Effect of Oxidative Stress Malondialdehyde and Antioxidant in Coronary Artery Diseases with/without Non-Alcoholic Fatty Liver in Karbala Heart Center

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ABSTRACT

Background: Up to one-third of adults worldwide may have non-alcoholic fatty liver disease (NAFLD), which is a public health issue. Numerous cohort studies have repeatedly demonstrated that the main causes of death in NAFLD patients are cardiovascular diseases (CVDs), and that NAFLD (especially in its more advanced stages) is associated with a higher risk of all-cause mortality than extrahepatic tumors and liver-related issues. An expanding body of evidence indicates that NAFLD is significantly associated with an increased risk of major CVD events and other cardiac issues. (i.e., cardiomyopathy, cardiac arrhythmias, and cardiac valvular calcification), independent of conventional cardiovascular risk factors. This article provides a summary of the research on the evidence pointing to a connection between NAFLD and a higher risk of cardiovascular disease, and the impact of MDA and Antoxidants in non-alcoholic fatty liver patients and patients with coronary arteries.

Objective: To know the extent to which non-alcoholic fatty liver disease effects and its relationship to coronary artery obstructive disease and assessment of MDA, TAC, GPX, and Selenium levels and their impact on both NAFLD and CAD.

Materials and Methods: This study was a case-control study that included 120 samples (male and female) and their serum samples were collected from Kerbala Heart Center / Kerbala Health Directorate l Kerbala - Iraq with ages ranging between 40 to 73 years. the number of patients with coronary arteries was 60 and the number of healthy people was 60 The number of patients was 37 with non-alcoholic fatty liver disease and 23 without NAFD people in both case and control. The serum withdrawn was kept at -20 °C., The liver function test, lipid profile, and albumin were determined at optimized conditions in the laboratory of the Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala by using the Automated Biochemistry analyzer to measure liver function and examine albumin, while the MDA, TAC, and GPX biomarkers was determined by Chemical methods Selenium measured by atomic absorption.

Results: The observed levels of MDA increased in non-alcoholic fatty liver disease, steatosis, and Coronary artery disease, Results indicated a significant difference in MDA level among groups, The mean levels of MDA in patient was (2.43 ± 0.90) which was significantly higher than for control group (2.15 ± 0.52), ($p \leq 0.001$). The level of MDA risk factor to liver inflammation and fibrosis, whereas, and increased risk factor of chronic coronary artery disease and antioxidants were protactive in CAD.
Conclusion: The results presented here contribute to the determination of the functions of the physiological MDA risk factor to the liver fat and chronic coronary artery obstructive disease, and antioxidant is decreased from NAFLD and CAD.
.**Keywords : Nonalcoholic fatty liver disease, Coronary artery disease, Malondialdehyde.**

الخلاصة

"تشير مجموعة متزايدة من الأدلة إلى أن NAFLD قد يعاني ما يصل إلى ثلث البالغين في جميع أنحاء العالم من مرض الكبد الدهني غير الكحولي(NAFLD) ، وهي مشكلة صحية عامة. أظهرت العديد من الدراسات الجماعية مرارًا وتكرارًا أن الأسباب الرئيسية للوفاة لدى مرضى NAFLD هي أمراض القلب والأوعية الدموية(CVDs) ، وأن NAFLD) خاصة في مراحله الأكثر تقدمًا) يرتبط بزيادة خطر الوفيات لجميع الأسباب مقارنة بالأورام خارج الكبد والقضايا المتعلقة بالكبد. تشير مجموعة متزايدة من الأدلة إلى أن NAFLD مرتبط بشكل كبير بزيادة خطر الإصابة بأمراض القلب والأوعية الدموية الرئيسية ومشاكل القلب الأخرى. (أي اعتلال عضلة القلب، وعدم انتظام ضربات القلب، والتكلس الصمامي القلبي)، بغض النظر عن عوامل الخطر القلبية الوعائية التقليدية. تقدم هذه المقالة ملخصًا للبحث حول الأدلة التي تشير إلى وجود صلة بين NAFLD وارتفاع خطر الإصابة بأمراض القلب والأوعية الدموية، وتأثير MDA و Antoxidants على مرضى الكبد الدهني غير الكحوليين ومرضى الشرايين التاجية.

