Evaluation of Advanced Glycation End Products, Oxidative stress, and Antioxidant Parameters in Patients with Atherosclerosis

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Abstract:

Objective: Cardiovascular diseases is the main reason of mortality in developed and developing nations. Atherosclerosis is the major source of morbidity and mortality. The main cause of coronary artery disease (CAD) is the atherosclerotic plaques formation. Advanced glycation end products (AGEs), such as N_{ε}-(carboxymethyl)-lysine (CML) and pentosidine (PTD) are implicated in vascular disease. The important risk factor for atherosclerosis is the imbalance between oxidative stress and antioxidants. This study is aimed to evaluate the serum AGE, oxidative stress and antioxidant parameters in atherosclerotic patients to compare with control group.

Methods: In the present study 80 subjects were conducted (50 patients with atherosclerosis and 30 healthy subjects, men and women). Serum CML, PTD, MDA, NO, SOD, CAT biomarkers and lipid profile parameters were evaluated using ELISA kits and spectrophotometrically methods using Cobas c 311 analyzer (Roche/Hitachi Cobas). Statistical analysis were performed using GraphPad Prism (version 9, independent student t-test).

Results: The serum levels of CML, PTD, MDA, NO, SOD, and CAT are significantly decreased in patients with atherosclerosis compared with healthy group (CML= 115.6 ± 3.446 *vs.* 132.5 ± 5.211 ng/ml) (p value = 0.0065), (PTD= 12.16 ± 0.2646 *vs.* 13.28 ± 0.4096 ng/ml) (p value = 0.0191), (MDA= 40.37 ± 0.9719 *vs.*

51.56 ± 2.600 ng/ml) (p value <0.0001), (NO= 17.35 ± 0.6476 *vs.* 20.41 ± 1.067 µmol/L) (p value = 0.0111), (SOD= 0.3323 ± 0.01157 *vs.* 0.4072 ± 0.02058 ng/ml) (p value = 0.0010), and (CAT= 0.4070 ± 0.02113 *vs.* 0.5321 ± 0.03498 ng/ml) (p value = 0.0017). The serum levels of CH, TG, LDL, and VLDL are not significantly increased in patients with atherosclerosis rather than healthy group (CH= 146.3 ± 8.737 *vs.* 143.4 ± 13.55 mg/dl) (p value = 0.8699), (TG= 170.7 ± 17.29 *vs.* 155.5 ± 27.15 mg/dl) (p value = 0.6485), (LDL= 77.22 ± 6.881 *vs.* 76.23 ± 13.31 mg/dl) (p value = 0.9446), and (VLDL= 32.59 ± 3.165 *vs.* 31.18 ± 5.388 mg/dl) (p value = 0.8199). While the serum level of High-density lipoprotein not significantly decreased in patients with atherosclerosis when compared with healthy group (HDL= 33.47 ± 1.536 *vs.* 38.93 ± 3.152 mg/dl) (p value = 0.0969).

Conclusion: The present study shows that the serum levels of advanced glycation end products (CML and PTD), oxidative stress parameters (MDA and NO) and antioxidant parameters (SOD and CAT) in patients with atherosclerosis are significantly decreased. Whereas, the serum levels of lipid profile parameters with exception of HDL in patients with atherosclerosis are not significantly increased.

Keywords: Advanced Glycation End products (AGEs), Cardiovascular Disease, Atherosclerosis, Oxidative Stress (OS), Antioxidants.

1. Introduction:

Cardiovascular Disease (CVD): Disorders the heart and blood vessels include one of the main reasons for death worldwide [1]. The terms "heart disease" and "cardiovascular disease" are frequently utilized interchangeably. The CVD refers to the condition depicted by narrowed or blocked blood vessels, which can cause in a chest pain (angina), stroke, or heart attack. Other heart conditions, including conditions affecting the heart's muscle, beating rhythm, or valves, and infections are also classified as types of heart disease [2]. Among the many diseases which fall under the umbrella of heart disease are atherosclerosis, heart valve disease, coronary artery disease (CAD) (angina, and heart attack), heart defects and heart infections

(congenital heart defects), and heart rhythm problems (arrhythmias). The most prevalent clinical symptom which denotes the final stages of a variety of various cardiac disorders diseases is Heart failure [3].

