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Evaluation of Oxidative Status and Antioxidants in Patients with Coronary Artery Disease

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ABSTRACT

Background: Oxidative stress is the result of enhanced production of reactive oxygen species (ROS), which are the key molecules in the signaling pathways implicated in vascular inflammation in atherogenesis, starting from the initiation of fatty streak formation to lesion progression and plaque rupture. ROS are established to damage the fundamental biomolecules in cells including DNA, proteins and lipids.

Objective: To evaluate the relationship between plasma levels of 8-isoprostaglandin F2-alpha as a marker of oxidative status and the severity of coronary artery disease (CAD) whether or not accompanied with hypertension and/or dyslipidemia.

Patients and Methods: This study was carried out from October 2020 to October 2021 at Medical Biochemistry Department, Faculty of Medicine, Al-Azhar University, Cardiac Department in Al-Azhar University Hospital, Egyptian National Heart Institute, Cardiac Department of Police Hospital and Cardiac Department of Minia University Hospital, on one hundred and twenty-five subjects.

Results: There was high statistically significant difference between the studied groups as regard systolic BP, diastolic BP and mean arterial pressure blood (MAPB). There was high statistically significant difference between studied groups as regard TC, LDL and triglycerides (TAG). There was high statistically significant difference between studied groups as regard 8-Isoprostane and blood vitamin C. There was high statistically significant difference between studied groups as regard coronary artery disease (CAD) severity.

Conclusion: Oxidative stress may play an important role in the pathogenesis of CAD. A high 8-isoPGF2α is a strong and independent risk factor for presence of CAD.

Keywords: Oxidative status, Antioxidants, Coronary artery diseases, reactive oxygen species.

INTRODUCTION

Atherosclerosis, the formation of plaque inside the arteries, is the main cause of CAD [(1)].

Several pathological events contribute to atherosclerosis, including endothelial dysfunction, extensive lipid deposition in the tunica intima, exacerbated innate and adaptive immune responses, vascular smooth muscle cell proliferation and remodeling of the extracellular matrix [(2)].

Two major hypotheses have been proposed to describe the origin of atherosclerosis:

(i) the thrombogenic theory, which suggests that thickening of the intima layer of vessels is a result of the organization of fibrin by fibroblasts, associated with secondary lipid enrichment; and (ii) the lipogenic theory, which suggests that the deposition of lipid inside the arterial walls is caused by an imbalance between the mechanisms responsible for lipid accumulation and removal [(3)].

Several lines of study have indicated a role for oxidative stress in atherosclerosis and cardiovascular diseases (CVDs)[(4)]. Oxidative stress is the result of enhanced production of reactive oxygen species (ROS), which are the key molecules in the signaling pathways implicated in vascular inflammation in atherogenesis, starting from the initiation of fatty streak formation to lesion progression and plaque rupture [(5)].

Reactive oxygen species (ROS) are established to damage the fundamental biomolecules in cells including DNA, proteins and lipids [(6)]. A previous report demonstrated that oxidative modification of low-density lipoprotein (LDL) is a key mechanism rendering lipoproteins atherogenic [(7)].

It has been reported that lipid peroxidation produces unsaturated aldehydes including acrolein and malondialdehyde (MDA), which exert toxic effects due to their reactivity with nucleophile compounds and their ability to produce protein and DNA adducts without prior metabolic activation. These aldehydes are considered to function as mediators of inflammation and vascular dysfunction [(8)].

There are a several key cellular and circulating antioxidant systems, including the superoxide dismutases, glutathione peroxidases, and catalase that collectively reduce superoxide/hydrogen peroxide (or lipid hydroperoxides) to water (or lipid hydroxides). There are also many important small-molecule antioxidants such as α-tocopherol, ascorbic acid, β-carotene, and reduced glutathione (GSH). Thus, when antioxidant activity is decreased or small molecule antioxidant availability is limited, oxidant stress may occur as a result of diminished net antioxidant capacity [(9)].

AIMS OF THE STUDY

The study is aimed to evaluate the relationship between plasma levels of 8-isoprostaglandin F2-alpha (also called isoprostane F2- alpha) as a marker of oxidative status and the severity of coronary artery disease (CAD) whether or not accompanied with hypertension and/or dyslipidemia. In addition, also, we selected blood vitamin C as antioxidant and studied its

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relation to the severity of coronary artery disease (CAD).