الهدف: معرفة مدى تأثير مرض الكبد الدهني غير الكحولي وعلاقته بمرض انسداد الشريان التاجي، وتقييم مستويات

وتأثيرها على كل من مرض الكبد الدهني غير الكحولي ومرض انسداد الشرايين التاجية GPX,TAC,Se, MDA

المواد والطرق: كانت هذه الدراسة عبارة عن دراسة تحكم في الحالة شملت 120 عينة (ذكور وإناث) وتم جمع عينات مصل من مركز كربلاء للقلب/مديرية كربلاء الصحية في كربلاء - العراق التي تتراوح أعمارها بين 40 و 73 عامًا. كان عدد المرضى الذين يعانون من الشرايين التاجية 60 وعدد الأشخاص الأصحاء 60. كان عدد المرضى 37 مصابًا بمرض الكبد الدهني غير الكحولي و 23 مريضًا بدون NAFD في كل من الحالة والسيطرة. تم الاحتفاظ بالمصل المسحوب عند -20 درجة مئوية، اختبار وظائف الكبد، تم تحديد ملامح الدهون، والألبومين في الظروف المثلى في مختبر قسم الكيمياء والكيمياء الحيوية، كلية الطب، جامعة كربلاء باستخدام محلل الكيمياء الحيوية الآلي لقياس وظائف الكبد وفحص الألبومين، بينما تم تحديد المؤشرات الحيوية MDA و TAC و GPX بالطرق الكيميائية Selenium المقاسة بالامتصاص الذري.

النتائج: زادت مستويات MDA الملحوظة في أمراض الكبد الدهنية غير الكحولية، وداء الدهون، ومرض الشريان التاجي، أشارت النتائج إلى اختلاف كبير في مستوى MDA بين المجموعات، وكان متوسط مستويات MDA عند المريض (2.43 ± 0.90) والذي كان أعلى بكثير من المجموعة الضابطة (2.15 ± 0.52)، (.(0.001 ≥ pكان مستوى عامل خطر MDA لالتهاب الكبد والتليف، بينما كان عامل الخطر المتزايد لأمراض الشريان التاجي المزمنة ومضادات الأكسدة بروتينيًا في CAD

.الاستنتاج: النتائج المعروضة هنا تساهم في تحديد وظائف عامل الخطر الفسيولوجي لـ MDA لدهون الكبد ومرض انسداد الشريان التاجي المزمن، وانخفاض مضادات الأكسدة من NAFLD و.CAD.

الكلمات المفتاحية: مرض الكبد الدهني غير الكحولي، مرض الشريان التاجي، مالونلديالدهيد

INTRODUCTION

Coronary artery obstructive disease is a leading cause of death worldwide, both in low- to middle-income countries. It occurs at younger and older ages. Over time, this disease doubles in adults over 30 years of age **[1].** Coronary artery disease (CAD), also known as Ischemic heart disease (IHD), refers to a group of symptoms that are all closely related to one another and are caused by an imbalance between the blood supply and oxygen demand in the myocardium. One of four syndromes could develop into angina pectoris (chest pain), acute myocardial infarction, sudden cardiac death, or persistent ischemic heart disease with congestive heart failure, depending on the severity of coronary artery constriction and the myocardial reaction **[2].** Atherosclerosis refers to the buildup of plaque in arteries. Plaque in the arteries can burst from obstructions and impede blood flow, which can result in a heart attack. In the inner lining of the coronary artery wall, where lipoproteins interact with the extracellular matrix, inflammation and mild atherosclerotic plaques are produced [**3].** Cell death, extracellular lipid buildup in the artery wall of the lesion, and calcium deposition happen as the inflammation gets worse **[4].** The constriction of the coronary channel is caused by atherosclerotic plaque thickening **[5].** It causes ischemia by limiting blood flow and oxygen-rich blood supply to the heart muscles. Atherosclerotic lesions with dense fibrous caps and calcification but with relatively smaller lipid cores may slowly produce ischemia due to the gradual plaque volume development that encroaches on the coronary lumen diameter. However, some atherosclerotic lesions with larger lipid cores and thinner fibrous caps are more likely to rupture, releasing their contents into the coronary lumen and leading to the development of thrombus that can completely clog the lumen and limit myocardial blood flow **[4**]. Acute myocardial infarction (MI) is the result, in which the heart muscles are killed by a protracted lack of oxygen **[6].**