The main cardiovascular disease risk factors could be divided into two groups controllable (modifiable) or uncontrollable (non-modifiable). The controllable risk factors for cardiovascular diseases include hyperhomocysteinemia, diabetes mellitus (DM), physical inactivity, obesity, smoking, hyperlipidemia, and hypertension [4].

Atherosclerosis is a type of CVD categorized by inflammation and plaque formation in the artery walls. The majority of these plaques are made up of calcium, inflammatory cells, and lipids. The condition is characterized by a persistent inflammatory process that is aggravated by specific cardiovascular risk factors like obesity, diabetes, hypertension, and high basal cytokine levels. Especially, hypercholesterolemia is a prerequisite for atherogenesis [5]. During the formation of an atherosclerotic plaque, the expression of cellular adhesion molecules causes monocyte enlistment and macrophage infiltration in the arterial wall intima [6, 7]. Homocysteine is recognized as an independent risk factor for atherosclerosis [8]. Atherosclerotic plaques grow over time and are frequently symptomless in the early stages of the disease. Atherosclerosis was recognized to produce clinically significant complications in CVD such as CAD, myocardial infarction (MI), heart attack, stroke, peripheral vascular disease, and peripheral artery disease [9, 10]. Atherosclerotic cardiovascular disorders increase healthcare costs greatly, in addition to contributing to high worldwide morbidity and mortality. As a result, early, the invasive diagnosis could have a significant clinical influence on the survival of this patient population [10, 11].

Advanced Glycation End products (AGEs) are nucleic acids, proteins, or lipids modifications that are non-enzymatically glycosylated and oxidized after being exposed to reducing sugars; they can be produced exogenously through food and smoking, or they can be generated as endogenously within the body [12, 13]. Certain

circumstances, such as hyperglycemia, hyperlipidemia, and elevated oxidative stress (OS), might speed up the synthesis of AGEs. Atherosclerosis is exacerbated by the presence of AGEs in the blood vessels of diabetics. The availability and accrual of AGEs in a variety of cell types have an impact on the extracellular and intracellular structure and function. In addition to diabetes complications, the Maillard reaction, which begins with the glycation of protein and ends with the creation of AGEs, is involved in the development of cardiovascular, renal, and neurological disorders and a variety of malignancies [14].

Reactive oxygen species (ROS) like superoxide anion radical ($O_2^{\bullet\ -}$), alkoxyl radical ($LO^{\bullet}$), peroxyl radical ($LOO^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), or non-radicals like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) [15] and RNS include nitric oxide ($NO^{\bullet}$), nitrogen dioxide radical ($NO_2^{\bullet}$), or non-radical like peroxynitrite ($ONOO^-$) [16, 17], are highly reactive compounds which are produced from oxygen or nitrogen metabolism. They may be either non-radicals or free radicals [18]. Increasing level of oxidative stress can encourage damage to the cellular structure and potentially destroy tissues [19]. In lipids, nucleic acids, and proteins, oxidative stress determines structural changes and function regulation, oxygen free radicals attack biological molecules such as proteins, DNA, and lipids. Oxidative degradation of lipids yields malondialdehyde. carbonylation, thiol oxidation, fragmentation, side-chain oxidation, misfolding, and unfolding, are some examples of protein degradation processes that can lead to activity loss. 8- hydroxyl-2-deoxyguanosine is an index of DNA damage [20].

