Association of Serum Myeloperoxidase Level with Risk of Coronary Artery Disease in Patients with Type 2 Diabetes

Huda S. Abdulghani[1], Sura A. Abdulsattar[2], Essam N. Salman[3]

[1]M.Sc. Student, Department of Chemistry and Biochemistry, College of Medicine, Mustansiriyah University, Iraq, [2]Prof., [3]Assist. Prof., National Diabetes Center for Research and Treatment/Mustansiriyah University, Iraq

Abstract

Aim: This study aimed to investigate if the change of serum myeloperoxidase (MPO) level would be associated with the incidence of coronary artery disease (CAD) in the type 2 diabetic patients. Method: Eighty-eight (88) Iraqi subjects were grouped into three categories:(29) Type 2 diabetes mellitus patients and (29) diabetic patients with CAD compared with (30) healthy person. Serum Myeloperoxidase was performed by using ELISA kit. Fasting blood sugar and lipid profile were done by Roche/Hitachi cobas c 311device. Atherogenic index (AI) and Coronary risk index (CRI) were estimated.

Results: The results of myeloperoxidase (ng/mL) indicate highly significant increase ($p<0.01$) in the sera of type 2 diabetic (54.18±22.62) and diabetic with CAD (70.79±35.90) patients groups in comparison to that level in control group (29.94±11.11). Meanwhile indicate highly significant differences between T2DM group and diabetic with CAD group. The results of FBS, HDL, triglyceride, VLDL, CRI and AI indicate a significant ($p< 0.05$) differences between all studied groups. While the results of Cholesterol and LDL indicate no significant ($p\geq0.05$) differences between control, T2DM and diabetic with CAD groups.

Conclusion: Elevated level of MPO are association with the presence of Coronary Artery Disease in type 2 diabetic patients. These findings support a potential role of MPO as an inflammatory marker.

Keywords: *Type 2 Diabetes Mellitus, Lipid profile, Myeloperoxidase, coronary artery disease.*

Introduction

Diabetes is a major global health concern, it is a metabolic syndrome that manifests a grade of systemic inflammation, leads to an increase in all-cause mortality and contributes to the development of number of cardiovascular complications[(1,2)]. Cardiovascular diseases remain the leading cause of deaths in many countries globally, including coronary heart disease, stroke, high blood pressure, and arterial diseases. Notably, death rates among adults with both heart disease and diabetes mellitus are 2–4 times higher than those with heart disease alone, and the mortality rate of patients with heart disease >65 years of age is ~68% in conjunction with diabetes[(3,4)]. Clearly, diabetes very negatively impacts the progression and outcome of heart disease, thus understanding the interplay between the two is an important endeavor for advancing treatment strategies of patients with diabetic cardiac complication.

Oxidative stress and inflammation play important roles in the pathogenesis of destabilization of coronary artery disease (CAD) leading to acute coronary syndromes (ACS). Previously, there has been a renewed interest in MPO, a pro-inflammatory enzyme that is abundant in ruptured plaque[5]. Numerous clinical studies have assessed the utility of inflammatory biomarkers for characterization cardiovascular disease (CVD) severity and thus the risk of plaque rupture. Previous research has focused on aninflammatory markers of which myeloperoxidase (MPO), released systemically and locally by activated leukocytes, has shown great promise [6]. Prior study hypothesized that MPO can be used as a diagnostic aid and risk stratification tool in patients who present to the emergency department with ACS[7]. Myeloperoxidase (MPO) (EC 1.11.1.7) is a mammalian enzyme localized in granules of polymorphnuclear granulocytes and macrophages, MPO is a homodimeric

protein with a mass of 146 kDa, consisting of two 73 kDa identical and functionally independent monomers joined by a single disulfide bond at cysteine residue 153. Each monomer has 2 polypeptide chains: a glycosylated heavy chain consisting of 467 amino acid residues and a mass of 58.5 kDa and a light chain with 106 amino acid residues and a mass of 14.5 kDa[8,5]. Myeloperoxidase produces reactive oxidants and other free radicals either through its peroxidasecycle or through a halogenation cycle, depending up on the substrate availability. However, any excessive or unregulated production oftheses oxidants can lead to damage of host cells and result in several diseases [9].

Therefore, in this study, the relationship between MPO levels and the incidence of CAD in patients with type 2 diabetes was determined.