Oxidative stress plays an essential role in the pathogenesis of chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer. Long-term exposure to increased levels of pro-oxidant factors can cause structural defects at a mitochondrial DNA level, as well as functional alteration of several enzymes and cellular structures leading to aberrations in gene expression. The modern lifestyle associated with processed food, exposure to a wide range of chemicals, and lack of exercise play an important role in oxidative stress induction. However, the use of medicinal plants with antioxidant properties has been exploited for their ability to treat or prevent several human pathologies of which oxidative stress seems to be one of the causes **[7].**

Free radical oxidants such as reactive oxygen species, reactive nitrogen species, and reactive sulfur species are produced inside cells through various metabolic processes. The body is equipped with an antioxidant defense system that guards against oxidative damage caused by these reactive oxidants and plays a major role in protecting cells from oxidative stress and damage. Antioxidants such as glutathione (GSH), thioredoxin, ascorbic acid, and enzymes, for example, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) counter oxidative stress, and protect lipids, proteins, and DNA. Antioxidants such as tocopherols, ascorbic acid, carotenoids, flavonoids, and amino acids are also natural antioxidants present in foods. There is increasing demand and availability of designer foods fortified with antioxidants and probiotics that may be important in human health. The review article presents a brief overview of oxidants and antioxidant systems inside the human body including the role of probiotics and inflammation **[8].**

Lipid peroxidation often occurs in response to oxidative stress, where reactive oxygen species (ROS) cause the oxidation of lipids containing carbon-carbon double bonds in membrane lipid bilayers. ROS may be defined as “derivatives of molecular oxygen that occur as a normal attribute of aerobic life” **[10]**. Lipid peroxidation occurs in three major phases: initiation, chain propagation, and termination. A single initiation reaction will result in 200–400 propagation cycles, giving rise to unsaturated aldehydes 4-hydroxy-2-nominall and acrolein), dialdehydes (malondialdehyde and glyoxal), and ketoaldehydes (4-oxo-2-nominal and isoketals) **[9].** Some of them are highly reactive and are considered second toxic messengers, which disseminate and magnify oxidative damage **[11].**The

most commonly determined aldehydes so far are 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). The former is endowed with the highest biological activity and the latter, highly produced during lipid peroxidation, is commonly used as a measure of oxidative stress **[12].** Arachidonic acid (AA) is the main precursor of bicyclic endoperoxide, although other eicosanoids should be able to Ref **[13].**

Malondialdehyd**e** MDA and 4-HNE originate from polyunsaturated fatty acids when a carbon-carbon double bond is attacked by a free radical, resulting in the formation of unsaturated lipid radicals with H2O release. Further O2 capture will lead to the formation of peroxyl radicals and lipid hydroperoxides. On the one hand, the peroxyl radical can develop cyclization thanks to their cis-double bond homoallylic to the peroxyl group. The intermediate free radicals formed after cyclization can cyclize again to form bicycle endoperoxides, structurally related to prostaglandins, and undergo cleavage to produce MDA(Figure 1).