PATIENTS AND METHODS

This study was carried out from October 2020 to October 2021 at Medical Biochemistry Department, Faculty of Medicine, Al-Azhar University, Cardiac Department in Al-Azhar University Hospital, Egyptian National Heart Institute, Cardiac Department of Police Hospital and Cardiac Department of Minia University Hospital, on one hundred and twenty-five subjects, divided in to five groups: four groups include one hundred diagnosed as coronary artery disease patients by CT angiography, cardiac catheterization, changes in echocardiogram and elevated troponin levels (classified according to their blood pressure and lipid profile in to four group each group include twenty-five patients) and control group include twenty-five subjects. They were prospectively recruited for this study.

Ethical consent:

An approval of the study was obtained from Al-Azhar University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

SUBJECTS OF THE STUDY

1. **Patients:**

We classified the patients according to the association of hypertension and/or hyperlipidemia with CAD into four groups: (i) Normotensive normolipidemic CAD patients (25 cases). (ii) Hypertensive normolipidemic CAD patients (25 cases). (iii) Normotensive hyperlipidemic CAD patients (25 cases), and (iv) Hypertensive hyperlipidemic CAD patients (25 cases).

- Hypertension defined as resting systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg or in the presence of active treatment with antihypertensive agents [(10)].
- LDLc guidelines 6 (NCEP): optimal: 100 mg/dl, near optimal: 100-129 mg/dl, border line high: 130-159 mg/dl, high: 160- 189mg/dl and very high:> 190 mg/dl.
- Hyperlipidemia defined as LDL-C > 160 at age 20-39 years with ASCVD risk or family history of ASCVD or LDL-C≥ 70mg/dl- <190mg/dl at age 40-75 years without diabetes mellitus or with 10 years ASCVD risk or persistent elevated TAG≥ 175mg/dl [(11)].

2. **Controls:** We select 25 healthy subjects as a control group.

Exclusion criteria:

1- Evidence of hemodynamically significant valvular heart Disease.
2- Surgery or trauma within the previous month.
3- Known cardiomyopathy.
4- Diabetes mellitus.
5- Renal failure.
6- Known cancer.
7- Febrile conditions.
8- Use of oral anticoagulant therapy within the previous four weeks.

Specimen collection:

Ten ml venous blood samples would collect from each subject and transferred and dived into two Vacationer tubes one tube contain 5ml for lipid profile and vitamin C another contain 5ml on EDTA for 8 isoprostane f2 alpha assay. Centrifugation of samples for vitamin c and lipid profile was done and transferred its serum in to Eppendorf and stored at -20c° until assay time. The samples for 8 isoprostane f2 Alpha assay were centrifuged for 15 minutes at 1000×g and the supernatant stored at -20c° until analysis time.

Methods:

All patients and control groups are subjected to the following:

- Personal medical history concerning age, sex and family history of CAD.
- Clinical examination including measurement of blood pressure and body mass index (BMI). BMI was calculated using the following formula [(12)]: Weight (kg)/ (Height (m)) ^{2}.
- Estimation of the severity of the CAD and the extent of coronary narrowing using a score (0 - 4) which depends on the stenotic segments in the major coronary arteries, A score for the severity of the CAD was performed according to the number of extents of coronary narrowing in addition, the number of stenotic segments. The degree of stenosis in each segment was scored, 1 point for > 25-50 % reduction, 2 points for> 50-75 % reduction. 3 points for > 75-90 % reduction in vessel diameter, and 4 points for >90% stenosis. Scores for each segment was added and the extent score of coronary stenosis was represented as the sum of the scores of all segments[(13)].

Laboratory investigations

1- Determination of the levels of lipids (total cholesterol, low density lipoprotein and TAG) by enzymatic colorimetric assay.
2- Determination of the plasma levels of 8-Isoprostane by Competitive-ELISA.
3- Determination of the serum levels of vitamin C by Competitive-ELISA.