Method

The study included 88 Iraqi subjects were grouped into three categories: (29) Type 2 diabetes mellitus patients and (29) diabetic patients with CAD compared with (30) healthy person in term of non-diabetic, non-hypertensive and have no ischemic heart disease. Patients with Type 1 diabetes, gestational diabetes, chronic diabetic complication (nephropathy, retinopathy and neuropathy), type 2 diabetes taking insulin injection, malignancies were excluded. This studywas designed as case–control study and done at National Diabetes Center for Research and Treatment/Mustansiriyah-University.

Serum of Myeloperoxidase was performed using ELISA kit an enzyme immunoassay for quantitative in vitro diagnostic measurement, kit manufactured by Mybiosource. Fasting blood sugar and lipid profile were done by Roche/Hitachi cobas c 311device. Atherogenic index (AI) was estimated by AI = log TG \ HDL and Coronary risk index (CRI)estimated byCRI = TG \ HDL.

Results

The results of myeloperoxidase (ng/mL) indicate highly significant increase ($p<0.01$) in the sera of type 2 diabetic (54.18±22.62) and diabetic with CAD (70.79±35.90) patients groups in comparison to that level in control group (29.94±11.11). Meanwhile indicate highly significant differences between (T2DM) and (diabetic with CAD) groups. Table (1) showed the results of FBS, lipid profile, CRI and AI that demonstrated a highly significant ($P<0.01$) differences of FBS, HDL, triglyceride, CRI and AI, meanwhile the VLDL result indicate a significant ($p< 0.05$) differences of studied groups. While the results of Cholesterol and LDL indicate no significant ($p\geq0.05$) differences between control, T2DM and diabetic with CAD groups.

Table 1: Mean±SD levels of lipid profile, fasting blood sugar, CRI and AI.

Parameter	Control	T2DM	Diabetic with CAD	P value
FBG (mg/dl)	90.88±6.23 Range (88.51-93.25)	180.7±68.86 Range (154.53-206.91)	182.31±78.8 Range (152.88-211.74)	0.00
Cholesterol (mg/dl)	164.79±28.52 Range (153.94-175.64)	169.62±47.84 Range (151.42-187.82)	167.29±50.19 Range (148.54-186.03)	0.91
Triglyceride (mg/dl)	101.25±36.41 Range (87.40-115.10)	151.86±75.40 Range (123.18-180.54)	149.95±86.32 Range (117.72-182.19)	0.009
VLDL (mg/dl)	21.58±8.23 Range (18.44-24.71)	30.32±15.13 Range (24.56-36.08)	29.71±17.51 Range (23.17-36.25)	0.037
LDL (mg/dl)	97.75±24.11 Range (88.58-106.93)	109.62±38.02 Range (95.16-124.09)	103.06±35.83 Range (89.68-116.44)	0.400
HDL (mg/dl)	48.94±17.44 Range (42.31-55.58)	31.84±8.5 Range (28.58-35.11)	30.33±9.27 Range (26.87-33.79)	0.000
CRI	2.26±1.09 Range (1.85-2.66)	5.17±3.30 Range (3.91-6.43)	5.52±3.26 Range (4.27-6.76)	0.000
AI	0.31±0.19 Range (0.24-0.38)	0.63±0.24 Range (0.54-0.73)	0.66±0.27 Range (0.55-0.76)	0.000

As shown in Table (2), MPO of type 2 diabetic patients showed significant negative correlation with HDL ($p<0.05$) as shown in Figure (1). The overall analyze of all groups samples it was observed highly significant negative correlation with HDL as shown in Figure (2), while demonstrated a significant positive correlation with Tri as shown in Figure (3). Meanwhile CRI and AI were indicated a highly significant positive correlation with MPO as shown in Figure (4) and (5) respectively.

Table 2: Pearson correlation analysis of MPO(ng/mL) in studied groups

Parameters	T2DM	Diabetic with CAD	All Groups
FBS (mg/dl)	0.179	0.214	0.418**
CHO (mg/dl)	-0.257	0.106	-0.010
Tri (mg/dl)	-0.141	0.253	0.257*
HDL (mg/dl)	-0.433*	0.114	-0.372**
LDL (mg/dl)	-0.092	-0.104	-0.025
VLDL (mg/dl)	-0.145	0.241	0.215
CRI	0.142	0.224	0.366**
AI	0.055	0.269	0.391**