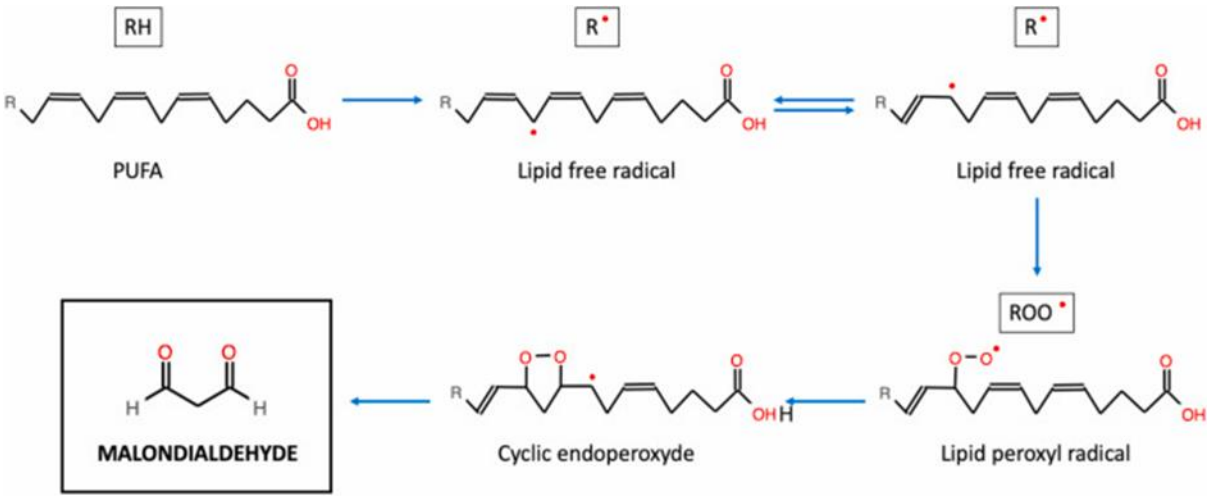

Fig. 1. MDA formation through lipid peroxidation **[14].**

MATERIALS AND METHODS

This study was Case-Control Study Where it included 120 samples of serum, patient samples were collected from Kerbala Heart Center, taking into account the Ethical approval of samples from the patient, Of both sexes And different weights They range in age from 40 to 73 years the number of patients 60 with Coronary artery disease among them non-alcoholic fatty liver disease was 37 and 23 without non-alcoholic fatty liver disease and the control group included 37 with NAFLD, 23 healthy people From relatives Also both sexes where The sample type was serum. have conducted a Mesencephalic Astrocyte-Derived Neurotrophic Factor, liver function tests, lipid profile, and albumins in the laboratory of the College of Medicine, University of Karbala in optimized conditions and the use of modern devices and tools. We used the Automated Bio Chemistry device to measure liver function and examine albumin and We did the MANF test by using the Eliza Sandwich technique.

SAMPLE COLLECTION:

Five milliliters of venous blood were drawn from fasting subjects by using a sterile disposable syringe. Blood was put into a gel tube and left at room temperature for nearly 5 minutes for clotting, then separated into four Eppendorf tubes and stored at -20 °C in the deep freezer. Two tubes were used to measure serum MDA, GPX and TAC by chemical methods, one tube was used to measure lipid profile, and one tube was to measure liver function test by using the colorimetric method.

Body mass index (BMI) was calculated by the following equation:

BMI (kg/m2) = weight / (height)2

Patients were classified into normal (BMI 18.5-24.9), overweight (25-29.9), and obese (30-34.9) **[15] (Donini *et al*., 2020)**.

RESULTS:

A total of 120 participants were included in this study 60 patients and 60 control, which were divided into subgroups based on Age, gender, and. The participant demographic characteristics and laboratory parameters of the study groups were summarized in Table (3.1).

(21.66%) the age range of participants was within (40 -50) years old, (45%) of the patient were within (51- 60) years, while (33.34%) of the patient were within the age range (More than 60). The gender distribution of the participants was (75%) male and (25 %) female. Also, the analysis of data illustrated that most patients (18.33%) had Metabolic syndrome, patients (83.33%) had a sedentary lifestyle, and 61.66% of the patients group had fatty liver all as shown in figure(2)