I- Determination of the serum levels of lipids (total cholesterol, low density lipoprotein and TAG):

A- Assay of serum level of total cholesterol: The determination of serum total cholesterol after enzymatic hydrolysis and oxidation by using Spectrum Company products. The colorimetric indicator is quinoneimine, which is generated from phenol and 4-aminoantipyrine by hydrogen peroxide under the catalytic action of peroxidase and the color was measured at 546 nm [(14)].

B- *Determination of serum level of HDL cholesterol:* Chylomicrons, VLDL and LDL were precipitated by adding phosphotungestic acid and magnesium ions to the samples by using Spectrum Company products. Centrifugation leaves only the HDL in the supernatant, their cholesterol content was determined enzymatically using cholesterol reagent [(15)].

C- *Determination of serum level of LDL cholesterol:* The serum level of LDL cholesterol was determined by the friedewald formula [(16)].

D- Determination of the serum triglycerides: Determination of the serum triglycerides after enzymatic splitting with lipoprotein lipase by using Spectrum Company products. Indicator is quinoneimine which is generated from 4-chlorophenol and 4-aminoantipyrine by hydrogen peroxide under the catalytic action of peroxidase to form a red color quinoneimine dye which was measured at 546 nm [(17)].

II- Determination of plasma level of 8 isoprostaneF2*α*: 8 isoprostane F2α Levels were measured using the ELISA method provided by Gamma trade, G-Biosciences, USA, Catalog Number: IT4758.

III-Determination of serum levels of vitamin C by Competitive-ELISA: Vitamin C Levels were measured using the ELISA method provided by Gamma trade, Abbexa LTD, Cambridge, UK Catalog No: abx156668.

Statistical Analysis:

Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. According to the type of data; qualitative data was represented as number and percentage, quantitative continues group data was represented by mean ± SD.

The following tests were used to test differences for significance; correlation by Pearson's correlation or Spearman's. Chi-square test: For categorical variables, to compare between different groups. Monte Carlo correction: Correction for chi-square when more than 20% of the cells have expected count less than 5.

F-test (ANOVA): For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) (LSD) for pairwise comparisons. Kruskal Wallis test: For abnormally distributed quantitative variables, to compare between more than two studied groups and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. ROC curve: Receiver operating characteristic (ROC), or simply ROC curve, is a graphical plot which illustrates the performance of a binary classifier system as its discrimination threshold is varied. It is created by plotting the fraction of true positives out of the positives (TPR = true positive rate) vs. the fraction of false positives out of the negatives (FPR = false positive rate), at various threshold settings. TPR is also known as sensitivity (also called recall in some fields), and FPR is one minus the specificity or true negative rate.

RESULTS

This table shows that CAD patients Group I, the mean of ages was 41.4 year (± 16.1 SD) with range (26.0 – 57.0), there were 15(60%) males, 10(40%) females, the mean of BMI was 26.2 (Kg/m^2) (± 2.1 SD). CAD patients Group II, the mean of ages was 43.0 (± 15.9 SD), there were 17(68%) males, 8(32%) females, the mean of BMI was 26.9 (± 2.5 SD). In CAD patients Group III, the mean of ages was 46.2 (±18.2 SD), there were 18(72%) males, 7(28%) females, the mean of BMI was 25.7 (Kg/m^2) (±2.3 SD). In CAD patients Group IV, the mean of ages was 43.6 (± 15.2 SD), there were 19(76%) males, 6(24%) females, the mean of BMI was 27.8 (Kg/m^2) (± 1.9 SD). In control group, the mean of ages was 44.2 (± 13.5 SD), there were 19(76%) males, 6(24%) females, the mean of BMI was 26.2 (± 2.7 SD). There was statistically significant difference between studied groups as regard BMI (Table 1).