*p<0.05, **p<0.01, No asterisk p≥0.05

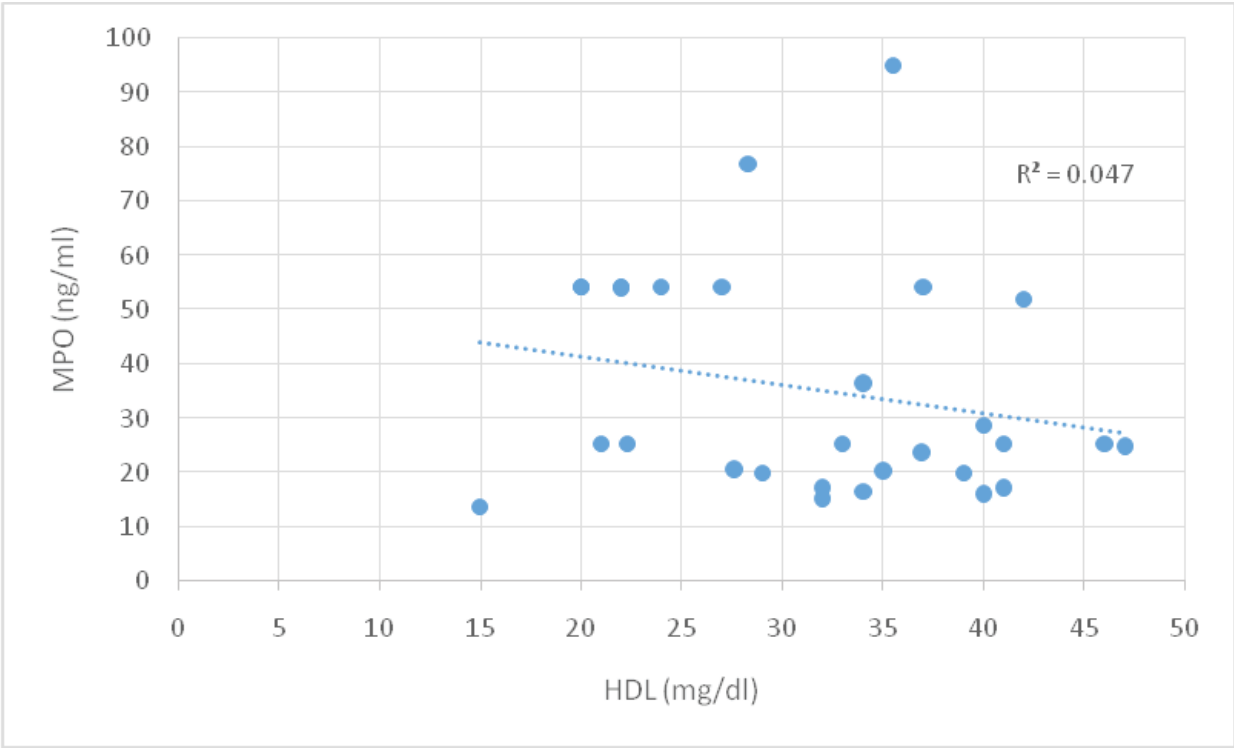

Figure 1: The correlation between serum MPO with HDL in T2DM.