(1) **(2)**

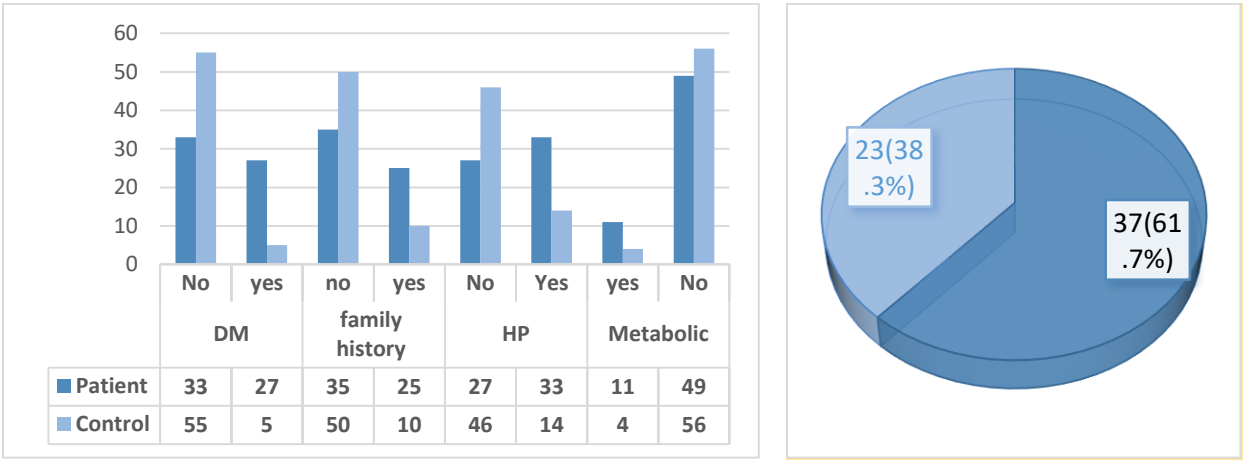

Figure 1: Baseline traits and demographic data Descriptive of the study's participants in patients compared to the control group, the number of participants (n= 120): Chronic disease

Figure 2: Baseline traits and demographic data descriptive of the study's participants in patients, the number of participant (n= 60): Fatty liver and non-fatty liver.

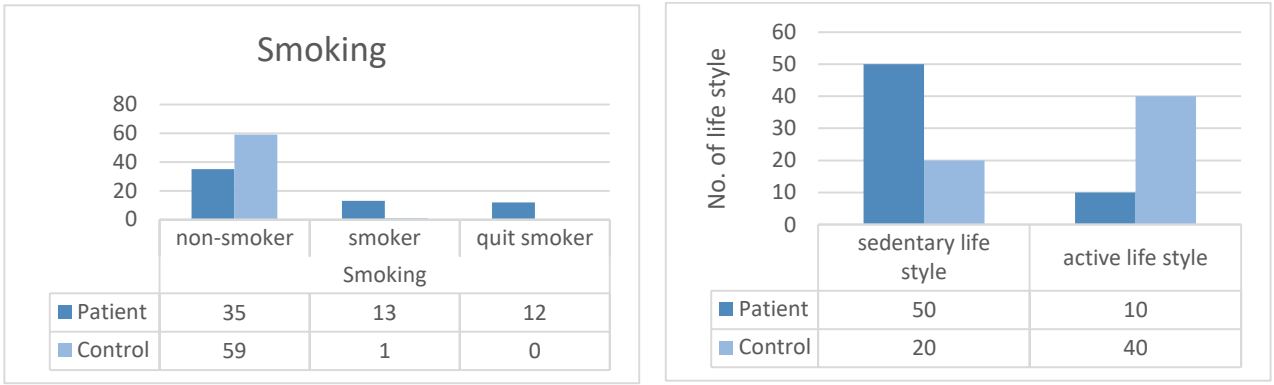

Figure 3: Baseline traits and demographic data Descriptive of the study's participants in patients compared to the control group, the number of participants (n= 120): (Smoking & Lifestyle) groups

Generally, patients with coronary were shown an increasing range level of the MDA when compared to the healthy control groups, while the range level of TAC, GPX, and Selenium were decreased compared to healthy control

Results indicated a significant difference in MDA level among groups.

The means and standard deviations were presented in (2.43±0.90). The Distribution of serum levels of TAC, GPX, and Selenium in patients compared to the healthy control group were (773.33±191.13),(359.82±115.69)and (71.41±24.16) presented in figure (4).