Antioxidants are chemical substances that have a wide range of uses and functions. They are considered to be beneficial to human health because they are found in foods such as fruits, vegetables, tea, and red wine. Antioxidants, on the other hand, prevent the oxidation of our body's proteins and nucleic acids. Antioxidants can also be employed as food additives to prolong the shelf life of food in the food business [21]. Antioxidants are classified into two classes: endogenous and

exogenous, endogenous antioxidants are glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), uric acid, and bilirubin [22]. Exogenous antioxidants are carotenoids, a plant polyphenol, vitamin E, and vitamin C [23].

2. Subjects and Methods:

For this study, blood samples of 80 individuals with coronary angiography at Surgical Specialty Hospital – Erbil Cardiac Center were taken. The study included 50 patients (25 females and 25 males) who had stenosis (diameter >50%) (coronary atherosclerosis disease) as the patient group. As a healthy group blood samples of 30 individuals (18 females and 12 males) were taken who had no stenosis. The patient and healthy groups were aged > 37 years old. Blood sample collection started in September 2021 and finished in November 2021.

Approximately five ml of blood was collected from each participant and placed in a collecting tube containing gel and clot activator. The blood sample was left without anticoagulant for a period to allow the blood clotting. Sera were collected by centrifugation for ten minutes and stored at -40˚C till analysis. The serum tests of CML, PTD, MDA, NO, CAT, and SOD were performed using ELISA kits (ZellBio GmbH assay kit) via a fully automated Enzyme-Linked Immunosorbent Assay (ELISA) of Germany origin. The sera lipid profile tests (CH, TG, HDL, LDL, and VLDL) were performed using Cobas c 311 analyzer (Roche/Hitachi Cobas), of Germany origin preloaded with the respective reagent kits. This was done at Research Center of Koya University and Kani Lab for Medical Analysis.

3. Statistical Analysis:

The current study's data were represented as mean, standard error of mean (Mean ± SE), differences in mean values between 2 groups were analyzed by two samples independent student’s t-test, and Pearson correlation was used to determine relationships, and the GraphPad Prism (version 9) was used as statistical software to analyze the data. The p value ($p<0.05$) level of significance was declared statistically significant. The area under the curve (AUC) for diagnostic accuracy in patients with

atherosclerosis was determined using ROC curve (Receiver Operating Characteristic) analysis.

4. Results and Discussion:

In the present study, the serum levels of CML in the coronary atherosclerotic patient′s group are (115.6 ± 3.446 ng/ml), and in healthy group are (132.5 ± 5.211 ng/ml), as shown in (Table 1 and Figure 1). The results of these data show that the concentrations of carboxymethyl-lysine in patients with atherosclerosis are significantly lower rather than in the healthy group. These data are in agreement with the data of a previous study that the level of CML is decreased in patients with atherosclerosis rather than to healthy group [24]. Whereas, these are in disagreement with the results of a previous study [25, 27], which showed that the level of CML in atherosclerotic patients is significantly higher than in healthy group.

Our study illustrates that the serum levels of PTD in patients with atherosclerosis and the healthy group are (12.16 ± 0.2646 and 13.28 ± 0.4096 ng/ml) respectively, as shown in (Table 1 and Figure 1). These results demonstrate that the serum levels of PTD in patients with atherosclerosis are significantly lower than that of healthy group. This is in agreement with the results of previous study that demonstrated the PTD level in serum of atherosclerosis patients is significantly lower than in healthy group [28]. Whereas, the results of this study are in disagreement with the study of [25, 26, 29] that demonstrated the PTD level in serum of atherosclerosis patients is significantly higher than healthy group.