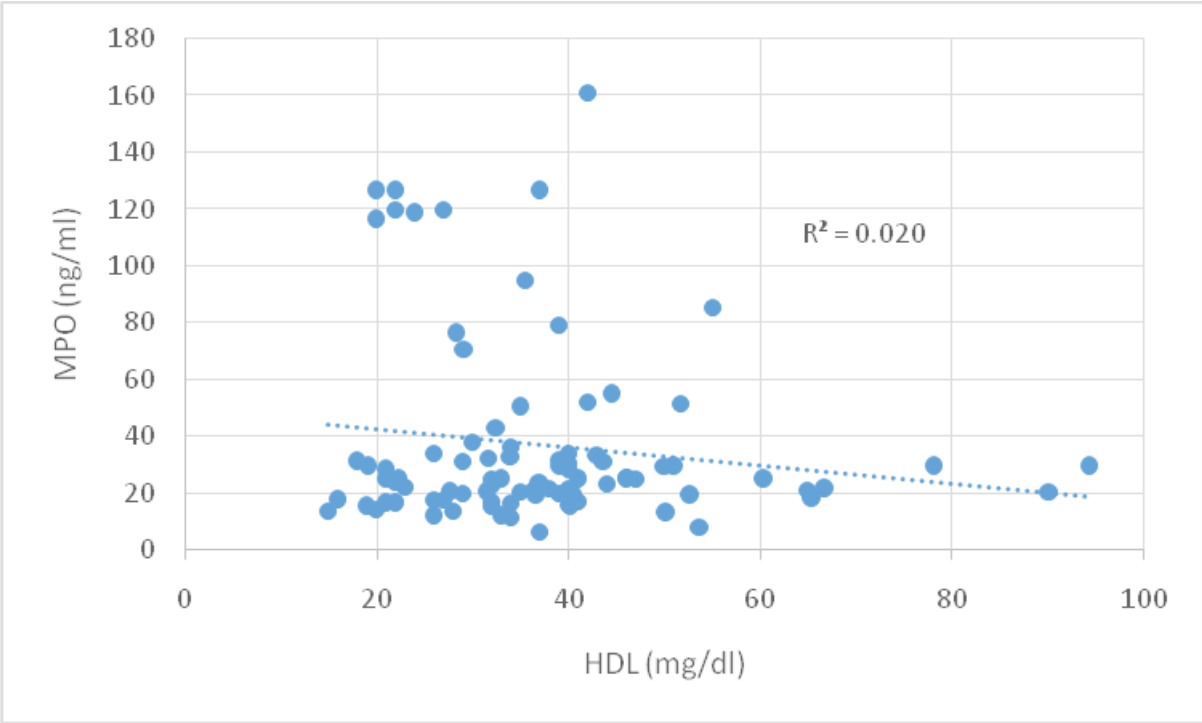

Figure 2: The correlation between serum MPO with HDL in all studied groups.

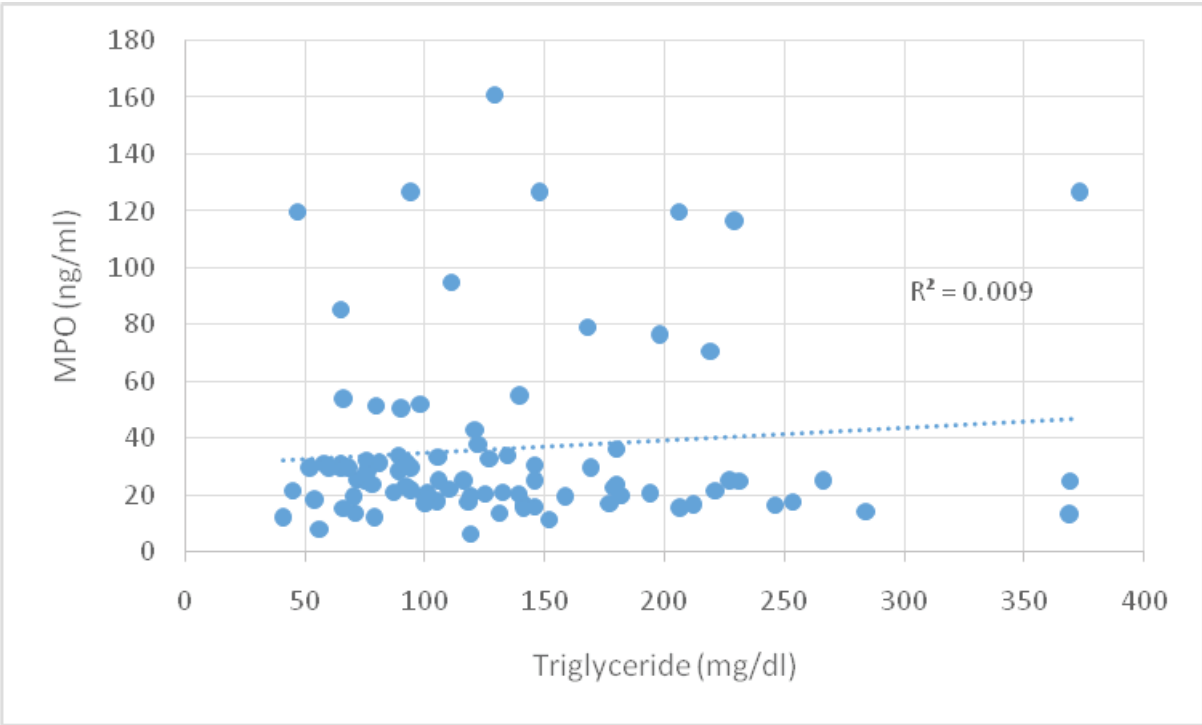

Figure 3: The correlation between serum MPO with Triglyceride in all studied groups.