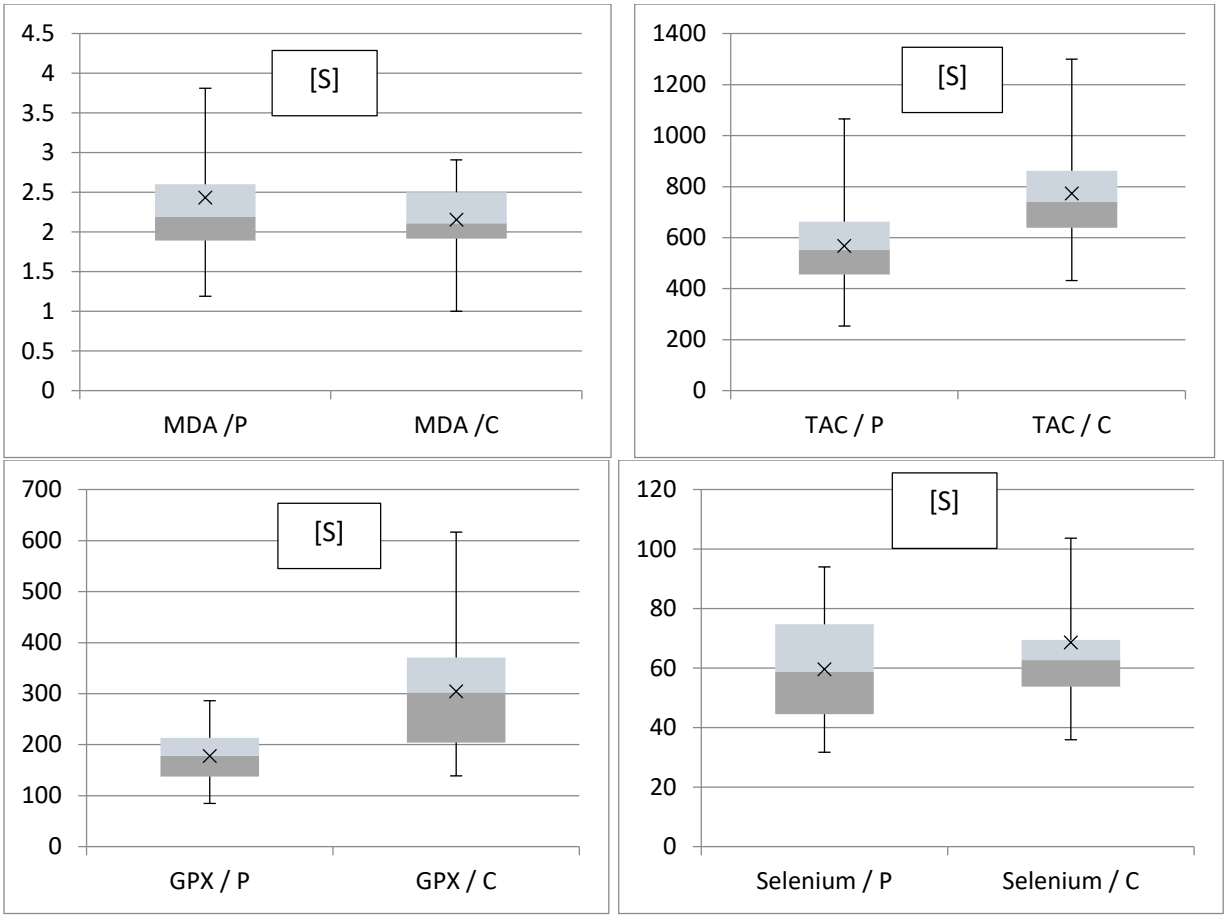

Figure 4: Boxplot of the Distribution of serum level of biochemical in coronary with patients and control groups (T-test was S= significant at p ≤ 0.05, NS= Non-significant).

Multinomial logistic regression was performed and forward logistic regression was adopted to analyze the results. The correlation coefficient was used for determining linear relationships between biochemical markers in patient groups compared to the control group. It was found that the biomarkers MDA in the patient was a risk factor and highly difference significant OR: 3.239; 95% CI: (0.504-20.821), while the TAC, GPX, and Selenium in the patient was a protective factor ((OR: 0. 999; 95% CI: (0.993-1.004), (OR: 0.974; 95% CI: (0.962-0.987) and (OR: 0.947; 95% CI: (0.904-0.992). Finally, all biomarkers were shown to be significantly correlated with coronary artery disease.

Table 1: Estimation the Associated of the analyzed factors in Patients Compared to the control

Variable	Groups	OR (Lower – upper)	P value
MDA µmol/l			
	Coronary with F.L	3.239(0.504-20.821)	**<0.001[S]**
	Coronary without F.L	2.87(0.425-19.399)	**<0.001[S]**
	Non-coronary with F.L	3.627(0.629-20.909)	**<0.001[S]**
	Non-coronary without F.L	1[a]	-
TAC µmol/l			
	Coronary with F.L	0.999(0.993-1.004)	0.617[NS]
	Coronary without F.L	0.998(0.992-1.005)	0.641[NS]
	Non-coronary with F.L	1.003(0.998-1.008)	0.287[NS]

