In present study it was found that the mean value of glutathione peroxidase in diabetic patient group (253.8 ± 56.3 U/ml) was higher than the mean value in non-diabetic healthy control group (97.6 ± 18.2 U/ml) (P<0.001). This significant increasing in the levels of glutathione peroxidase is in agreement with previous studies.

Many studies showed that the protein glycation and glucose oxidation occur by action of reactive oxygen species ROS or oxidative stress. ROS result in mitochondria due to the metabolic reactions; increasing in ROS can be measured by increasing production of anti- oxidant enzyme like glutathione peroxidase, and this high level of this enzyme indicate high level of oxidative stress that

occur in diabetic patient with poor glycemic control and high level of protein glycated HbA1c, so we can use glutathione peroxidase as oxidative stress marker as shown in our study.

So AGEs will lead to progression of atherosclerosis by change vessels wall hemostasis either by direct toxic effect to the cell by glycation and oxidation or by oxidative stress that form during formation of AGEs that involved in tissue damage.

All these changes that lead to inflammatory reaction by inflammatory cells and deposition of foam cells lead to thickening and narrowing of vascular radius and accelerate atherosclerosis. [29, 30]

## V. Conclusion

We can conclude that accelerated factors of atherosclerosis in diabetic patients is hyperglycemia in addition to dyslipidemia and increasing oxidative stress that lead to production of inflammatory factors and promote inflammatory reaction with blood vessels wall.

Diabetic hyperlipidemic vasculature is a key mediator for atherosclerosis

## References

1) Lim SS, Vos T, Flaxman AD, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions,1990–2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2224–2260.

2) Kangralkar, V.A., Patil, S.D., Bandivadekar, R.M., 2010. Oxidativestress and diabetes: a review. Int. J. Pharm. Appl. 1 (1), 38–45.

3) The Emerging Risk Factors Collaboration Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet 2010; 375: 2215-2222.

4) Bornfeldt KE, Tabas I. Insulin Resistance, Hyperglycemia, and Atherosclerosis. Cell Metab 2011; 14: 575.

5) Liang CP, Han S, Senokuchi T, Tall AR. The Macrophage at the Crossroads of Insulin Resistance and Atherosclerosis. Circ Res 2007; 100: 1546-1555.

6) Vasquez-Trincado C, Garcia-Carvajal I, Pennanen C, Parra V, Hill JA, Rothermel BA, Lavandero S. Mitochondrial dynamics, mitophagy and cardiovascular disease. J Physiol. 2016; 594: 509-5257). 7)Makino A, Scott BT, Dillmann WH. Mitochondrial fragmentation and superoxide anion production in coronary endothelial cells from a mouse model of type 1 diabetes. Diabetologia. 2010; 53: 1783-1794.

8) Matough, F.A., Budin, S.B., Hamid, Z.A., Alwahaibi, N., Mohamed,J., 2012. The role of oxidative stress and antioxidants in diabeticcomplications. Sultan Qaboos Univ. Med. J. 12 (1), 5–18.

9) Tiwari, B.K. et al, 2013. Markers of oxidative stress during diabetes mellitus. J. Biomarkers (Article ID 378790).

10) Shrikhande GV, Scali ST, da Silva CG, Damrauer SM, Csizmadia E, Putheti P, Matthey M, Arjoon R, Patel R, Siracuse JJ, Maccariello ER, Andersen ND, Monahan T, Peterson C, Essayagh S, Studer P, Guedes RP, Kocher O, Usheva A, Veves A, Kaczmarek E, Ferran C. O-glycosylation regulates ubiquitination and degradation of the antiinflammatory protein A20 to accelerate atherosclerosis in diabetic ApoE-null mice. PLoS One. 2010; 5: e14240. 10.1371/journal.pone.0014240

11) Makino A, Dai A, Han Y, Youssef KD, Wang W, Donthamsetty R, Scott BT, Wang H, Dillmann WH. O-GlcNAcase overexpression reverses coronary endothelial cell dysfunction in type 1 diabetic mice. Am J Physiol Cell Physiol. 2015; 309: C593-C599.