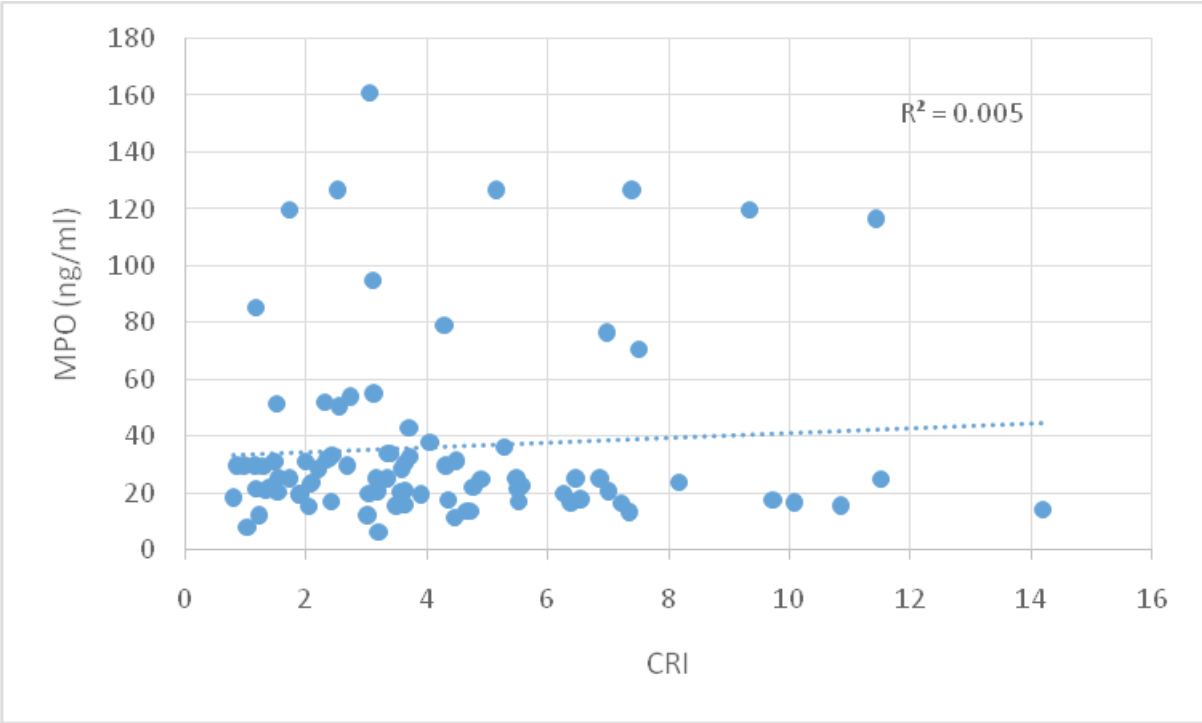

Figure 4: The correlation between serum MPO with CRI in all studied groups.