•ROC curve and AUC analysis for the MDA for

	Non-coronary without F.L	1[a]	-
GPX u/l			
	Coronary with F.L	0.974 (0.962-0.987)	**<0.001[S]**
	Coronary without F.L	0.963(0.947-0.979)	**<0.001[S]**
	Non-coronary with F.L	0.993(0.985-1.002)	0.124[NS]
	Non-coronary without F.L	1[a]	-
Selenium ppb			
	Coronary with F.L	0.947(0.904-0.992)	**<0.021[S]**
	Coronary without F.L	0.965(0.916-1.016)	0.171[NS]
	Non-coronary with F.L	0.992(0.961-1.024)	0.622[NS]
	Non-coronary without F.L	1[a]	-
p<0.05 [S]= Significant, [NS]= Non significant, 1[a] : reference category is Control			

Patients compared to the control group

Results of the receiver operating curve (ROC) curve and AUC analysis for the MDA diagnostic parameters. MDA was shown a good performance for prediction patients compared to the control group, data are presented in Table (2).

For MDA levels (sensitivity 30%, specificity 96.7%) at a level = 2.76, the p-values of the AUC were <0.05 and highly statistically significant

Table 2: Receiver operating characteristic curve showing sensitivity and specificity of MANF & MDA in patients compared to the control group

Variable	AUP	Sensitivity %	Specificity %	Youden index	Cut-off points	CI (95%)	PPV	NPV
MDA	55.30%	30%	96.70%	0.2	2.76	0.522-0.733	70%	58.82 %

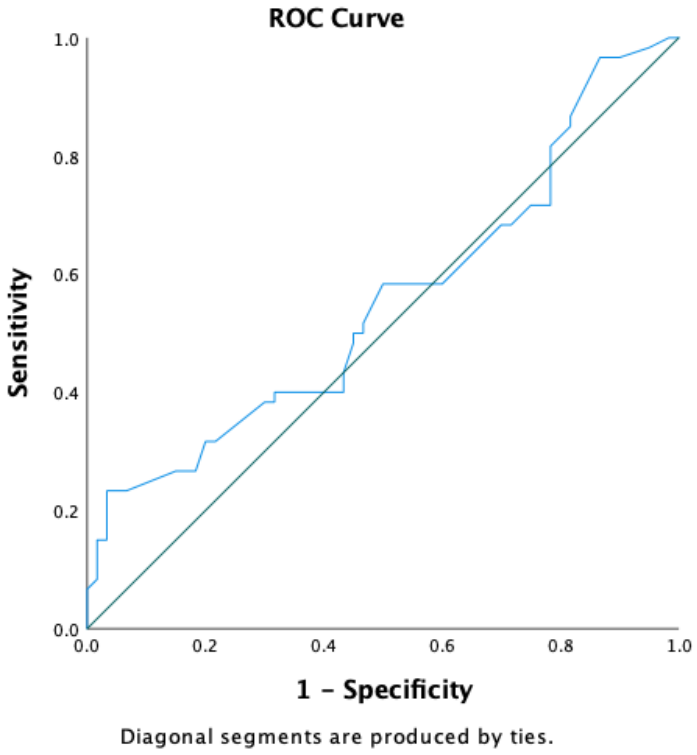

Figure 1: Receiver operating characteristics (ROC) curve analysis of MDA levels in the patient with control groups