Table (1): Comparison between the studied different groups according to demographic data

Demographic data	CAD patients (n = 100)								Control (n = 25)		Test of Sig.	P
	Group I (n =25)		Group II (n =25)		Group III (n =25)		Group IV (n =25)					
Age (years)												
Min. – Max.	26.0 – 57.0		27.0 – 60.0		28.0 – 65.0		29.0 – 58.0		30.0 – 58.0		F= 0.305	0.873
Mean ± SD.	41.4 ± 16.1		43.0 ± 15.9		46.2 ± 18.2		43.6 ± 15.2		44.2 ± 13.5			
Gender	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Male	15	60.0	17	68.0	18	72.0	19	76.0	19	76.0	χ^2= 2.150	0.708
Female	10	40.0	8	32.0	7	28.0	6	24.0	6	24.0		
BMI (Kg/m^2)												
Mean ± SD.	26.2 ± 2.1		26.9 ± 2.5		25.7 ± 2.3		27.8 ± 1.9		26.2 ± 2.7		F= 3.227*	0.015*
p_0	1.000		0.748		0.961		0.085					
Sig. bet. Groups	p_1=0.795,p_2=0.940,p_3=0.104,p_4=0.331,p_5=0.660,p_6=0.013*											

χ^2: Chi square test **IQR:** Inter Quartile Range **SD**: Standard deviation
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**
p: p value for comparing between the studied different groups
p_0: p value for comparing between Control and each other groups
p_1: p value for comparing between **Group I** and **Group II**
p_2: p value for comparing between **Group I** and **Group III**
p_3: p value for comparing between **Group I** and **Group IV**
p_4: p value for comparing between **Group II** and **Group III**
p_5: p value for comparing between **Group II** and **Group IV**
p_6: p value for comparing between **Group III** and **Group IV**
*****: Statistically significant at $p \leq 0.05$

Group I: Normotensive normolipidemic CAD patients
Group II: Hypertensive normolipidemic CAD patients
Group III: Normotensive hyperlipidemic CAD patients
Group IV: Hypertensive hyperlipidemic CAD patients

This table shows that, the mean of systolic BP in patients Group I was 119.2 (± 8.6 SD), the mean of diastolic BP was 72.4 (± 7.8 SD), the mean of MAPB was 88.0 (±5.7 SD). Among patients Group II, the mean of Systolic BP was 170.0 (± 14.1 SD), the mean of diastolic BP was 103.2 (± 8.5 SD), the mean of MAPB was 125.5 (± 7.9 SD). The mean of systolic BP in patients Group III was 119.6 (± 8.4 SD), the mean of diastolic BP was 68.4 (± 9.0 SD), the mean of MAPB was 85.5 (± 7.1 SD). Among patients Group IV, the mean of systolic BP was 161.2 (± 17.4 SD), the mean of diastolic BP was 100.8 (± 10.0 SD), the mean of MAPB was 120.9 (± 9.0 SD). The control group, showed that the mean of systolic BP was 117.2 (± 7.9 SD), the mean of diastolic BP was 71.2 (± 7.3 SD), the mean of MAPB was 86.5 (± 5.5 SD). There was high statistically significant difference between studied groups as regard systolic BP, diastolic BP and MAPB (Table 2).

Table (2): Comparison between the studied different groups according to examination

Examination	Patients (n = 100)				Control (n = 25)	H	P
	Group I (n =25)	Group II (n =25)	Group III (n =25)	Group IV (n =25)			
Systolic BP						92.855*	<0.001*
Mean ± SD	119.2 ± 8.6	170.0 ± 14.1	119.6 ± 8.4	161.2 ± 17.4	117.2 ± 7.9		
p_0	0.635	<0.001*	0.561	<0.001*			
Sig. bet. Groups	p_1<0.001*,p_2=0.915,p_3<0.001*,p_4<0.001*,$p_5$0.435,p_6<0.001*						
Diastolic B						93.830*	<0.001*
Mean ± SD	72.4 ± 7.8	103.2 ± 8.5	68.4 ± 9.0	100.8 ± 10.0	71.2 ± 7.3		
p_0	0.752	<0.001*	0.504	<0.001*			
Sig. bet. Groups	p_1<0.001*,p_2=0.325,p_3<0.001*,p_4<0.001*,p_5=0.768,p_6<0.001*						
MAPB						91.302*	<0.001*
Mean ± SD.	88.0 ± 5.7	125.5 ± 7.9	85.5 ± 7.1	120.9 ± 9.0	86.5 ± 5.5		
p_0	0.610	<0.001*	0.730	<0.001*			
Sig. bet. Groups	p_1<0.001*,p_2=0.393,p_3<0.001*,p_4<0.001*,p_5=0.464,p_6<0.001*						