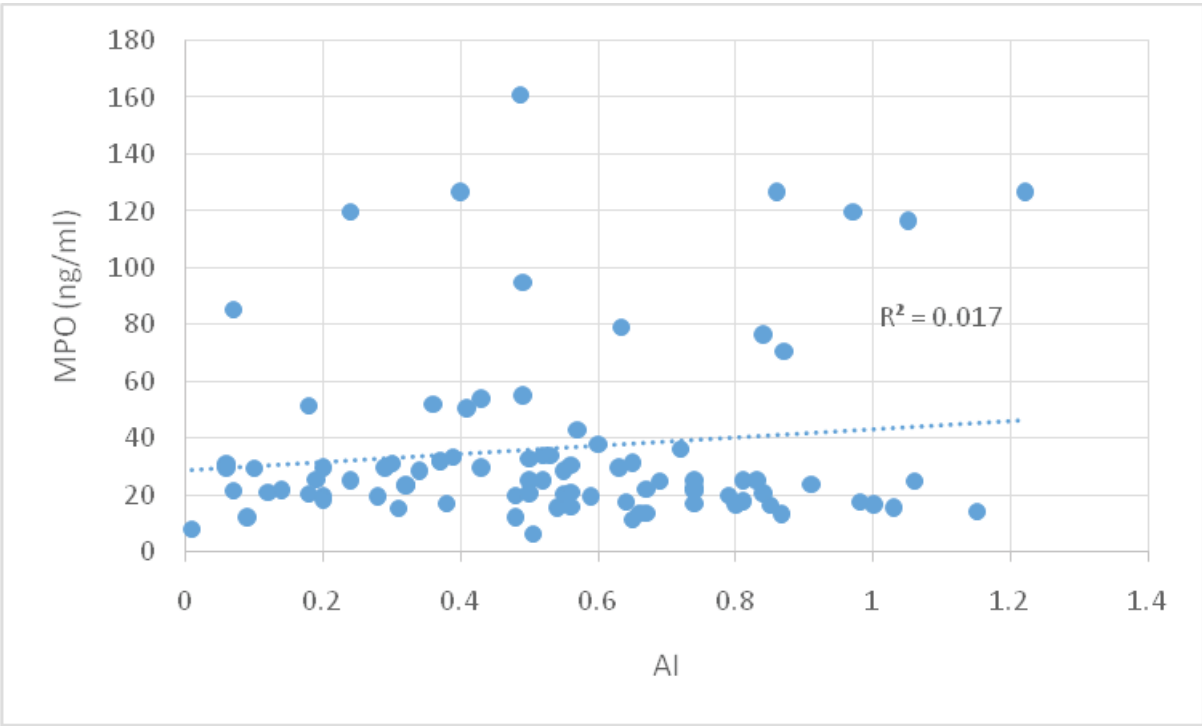

Figure 5: The correlation between serum MPO with AI in all studied groups.

Discussion

In this study,the results of myeloperoxidase indicate a highly significant increase in the sera of T2DM group and diabetic with CADgroup in comparison to that level in controlgroup. This finding was agreement with song p, et al. study, which indicate thatplasma MPO level was positively correlated with the degree of coronary artery stenosis in type 2

diabetic patients[10]. Also agree with I. V. Gorudko, et al., study which reported that myeloperoxidase is increased activity in the blood can be an additional marker of oxidative stress and cardiovascular risk in patients with diabetes mellitus[11]. Diabetic patients are prone to atherosclerosis, which is the major cause of cardiovascular diseases (CVD), neutrophils and monocytesplay a key role in atherosclerosis, leading to chronic inflammatory problems. Different events andsequences occur during CVD, which include endothelial dysfunction besides the formation and rupture of atherosclerotic plaque. In the arterial wall sub endothelial region, all of these stages occurduring inflammation, which ultimately leads to the accumulation and deposition of altered lipids [12]. Atherosclerosis leads to the accumulation of cholesterol and cholesteryl esters on arterial walls,which derived from LDL. In addition to this, LDL retention on these walls triggers an immuneresponse, resulting in a cascade of production of oxidants and inflammation[13] Plasma LDLinteracts with circulating MPO, which has been reported to be higher in patients suffering from atherosclerosis. HOCl reactswith LDL, which promotes atherogenesis[14]. High glucose stimulates the production of hydrogen peroxide. This hydrogen peroxide which is a physiological substrate for Myeloperoxidase is converted to hypochlorous acid. Thus, high glucose results in increase in Myeloperoxidase activity[15].

The results of cholesterol and LDL indicate no significant increase between studied groups. This finding disagreement with Rothangpui, et al., who shows LDL cholesterol level are higher among the diabetics with cardiomyopathy compared with those without cardiomyopathy[16]. Also, disagree with Po-Chung Cheng, et al., who observed an inverse correlation between plasma LDL-cholesterol and heart function in individuals with T2DM. Patients with higher levels of plasma LDL cholesterol had worse left ventricular function[17]. Plasma LDL-cholesterol may be a modifiable risk factor of heart failure in diabetes.P. R. Lawler, et al, study that showed cardiovascular disease events prevalent among individuals with low or normal LDL, both pretreatment and during statin therapy[18] and Kenneth R Feingold & Carl Gthey were reported thatIn Type 2 diabetes, poor glycemic control increases triglyceride levels and decreases HDL cholesterol levels with only modest effects on LDL cholesterol levels [19]. The results of Triglyceride and VLDL in the sera of T2DMgroup and diabetic with CADgroup indicate a significant increase in comparison to that level in the controlgroup. These finding agreements withAdam Shaver, et al., who's indicated thattriglycerides are elevated in diabetic patients due to an increased dependence on fatty acid metabolism, and demonstrated that diabetic patients with CVD patients had significantly elevated triglyceride levels[20]. Triglyceride are negatively associated with atheroprotective HDL- cholesterol. The most obvious lipid defect in uncontrolled diabetes is the elevated level of triglycerides [21], Increasing TG in fasting status could be marker for increased level of remnant lipoprotein particles, which could be directly atherogenic, and they could penetrate into the vessel wall and cause inflammation [22].