The decreased level of serum CML and PTD could due to the medication for the atherosclerosis patients since atherosclerotic patients were receiving regular atherosclerosis medication, who are getting the lipid-lowering agents for example atorvastatin (inhibits the key enzyme activity in the biosynthesis of cholesterol, namely HMG-CoA reductase), and this causes also to reduce serum level of CML [24, 30], metformin administration leads to reduce serum levels of PTD [25]. In the case of this study the atherosclerotic patients administered different medicine among them aspirin, antiplatelet agents, piax (prevents blood clotting), and Plavix

(Clopidogrel: reduces the atherosclerotic plaque formation) [31, 32], aminoguanidine (pimagedine) and pyridoxamine (prevent the diabetic complication, they have anti-oxidant property), they prevent the formation of AGEs [33, 34]. The healthy group′s serum levels of CML and PTD are higher since they do not have any serious chronic illnesses and are not taking any medicines on a regular basis.

The data of the current study show that the serum levels of MDA in coronary atherosclerotic patients is (40.37 ± 0.9719 ng/ml), and in healthy group is (51.56 ± 2.600 ng/ml), which is present in (Table 1 and Figure 1). These results show that the serum levels of MDA are significantly lower in atherosclerotic patients rather than healthy group. This study is in agreement with the study of [35, 36] that demonstrated the MDA level in serum of atherosclerosis patients is significantly lower than healthy group, this could due to the medication by the atherosclerosis patients, who are getting the lipid-lowering agents (statins) [36]. Whereas, the result of this study is in disagreement with the results of previous study that demonstrated the MDA level in serum of atherosclerosis patients is significantly higher than in healthy group [37, 38].

Our study shows that the serum levels of NO is (17.35 ± 0.6476 μmol/L) in coronary atherosclerotic patient group, whereas, it is (20.41 ± 1.067 μmol/L) in healthy group, which is shown in (Table 1 and Figure 1). These results show that patients with atherosclerosis had serum levels of NO that are significantly lower than those of the healthy group. Our study is in agreement with the study of [39, 40] that demonstrated the NO level in serum of atherosclerosis patients is significantly lower than healthy group because they use statins as medication. Whereas, the data of the present study is in disagreement with the results of previous study that it demonstrated the NO level in serum of atherosclerosis patients is tend to be significantly higher than in healthy group, particularly the patients who are obese BMI>25 kg/m^2 and hypertensive patients they received angiotensin-converting enzyme (ACE) inhibitors [26, 41].

Our study demonstrates that the serum levels of SOD is (0.3323 ± 0.01157 ng/ml) in coronary atherosclerotic patient group, whereas, it is (0.4072 ± 0.02058 ng/ml) in healthy group, which is shown in (Table 1 and Figure 1). These data exhibit that the serum levels of SOD are significantly lower in patients with atherosclerosis compared to healthy group. The results of the extant study illustrate the serum levels of CAT (0.4070 ± 0.02113 ng/ml) in coronary atherosclerotic patients, while it is (0.5321 ± 0.03498 ng/ml) in healthy group, which is shown in (Table 1 and Figure 1). The data exhibit that the serum levels of CAT is significantly lower in patients with atherosclerosis rather than to healthy group. Our study is in agreement with the results of previous study that demonstrated antioxidants (SOD and CAT) levels in serum of atherosclerosis patients is significantly lower than healthy group [26, 39, 42].

The present study illustrates that the serum levels of CH, TG, HDL, LDL, and VLDL in coronary atherosclerotic patient group are (146.3 ± 8.737, 170.7 ± 17.29, 33.47 ± 1.536, 77.22 ± 6.881, and 32.59 ± 3.165 mg/dL), respectively, while in healthy group are (143.4 ± 13.55, 155.5 ± 27.15, 38.93 ± 3.152, 76.23 ± 13.31, and 31.18 ± 5.388 mg/dL), respectively, as shown in (Table 1 and Figure 1). These results show that the serum levels of CH, TG, LDL, and VLDL in patients with atherosclerosis are not significantly higher compared to healthy group. Whereas, the serum levels of HDL in patients with atherosclerosis is not significantly lower compared to the healthy group.