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

IQR: Inter Quartile Range **SD**: Standard deviation

p: p value for comparing between the studied different groups
p_0: p value for comparing between **Control and each other groups**
p_1: p value for comparing between **Group I** and **Group II**
p_2: p value for comparing between **Group I** and **Group III**
p_3: p value for comparing between **Group I** and **Group IV**
p_4: p value for comparing between **Group II** and **Group III**
p_5: p value for comparing between **Group II** and **Group IV**
p_6: p value for comparing between **Group III** and **Group IV**
*: Statistically significant at $p \leq 0.05$

Group I: Normotensive normolipidemic CAD patients
Group II: Hypertensive normolipidemic CAD patients
Group III: Normotensive hyperlipidemic CAD patients
Group IV: Hypertensive hyperlipidemic CAD patients

This table shows that among patients Group I, the mean of TC was 148 (± 25.7 SD), the mean of LDL was 63.07 (± 5.08 SD), the mean of TAG was 100.8 (± 26.9 SD). In patients Group II, the mean of TC was 152.6 (± 25.1 SD), the mean of LDL was 63.6 (± 4.3 SD), the mean of TAG was 107.3 (± 27.04 SD). The mean of TC in patients Group III was 278.8 (± 22.8 SD), the mean of LDL was 191.1 (± 20.4 SD), the mean of TAG was 239.4 (± 21.2 SD). The mean of TC was 282.4 (± 22.3 SD), the mean of LDL was 187.1 (± 17.7 SD), the mean of TAG was 237.7 (± 19.1 SD) in Patients Group IV. Among control group, the mean of TC was 141.08 (± 23.6 SD), the mean of LDL was 60.84 (± 6.04 SD), the mean of TAG was 101.1(± 31.03 SD). There was high statistically significant difference between studied groups as regard TC, LDL and TAG (Table 3).

Table (3): Comparison between the studied different groups according to lab investigation

Lab investigation	Patients (n = 100)				Control (n = 25)	Test of Sig.	P
	Group I (n =25)	Group II (n =25)	Group III (n =25)	Group IV (n =25)			
TC (mg/dl)							
Mean ± SD.	148 ± 25.7	152.6 ± 25.1	278.8 ± 22.8	282.4 ± 22.3	141.08 ± 23.6	H= 91.720*	<0.001*
p_0	0.750	0.773	<0.001*	<0.001*			
Sig. bet. Groups	p_1=0.544,p_2<0.001*,p_3<0.001*,p_4<0.001*,p_5<0.001*,p_6=0.788						
LDL (mg/dl)							
Mean ± SD.	63.07 ± 5.08	63.6 ± 4.3	191.1 ± 20.4	187.1 ± 17.7	60.84 ± 6.04	F= 81.630*	<0.001*
p_0	1.000	1.000	<0.001*	<0.001*			
Sig. bet. Groups	p_1=0.999,p_2<0.001*,p_3<0.001*,p_4<0.001*,p_5<0.001*,p_6=0.966						
TAG (mg/dl)							
Mean ± SD.	100.8 ± 22.9	107.3 ± 27.04	239.4 ± 21.2	237.7 ± 19.1	101.1 ± 3.03	F= 113.306*	<0.001*
p_0	0.627	0.775	<0.001*	<0.001*			
Sig. bet. Groups	p_1=0.084,p_2<0.001*,p_3p_2<0.001*,p_4p_2<0.001*,p_5p_2<0.001*,p_6=0.998						

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**
H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

IQR: Inter Quartile Range SD: **Standard deviation**
p: p value for comparing between the studied different groups
p_0: p value for comparing between **Control and each other groups**
p_1: p value for comparing between **Group I** and **Group II**
p_2: p value for comparing between **Group I** and **Group III**
p_3: p value for comparing between **Group I** and **Group IV**
p_4: p value for comparing between **Group II** and **Group III**
p_5: p value for comparing between **Group II** and **Group IV**
p_6: p value for comparing between **Group III** and **Group IV**
*: Statistically significant at $p \leq 0.05$
Group I: Normotensive normolipidemic CAD patients
Group II: Hypertensive normolipidemic CAD patients
Group III: Normotensive hyperlipidemic CAD patients
Group IV: Hypertensive hyperlipidemic CAD patients