Results of HDL demonstrated a highly significant decrease in T2DMgroup and diabetic with CADgroup in comparison to that level in controlgroup. This result was in agreement with Rothangpui, et al., who indicated that serum HDL-cholesterol was lower among the diabetics with cardiomyopathy compared with those without cardiomyopathy[16]. In addition, HDL decreased in many CVD patients, blood level of HDL < 40 mg\dl may be an effective warning sign for atherosclerotic development [23]. High-density lipoprotein cholesterol is inversely associated with risk of coronary heart disease[24], associated to the anti-inflammatory, anti-thrombotic and anti-oxidant properties as well as to the ability to support endothelial physiology [25]. Raising plasma HDL-cholesterol through weight loss and a healthy diet, by an increased physical activity and, if required, by proper pharmacotherapy is therefore a legitimate therapeutic target for the optimal prevention of CHD in a large proportion of high-risk patients[26].

The results of Coronary risk and atherogenic index of the studied groups indicated a highly significant increase of levels in sera of T2DM group and diabetic with CAD group in comparison to that in (control) group. The result was in agreement with Domingo O Beltran, et al., who reported that high level of CRI and AI were associated with all-cause mortality and risk of hospitalization due to coronary heart disease[27], also agree with Harini D Nimmanapalli, et al., study that demonstrated atherogenic

indices were found to be significantly different and The ratios contribute significantly to the estimation of CVD risk in type 2 diabetes mellitus[28]. Diabetic dyslipidemia that characterized by an increased TG level and also decreased HDL-C value, a main feature of lipoproteins abnormalities in diabetic patients and in turn affects the results of CRI and AI, which lead to increase risk factors for developing CVD [29]. There is correlation between AI and lipoprotein particle size, therefore AI could be considered as an indicator of atherogenic lipoprotein status and used as a diagnostic indicator when other atherogenic parameters appear normal.[30]

Conclusion

Elevated level of MPO are association with the presence of Coronary Artery Disease in type 2 diabetic patients. These findings support a potential role of MPO as an inflammatory marker.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine and all experiments were carried out in accordance with approved guidelines.

Reference

1. Duncan BB, Schmidt M, Pankow JS, Ballantyne CM, Couper D, Vigo A, Hoogeveen R, Folsom AR, Heiss G. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes. 2003;52(7):1799-805.
2. Gaurav S. Gulsin, Lavanya Athithan, Gerry P. McCann. Diabetic cardiomyopathy: prevalence, determinants and potential treatments. Ther Adv Endocrinol Metab. 2019; 10: 2042018819834869.
3. Anamika B, Douglas G. Tilley. The Role of Leukocytes in Diabetic Cardiomyopathy. Front Physiol. 2018; 9: 1547.
4. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, et al. Heart Disease and Stroke Statistics-2018 Update: A Report from the American Heart Association. Circulation. 2018 Mar 20;137 (12):e67-e492.
5. Kevin M, Rosemary K, Bruce S. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemie myocardium. Journal of Pharmacological Method, 1985;14(3): 157–167.
6. Koeth RA, Haselden V, Tang WH. Myeloperoxidase in cardiovascular disease. Adv Clin Chem. 2013;62:1-32.
7. Alberto D Rodriguez, Pedro A Gonzalez. Current role of myeloperoxidase in routine clinical practice. Journal Expert Review of Cardiovascular Therapy, V2011;9(2).
8. Gjin N, Myeloperoxidase – A bridge linking inflammation and oxidative stress with cardiovascular disease. Clinica Chimica Acta, Volume 493, June 2019; 36-51.
9. Yoji Kato. Neutrophil myeloperoxidase and its substrates: formation of specific markers and reactive compounds during inflammation. J ClinBiochemNutr. 2016 Mar; 58(2): 99–104.
10. Song P, Xu J, Song Y, Jiang S, Yuan H, Zhang X. Association of Plasma Myeloperoxidase Level with Risk of Coronary Artery Disease in Patients with Type 2 Diabetes. Dis Markers. 2015;2015:761939.
11. IV Gorudko, VAKostevich, AVSokolov, EV. Shamova, IV Buko, EEKonstantinova, VB. Vasiliev SN Cherenkevich, OM Panasenko. Functional Activity of Neutrophils in Diabetes Mellitus and Coronary Heart Disease: Role of Myeloperoxidase in the Development of Oxidative Stress. Bulletin of Experimental Biology and Medicine, November 2012; 154(Issue 1) 23–26.
12. Elena Galkina, Klaus Ley. Immune and Inflammatory Mechanisms of Atherosclerosis. Annual Review of Immunology, 2009;27:165-197
13. Roland Stocker, John F. Keaney, JrRole of Oxidative Modifications in Atherosclerosis. Physiological Reviews Oct. 1, 2004;84(Issue 4.).
14. Delporte C, Boudjeltia KZ, Noyon C, Furtmüller PG, Nuyens V, et al. Impact of myeloperoxidase-LDL interactions on enzyme activity and subsequent posttranslational oxidative modifications of apoB-100.J Lipid Res. 2014;55(4):747-57.
15. Sujatha R, Gayathri B.Correlation between lipid profile and myeloperoxidase levels in type-2 diabetes mellitus. International Journal of Medical Research and Review, 2016; 4(09).
16. Rothangpui S, Premchand S, Lallan P, Romeo K. Diabetic cardiomyopathy in Manipur. Indian J Endocrinol Metab. 2011 Jul-Sep; 15(3): 204–208.