DISCUSSION

Through our current study, we focused on chronic coronary artery obstructive disease because this disease is a leading cause of death worldwide, as this disease multiplies in adults over the age of 30 years**[1]**.where we found in this that men are more affected than women, where the rate of injury with CAD of men 75 Percentage**,** where every three men correspond to the injury of one woman, as well as the rate of men with NAFLD is higher than women, where the proportion of men was 75.7 Percentage and women 24.3 Percentage as other studies have shown that men are higher incidence of coronary artery disease and NAFLD than women before menopause, due to the presence of estrogen, which is a protective factor for women before menopause women are more affected by CAD and NAFLD in older women**[16,17,18].** Through our study, we examined the effect of non-alcoholic liver fat disease on coronary artery disease. In this study, we found that 61.7 percent of chronic coronary artery patients suffer from NAFLD, and this large percentage indicates an association between or a risk factor for coronary artery disease, and this is what a previous study showed in 2019 that NAFLD is associated with cardiovascular disease **[19].** Another study in 2021 showed that the pathophysiology behind NAFLD association with cardiovascular disease is not fully understood and may include other pathways besides insulin resistance **[20].** Through this study, we noticed the effect of risk factors for coronary artery disease, including type II diabetes, and we found that there are a number of people with chronic coronary artery disease suffering from type II diabetes This indicates that diabetes is a risk factor for coronary artery disease, in addition to other risk factors such as smoking, lifestyle, medical history and high blood pressure, which are considered a risk indicator for CAD as other studies have shown **[21,22,23].** Through our study, we also addressed the effect of oxidative stress represented by the level of Malondialdehyde MDA the final product of fat oxidation (lipide peroxidation) and did not have an increase in its levels in patients compared to control as The oxidative stress induced by uncontrolled production of ROS and altered antioxidant systems has a key role in the pathogenesis of ACVDs, such as CAD, HF, or heart valve disorders among others. Additionally, the fact that the heart is a highly oxidative organ makes it more susceptible to the accumulation of lipid peroxidation and lipoxidation products contributing to the onset of ACVDs. Indeed, the excessive production of ROS stimulates the oxidative modification of LDL and the loss of HDL cardioprotective properties, increasing the inflammatory response and deregulating cell growth and vascular tone. and core-aldehydes promote the inflammatory process underlying atherosclerosis, macrophage recruitment endothelial dysfunction, extracellular matrix deposition, and platelet aggregation accelerating atherosclerosis and compromising atherosclerotic plaque stability. In the case of heart failure, both lipid peroxidation products and the further adducted proteins have been associated with cardiac hypertrophy, apoptosis, or contractile dysfunction, thus playing a role in the development and progression of the pathology As a previous study showed **[24],** Through this study, we have not seen the effect of antioxidants and its relationship with coronary artery disease, where we have noticed low levels of antioxidants represented by (GPX,

TAC, Se). This is because the decrease in antioxidant levels leads to an imbalance between the oxidant represented by MDA and antioxidant and thus leads to an increase in free radicals ROS causing the disease **[25].** A previous study found that patients with cardiovascular disease had lower GPX levels compared to healthy people **[26].** There are other studies that have shown that TAC level is decreased in CAD **[27].** As for selenium, studies have not yet found any effect on coronary artery disease **[28].** The main function of primary antioxidants is to donate hydrogens to the lipid-free radical, which turns itself into a free radical. The antioxidant free radical then can react with other lipid peroxide radicals or other antioxidant free radicals to finish the reaction. Several primary antioxidants are endogenous in food systems **[29].** we found lower GPx concentrations in obese versus non-obese patients. This is confirmed by previous studies **[30].** we observed in these studies that the results between BMI and antioxidant levels were contradictories Although the relationship between increased oxidative stress and the development of disease and complication in obesity has been revealed in numerous studies, there are limited studies investigating the relationship between the dietary antioxidant levels and serum TAC levels of the obese individuals of which a study in 2020 corresponds to our current study **[31].** As for selenium, previous studies have agreed with the results of our current study, where obesity affects the decrease in the level of selenium, including a study in ng role for selenium supplementation in Brazilian patients with obesity, especially for those using statins **[32]** When comparing the results of patients taking blood lipid-lowering treatments with patients without treatments, Brazil that showed the magnitude of obesity in the Brazilian population, the influence of obesity in selenium deficiency, the role of the environment as a determinant factor for selenium deficiency, and how the combination of these factors could increase the risk of muscle symptoms derived from statin use. There is potentially a promising found that antioxidant levels were higher with treatment than with patients without treatment and MDA level had decreased with treatment than in patients without treatment, and this indicates that lipid reduction therapy reduced lipid oxidation and reduced bad cholesterol by reducing the release of free radicals and thus reduced the association of antioxidants with free radicals, so the level of antioxidant increased in patients. With treatment, this increase was noticeable and statistically significant As previous studies have shown). Excessive ROS can be generated in vascular cells from NAD(P)H oxidase (Nox), nitric oxide synthases (NOS) uncoupling, and mitochondria, which cause oxidative modifications of low-density lipoprotein (LDL) [**33, 34].**

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