This table shows that among patients Group I, the mean of 8-Isoprostane was 490.2 (± 295.5 SD), the mean of blood vitamin C was 0.7 (± 0.3SD). Among, the mean of 8-Isoprostane patients Group II was 552.6(± 298.0 SD), the mean of blood vitamin C was 0.6 (± 0.2 SD). Among patients Group III, the mean of 8-Isoprostane was 617.2 (± 340.5 SD), the mean of blood vitamin C was 0.5 (± 0.2 SD). Among patients Group IV, the mean of 8-Isoprostane was 693.1 (± 301.3 SD), the mean of blood vitamin C was 0.3(± 0.2SD). Among control group, the mean of 8-Isoprostane was 17.7 (± 9.6 SD), the mean of blood vitamin C was 1.3 (± 0.4 SD). Comparison between the different groups as regard to plasma level of 8-Isoprostane (pg/ml) show high statistically significant difference between those studied groups (p value <0.001*). Comparison between the different groups as regard serum level of vitamin c (mg/dl) show high statistically significant difference between those studied groups (p value <0.001*) (Table 4).

Table (4): Comparison between the studied different groups according to lab investigation "continue"

Lab investigation	Patients (n = 100)				Control (n = 25)	Test of Sig.	p
	Group I (n =25)	Group II (n =25)	Group III (n =25)	Group IV (n =25)			
8-Isoprostane (pg/ml)							
Mean ± SD.	490.2 ± 95.5	552.6 ± 98.0	617.2 ± 40.5	693.1 ± 31.3	17.7 ± 9.6	F= 23.105*	<0.001*
p_0	<0.001*	<0.001*	<0.001*	<0.001*			
Sig. bet. Groups	p_1=0.931,p_2=0.078,p_3=0.486,p_4=0.382,p_5=0.922,p_6=0.868						
Blood vitamin C (mg/dl)							
Mean ± SD.	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.02	1.3 ± 0.4	H= 23.834*	<0.001*
p_0	0.041*	<0.001*	0.007*	<0.001*	<0.001*		
Sig. bet. Groups	p_1=0.068,p_2=0.376,p_3=0.013*,p_4=0.347,p_5=0.507,p_6=0.109						

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**
H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**
IQR: Inter Quartile Range SD: **Standard deviation**

p: p value for comparing between the studied different groups
p_0: p value for comparing between **Control and each other groups**
p_1: p value for comparing between **Group I** and **Group II**
p_2: p value for comparing between **Group I** and **Group III**
p_3: p value for comparing between **Group I** and **Group IV**
p_4: p value for comparing between **Group II** and **Group III**
p_5: p value for comparing between **Group II** and **Group IV**
p_6: p value for comparing between **Group III** and **Group IV**
*: Statistically significant at $p \leq 0.05$

Group I: Normotensive normolipidemic CAD patients
Group II: Hypertensive normolipidemic CAD patients
Group III: Normotensive hyperlipidemic CAD patients
Group IV: Hypertensive hyperlipidemic CAD patients

This table shows that among patients Group I, according to CAD severity, 9(36.0%) were > 25-50 % reduction, 10(40.0%) were > 50-75 % reduction, 6(24.0%) were > 75-90 % reduction, 0(0.0%) were >90%, the mean of CAD severity was 1.9 (± 0.8 SD). Among patients Group II, according to CAD severity, 8(32.0%) were > 25-50 % reduction, 5(20.0%) were > 50-75 % reduction, 10(40.0%) were > 75-90 % reduction, 2(8.0%) were >90%, the mean of CAD severity was 2.2 (± 1.0 SD). Among patients Group III, according to CAD severity, 5(20.0%) were > 25-50 % reduction, 6(24.0%) were > 50-75 % reduction, 11(44.0%) were > 75-90 % reduction, 3(12.0%) were >90%, the mean of CAD severity was 2.5 (± 1.0 SD). Among patients Group IV, according to CAD severity, 0(0.0%) were > 25-50 % reduction, 7(28.0%) were > 50-75 % reduction, 11(44.0%) were > 75-90 % reduction, 7(28.0%) were >90%, the mean of CAD severity was 3.0 (± 0.8 SD). There was high statistically significant difference between studied groups as regard CAD severity (Table 5).