The results of the present study are in agreement with the previous study that demonstrated the CH, TG, LDL, and VLDL level in serum of atherosclerosis patients are higher than the healthy group. Whereas, the HDL serum levels of atherosclerosis patients are lower than the healthy group [26, 42, 43]. The differences are not significant because atherosclerotic patient's advent of the statin drug class (HMG-CoA reductase inhibitors) provided a much more effective approach to lowering lipid profile parameters and laid to rest the concerns raised by the earlier trials [44].

Table (1) Comparing the serum levels of CML, PTD, MDA, NO, SOD, CAT, and lipid profile parameters in patients with atherosclerosis and healthy group:

Parameters	Patient Group	Healthy Group	P value
CML (ng/ml)	115.6 ± 3.446	132.5 ± 5.211	0.0065
PTD (ng/ml)	12.16 ± 0.2646	13.28 ± 0.4096	0.0191
MDA (ng/ml)	40.37 ± 0.9719	51.56 ± 2.600	<0.0001
NO (µmol/L)	17.35 ± 0.6476	20.41 ± 1.067	0.0111
SOD (ng/ml)	0.3323 ± 0.01157	0.4072 ± 0.02058	0.0010
CAT (ng/ml)	0.4070 ± 0.02113	0.5321 ± 0.03498	0.0017
CH (mg/dl)	146.3 ± 8.737	143.4 ± 13.55	0.8699
T.G. (mg/dl)	170.7 ± 17.29	155.5 ± 27.15	0.6485
HDL (mg/dl)	33.47 ± 1.536	38.93 ± 3.152	0.0969
LDL (mg/dl)	66.14 ± 4.869	76.23 ± 13.31	0.3778
VLDL (mg/dl)	32.59 ± 3.165	31.18 ± 5.388	0.8199

The values are expressed as (Mean ± SE).