12) Joshi MB, Lad A, Bharath Prasad AS, Balakrishnan A, Ramachandra L, Satyamoorthy K. High glucose modulates IL-6 mediated immune homeostasis through impeding neutrophil extracellular trap formation. FEBS letters 2013; 587: 2241-2246.

13)Zimmer S, Grebe A, Latz E. Danger signaling in atherosclerosis. Circ Res 2015; 116: 323-340.

14) Demer LL and Tintut Y. Interactive and multifactorial mechanisms of calcific vascular and valvular disease.

15) Nahrendorf M, Swirski FK. Immunology. Neutrophil-macrophage communication in inflammation and atherosclerosis. Science. 2015; 349: 237-238.

16) Nambi V, Chambless L, Folsom AR, et al. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: The ARIC (Atherosclerosis Risk In Communities)study. J Am Coll Cardiol 2010; 55: 1600–1607.

17) Yla¨ -Herttuala, S., 1999. Oxidized LDL and atherogenesis. Ann. N.Y.Acad. Sci. 874, 134–137.

18)King G.L. Shiba T. Oliver J. Inoguchi T. Bursell S.E. Cellular and molecular abnormalities in the vascular endothelium of diabetes mellitus Annu. Rev. Med. 1994 45 179 188.

19) Koya D. King G.L. Protein kinase C activation and the development of diabetic complications Diabetes 1998 47 859 866.

20)Patricia P.M. Reactive species and diabetes: counteracting oxidative stress to improve health. Curr. Opin. Pharmacol. 2009;9:771–779.

21)Bornfeldt KE, Tabas I. Insulin Resistance, Hyperglycemia, and Atherosclerosis. Cell Metab 2011; 14: 575-585.

22)Guiotto, Andrea; Calderan, Andrea; Ruzza, Paolo; Borin, Gianfranco (1 September 2005). "Carnosine and Carnosine-Related Antioxidants: A Review". Current Medicinal Chemistry. **12** (20): 2293–2315.

23)Rahbar, S; Figarola, JL (2013-03-25). "Novel inhibitors of advanced glycation endproducts". Arch. Biochem. Biophys. **419** (1): 63–79.

24)M.E. Rać, K. Safranow, M. Rać, G. Kurzawski, A. Krzystolik, D. Sagasz-Tysiewicz, K. Jakubowska, W. Poncyljusz, D. ChlubekCD36 gene is associated with thickness of atheromatous plaque and ankle-brachial index in patients with early coronary artery disease Kardiol. Pol., 70 (2012), pp. 918-923.

25)H. Sawada, Y. Saito, N. NoguchiEnhanced CD36 expression changes the role of Nrf2 activation from anti-atherogenic to pro-atherogenic in apoE-deficient mice Atherosclerosis, 225 (2012), pp. 83-90, 10.1016/j.atherosclerosis.2012.08.023.

26) Klein R.L. Laimins M. Lopes-Virella M.F. Isolation, characterization, and metabolism of the glycated and nonglycated subfractions of low-density lipoproteins isolated from type I diabetic patients and nondiabetic subjects Diabetes 1995 44 1093 1098.

27)Prasad, Anand; Bekker, Peter; Tsimikas, Sotirios (2012). "Advanced Glycation End Products and Diabetic Cardiovascular Disease". Cardiology in Review. 20 (4): 177–183.

28) Di Marco, Elyse; Gray, Stephen P.; Jandeleit-Dahm, Karin (2013). "Diabetes Alters Activation and Repression of Pro- and Anti-Inflammatory Signaling Pathways in the Vasculature". Frontiers in Endocrinology. 4: 68.

29)Jinnouchi Y, Sano H, Nagai R, Hakamata H, Kodama T, Suzuki H, Yoshida M, Ueda S, Horiuchi S. Glycoaldehide-modified low density lipoprotein leads macrophages to foam cells via the macrophage scavenger receptor. J Biochem 1998; 123: 1208-1217.

30) Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 1990; 173: 932-939.