Table (5): Comparison between the studied different groups according to CAD severity

CAD Severity	Patients (n = 100)								Test of Sig.	p
	Group I (n =25)		Group II (n =25)		Group III (n =25)		Group IV (n =25)			
	No.	%	No.	%	No.	%	No.	%		
> 25-50 % reduction	9	36.0	8	32.0	5	20.0	0	0.0	χ^2= 22.867*	MCp= 0.004*
> 50-75 % reduction	10	40.0	5	20.0	6	24.0	7	28.0		
> 75-90 % reduction	6	24.0	10	40.0	11	44.0	11	44.0		
>90%	0	0.0	2	8.0	3	12.0	7	28.0		
Mean ± SD.	1.9 ± 0.8		2.2 ± 1.0		2.5 ± 1.0		3.0 ± 0.8		H= 17.340*	<0.001*
Sig. bet. Groups	p_1=0.163,p_2=0.025*,p_3<0.001*,p_4=0.397,p_5=0.008*,p_6=0.068									

χ^2: **Chi square test** MC: **Monte Carlo**

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test).**

IQR: Inter Quartile Range SD: **Standard deviation**

p: p value for comparing between the studied different groups

p_1: p value for comparing between **Group I** and **Group II**

p_2: p value for comparing between **Group I** and **Group III**

p_3: p value for comparing between **Group I** and **Group IV**

p_4: p value for comparing between **Group II** and **Group III**

p_5: p value for comparing between **Group II** and **Group IV**

p_6: p value for comparing between **Group III** and **Group IV**

*: Statistically significant at $p \leq 0.05$

Group I: Normotensive normolipidemic CAD patients

Group II: Hypertensive normolipidemic CAD patients

Group III: Normotensive hyperlipidemic CAD patients

Group IV: Hypertensive hyperlipidemic CAD patients

DISCUSSION

In this study, we aimed to evaluate the relationship between plasma levels of 8-isoprostaglandin F2-alpha (also called isoprostane F2-alpha) as a marker of oxidative status and the severity of coronary artery disease (CAD) whether or not accompanied with hypertension and/or dyslipidemia. In addition, we selected blood vitamin C as antioxidant and we studied its relation to the severity of CAD.

An imbalance between production and neutralization of ROS[19,20]. There are reports describing the differences in PGF2 and PGE2 concentrations in affected tissues [18,21]. The levels of 8-iso-PGF2, determined in the present study, were high, this could be due to an increase in lipid peroxidation [18]. The activity of phospholipase A2 measured in affected tissues was lower in retained than normal [22]. The levels of tissue arachidonic acid determined in the same experiment were also lower, while the levels of linoleic acid were higher than normal. It may confirm the disturbances in arachidonic acid cascade resulting in alterations in prostaglandin levels [22]. May also confirm the present study because the concentrations of previously examined 18:2 and 20:4 fatty acids were lower in patients than normal. The differences in the concentrations of determined tissue fatty acids between normal and cardiovascular patients reflect the alterations in the concentrations of 8-iso-PGF2 described here. **Touzard *et al.*** [23] showed that the secretion of 8-iso-PGF2 by oxidative stressed tissues. It is worth mentioning that in vitro experiments of **Jamioł *et al.*** [24] showed the same previously result predominance of PGF production by oxidative stressed tissues. **Baydar *et al.*** [25] reported that total plasma antioxidant activity decreased in oxidative stressed tissues. They described the differences in red blood cell defense mechanisms against ROS between normal and patients. Such differences were measured by the activity of enzymatic and the concentration of non-enzymatic anti oxidative defense systems [26].

This study showed that among CAD patients Group I, the mean of ages was 41.4 year (± 16.1 SD), there were 15(60%) males, 10(40%) females, the mean of BMI was 26.2(Kg/m^2) (± 2.1 SD). Among CAD patients Group II, the mean of ages was 43.0 (± 15.9 SD), there were 17(68%) males, 8(32%) females, the mean of BMI was 26.9 (± 2.5 SD). Among CAD patients Group III, the mean of ages was 46.2 (± 18.2 SD), there were 18(72%) males, 7(28%) females, the mean of BMI was 25.7 (± 2.3 SD). Among CAD

patients Group IV, the mean of ages was 43.6 (± 15.2 SD), there were 19(76%) males, 6(24%) females, the mean of BMI was 27.8 (± 1.9 SD). Among control group, the mean of ages was 44.2 (± 13.5 SD), there were 19(76%) males, 6(24%) females, the mean of BMI was 26.2 (± 2.7 SD). There was statistically significant difference between studied groups as regard BMI.