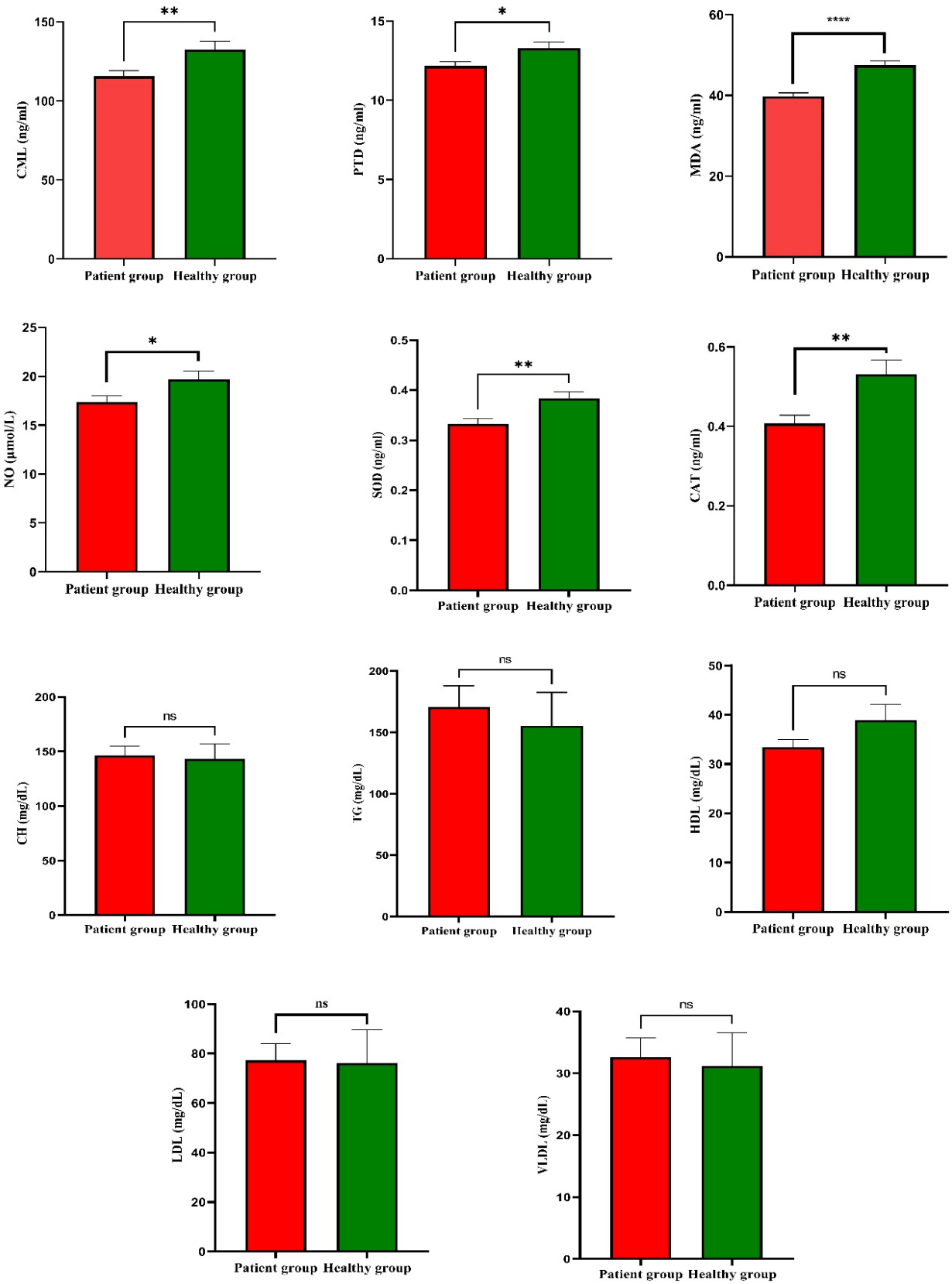

Figure 1: Serum levels of CML, PTD, MDA, NO, SOD, CAT, CH, TG, HDL, LDL, and VLDL in patients with atherosclerosis and healthy group.

The data of our study show that there are positive and significant correlations between CML and MDA, NO, and SOD. The p values for MDA (p= 0.0016), NO (p= 0.0079), and SOD (p<0.0001). And there are positive but not significant correlations among serum CML and CAT, CH, TG, HDL, LDL, and VLDL which p values for CAT (p= 0.3146), CH (p= 0.3104), TG (p= 0.5063), HDL (p= 0.1759), LDL (p= 0.2791), and VLDL (p= 0.8301) as shown in (Table 2).

Table (2) Correlations analysis between CML with MDA, NO, SOD, CAT, CH, TG, HDL, LDL, and VLDL in patients with atherosclerosis:

Parameters	Correlation coefficient (r) (Pearson correlation)	P value
CML and MDA	0.4350	0.0016
CML and NO	0.3789	0.0079
CML and SOD	0.5826	<0.0001
CML and CAT	0.1467	0.3146
CML and CH	0.1765	0.3104
CML and TG	0.1240	0.5063
CML and HDL	0.2414	0.1759
CML and LDL	0.2079	0.2791
CML and VLDL	0.0409	0.8301