17. Po-Chung Cheng, Shang-Ren Hsu, Jung-Chi Li, Ching-Pei Chen, Szu-Chi Chien, Shih-Te Tu, Yun-Chung Cheng, Yu-Hsiu Liu, Jeng-Fu Kuo. Plasma Low-Density Lipoprotein Cholesterol Correlates With Heart Function in Individuals With Type 2 Diabetes Mellitus: A Cross-Sectional Study. Front. Endocrinol.2019.
18. PRLawler. Atherogenic lipoprotein determination of cardiovascular disease and residual risk among individuals with low-density lipoprotein cholesterol. J. Am. Heart Assoc., 2017; 6(7): 1-17.
19. Kenneth R, Carl G. Role of Glucose and Lipids in the Cardiovascular Disease of Patients with Diabetes. Endotext, 2019.
20. Adam S, Alexandra N, Ellen T, Amrita M, Kristen P. Role of Serum Biomarkers in Early Detection of Diabetic Cardiomyopathy in the West Virginian Population. Int J Med Sci 2016; 13(3):161-168.
21. GH Tomkin, D Owens. Diabetes and dyslipidemia: characterizing lipoprotein metabolism. Diabetes Metab. Syndr. Obes. 2017; 10: 333–343.
22. AVarbo and BG Nordestgaard. Remnant cholesterol and ischemic heart disease. Curr. Opin. Lipidol., 2014;25(4): 266-273.
23. Musunuru K. Atherogenic dyslipidemia: cardiovascular risk and dietary intervention. Lipids, 2010;45(10): 907-914.
24. Saleheen D, Scott R, Javad S, Zhao W, Rodrigues A, Picataggi A, Lukmanova D, Mucksavage ML, Luben R, Billheimer J, Kastelein JJ, Boekholdt SM, Khaw KT, Wareham N, Rader DJ. Association of HDL cholesterol efflux capacity with incident coronary heart disease events: A prospective case-control study. Lancet Diabetes Endocrinol., 2015;3(7): 507-513.
25. Pirillo, Angela; Catapano, Alberico L.; Norata, Giuseppe Danilo. Biological Consequences of Dysfunctional HDL. Current Medicinal Chemistry, 2019;26(9): 1644-1664(21).
26. Després JP, Lemieux I, Dagenais GR, Cantin B, Lamarche B. HDL-cholesterol as a marker of coronary heart disease risk: the Québec cardiovascular study. Atherosclerosis. 2000 Dec; 153 (2):263-72
27. Domingo O Beltran, Vicente F Gil-Guillen, Josep R, Jose M Martin-Moreno, Vicente P Carratala, et al., Lipid profile, cardiovascular disease and mortality in a Mediterranean high-risk population: The ESCARVAL-RISK study. PLoS One. 2017; 12(10): e0186196.
28. Harini D, Ambika D, Prabath K, Vani N. Lipid ratios, atherogenic coefficient and atherogenic index of plasma as parameters in assessing cardiovascular risk in type 2 diabetes mellitus. IJRMS, 2016;4(7).
29. Tariq M. Comparative Studyfor Atherogenic Index of Plasma (AIP) inpatients with Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus, Betathalassemia, and Hypothyroidism. Chemistry department/College of Education Ibn Al-Haitham/ Baghdad University, J. Chem. Res., 2012; ISSN: 2249-0329.
30. Shabnam N, Mohammad K, Majid K Rezaiyan, Maryam A, Mohammadreza J, Gholamhasan K, Maliheh D. Atherogenic index of plasma (AIP): a marker of cardiovascular disease. Med. J. Islam. Repub. Iran, 2015;29: 240.