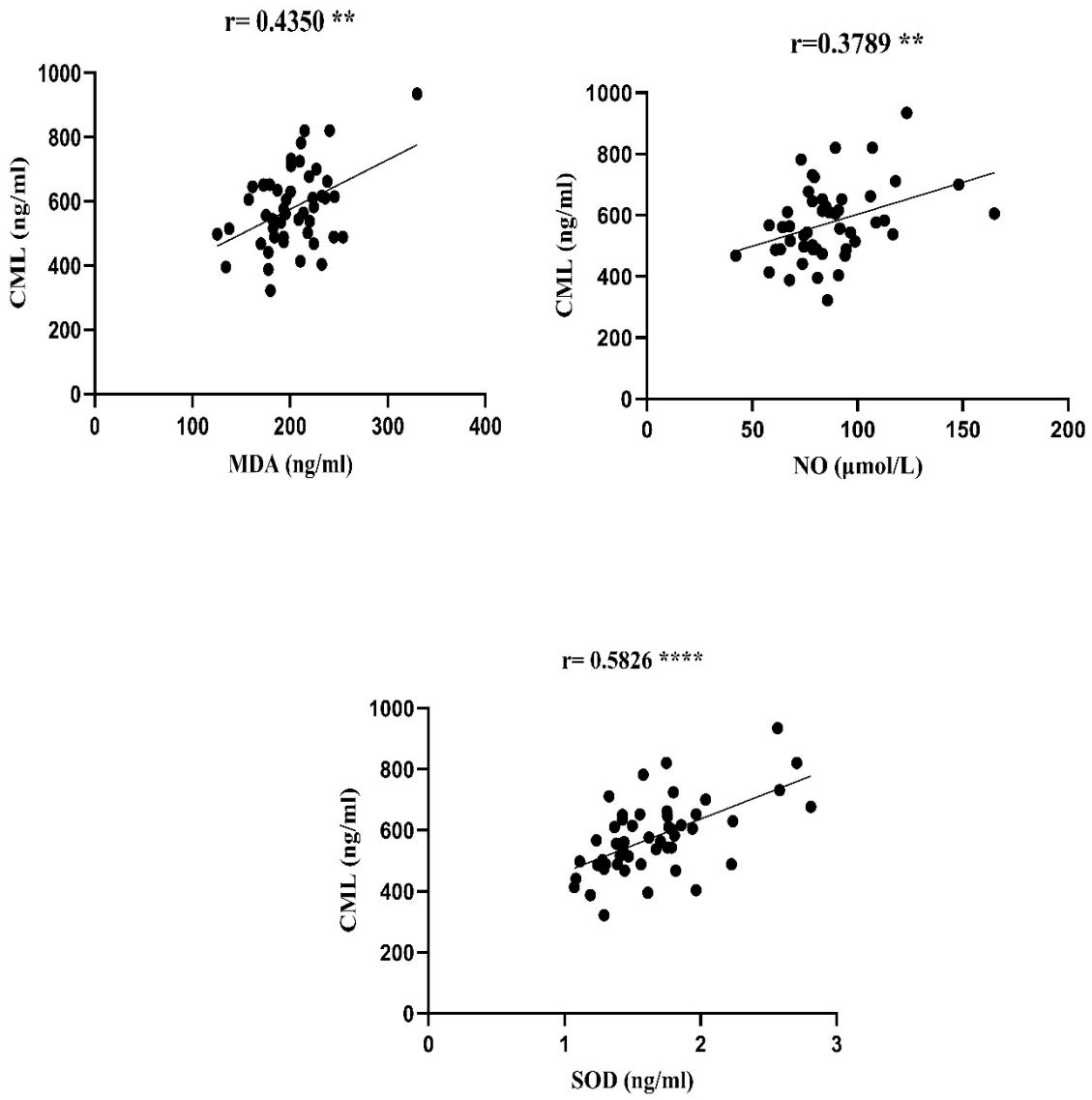

Figure 2: Correlation between CML with MDA, NO, and SOD.

The data of our study show that there is positive and significant correlation between PTD and LDL (p = 0.0403). Even though there are positive but not significant correlations between serum PTD with MDA, NO, SOD, CAT, CH, TG, HDL, and VLDL. The p values are (0.0574, 0.7067, 0.8715, 0.1332, 0.0936, 0.3131, 0.3513, and 0.1609) respectively, as seen in (Table 3).

Table (3) Correlations analysis between CML with MDA, NO, SOD, CAT, CH, TG, HDL, LDL, and VLDL in patients with atherosclerosis:

Parameters	Correlation coefficient (r) (Pearson correlation)	P value
PTD and MDA	0.2733	0.0574
PTD and NO	0.0563	0.7067
PTD and SOD	0.0237	0.8715
PTD and CAT	0.2199	0.1332
PTD and CH	0.2922	0.0936
PTD and TG	0.1906	0.3131
PTD and HDL	0.1703	0.3513
PTD and LDL	0.3898	0.0403
PTD and VLDL	0.2673	0.1609

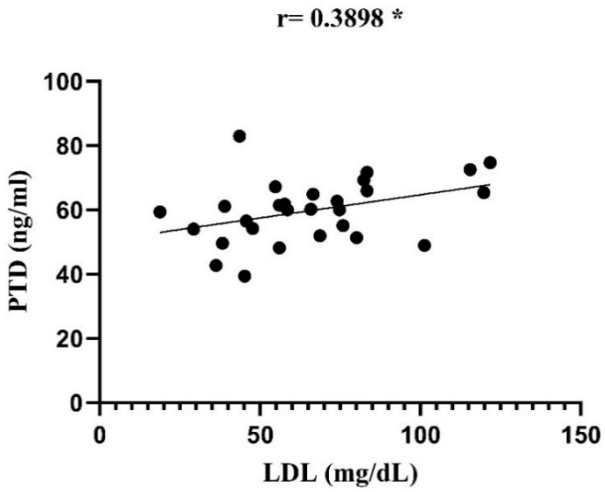

Figure 3: Correlation of PTD with LDL.

For determining the diagnostic accuracy of CML, PTD, MDA, NO, CH, TG, HDL, LDL, and VLDL, the ROC curve analysis was suggested. The AUC value in serum of CML is 0.6861. The S.E value is 0.06251 and the 95% CI value is 0.5636 to 0.8086, (p= 0.0067). While the AUC value in serum of PTD is 0.6606. The S.E value is 0.06666 and the 95% CI value is 0.5300 to 0.7913, (p=0.0211), they exhibit that

both of them are good but not potential biomarkers for atherosclerosis. The data exhibit that MDA could be a good biomarker with an AUC value of 0.8220 for atherosclerosis. The S.E value is 0.04739, the 95% CI value are 0.7291 to 0.9149, and ($p<0.0001$). While the AUC value in serum NO is 0.6690. The S.E, 95%CI, and p values are 0.06493, 0.5417 to 0.7962, and 0.0156 respectively. These data show that the serum NO is good but not a potential biomarker for atherosclerosis as shown in (Table 4).

Table (4) ROC curve analysis was used to determine the diagnostic accuracy of CML, PTD, MDA, NO, CH, TG, HDL, LDL, and VLDL in patients with atherosclerosis:

Parameters	AUC	S.E	95%CI	P value
CML (ng/ml)	0.6861	0.06251	0.5636 to 0.8086	0.0067
PTD (ng/ml)	0.6606	0.06666	0.5300 to 0.7913	0.0211
MDA (ng/ml)	0.8220	0.04739	0.7291 to 0.9149	<0.0001
NO (µmol/L)	0.6690	0.06493	0.5417 to 0.7962	0.0156
CH (mg/dL)	0.5039	0.1020	0.3039 to 0.7039	0.9692
TG (mg/dL)	0.5528	0.1068	0.3434 to 0.7622	0.6066
HDL (mg/dL)	0.6391	0.09707	0.4489 to 0.8294	0.1711
LDL (mg/dL)	0.5372	0.1145	0.3127 to 0.7617	0.7144
VLDL (mg/dL)	0.5333	0.1093	0.3191 to 0.7475	0.7462

5. Conclusion:

The present study demonstrates that serum levels of AGE, oxidative stress, and antioxidant parameters in patients with atherosclerosis are significantly decreased compared to the healthy group. The serum level of lipid profile with exception of HDL in patients with atherosclerosis are not significantly increased rather than in the healthy group, this could due to the medication for the atherosclerosis patients. MDA has proven to be a good biomarker for atherosclerosis.

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