In agreement with our results **Serdar *et al.*** [27] showed that there were no significant differences in age and family history between the groups. But it also showed no significant differences in BMI which is in contrast to our results. It included 54 controls (25 men and 29 women, ages between 32 and 78 years) and 154 /CAD patients (122 men and 32 women, ages between 39 and 81 years). Although the presentation of conventional risk factors was higher in the CAD group, significant differences were observed only in dyslipidemic, diabetic and smoking subjects.

Matching our results, **Kals *et al.*** [28] showed that there was no significant difference between the patients and the controls in age. However, there was a significant difference in BMI.

Wang *et al.* [29] showed that of the total 241 patients who were examined by angiography, 169 were CAD (+) and 72 were free of significant CAD. In the CAD (+) group, 65 had 1-vessel disease, 61 had 2-vessel disease, and 43 had 3-vessel disease. Patients with CAD, as compared to the patients without CAD, were of older age and had a smaller proportion of females and a higher proportion of subjects with hypertension and diabetes.

Similar results were reported by **Wolfram *et al.*** [30] who showed that age, the prevalence of male gender, and current smoking status were comparable in all groups.

Our study showed that among patients Group I, the mean of systolic BP was 119.2 (± 8.6 SD), the mean of diastolic BP was 72.4 (± 7.8 SD), the mean of MAPB was 88.0 (±5.7 SD). Among patients Group II, the mean of systolic BP was 170.0 (± 14.1 SD), the mean of diastolic BP was 103.2 (± 8.5 SD), the mean of MAPB was 125.5 (± 7.9 SD). Among patients Group III, the mean of systolic BP was 119.6 (± 8.4 SD), the mean of diastolic BP was 68.4 (± 9.0 SD), the mean of MAPB was 85.5 (± 7.1 SD). Among patients Group IV, the mean of Systolic BP was 161.2 (± 17.4 SD), the mean of diastolic BP was 100.8 (± 10.0 SD), the mean of MAPB was 120.9 (± 9.0 SD). Among control group, the mean of systolic BP was 117.2 (± 7.9 SD), the mean of diastolic BP was 71.2 (± 7.3 SD), the mean of MAPB was 86.5 (± 5.5 SD). There was high statistically significant difference between studied groups as regard systolic BP, diastolic BP and MAPB.

In agreement with our results, **Vassalle *et al.*** [31] showed that there is a significant difference in 8-isoPGF2α levels was observed between patients with and without hypertension (394.2 ± 42.7 and 232.7 ± 25.1 pg /ml, $P < 0.01$, respectively)

Gross *et al.* [32] showed that partial correlations of F2-isoprostanes, adjusted for race and sex, were r = 0.12 with systolic blood pressure, and r = 0.09 with current smoking ($P < 0.001$ for each correlation), but there were no significant correlations with LDL-C.

Wang *et al.* [29] showed that the analysis in the total population revealed a significant difference in 8-iso-PGF2 levels in patients with hypertension relative to non-hypertensive patients ($P < 0.001$), in patients with and without diabetes ($P < 0.05$).

In the present study we found that among patients Group I, the mean of TC was 148 (± 25.7 SD), the mean of LDL was 63.07 (± 5.08 SD), the mean of TAG was 100.8 (± 26.9 SD), among patients Group II, the mean of TC was 152.6 (± 25.1 SD), the mean of LDL was 63.6 (± 4.3 SD), the mean of TAG was 107.3 (± 27.04 SD), among patients Group III, the mean of TC was 278.8 (± 22.8 SD), the mean of LDL was 191.1 (± 20.4 SD), the mean of TAG was 239.4 (± 21.2 SD), among patients Group IV, the mean of TC was 282.4 (± 22.3 SD), the mean of LDL was 187.1 (± 17.7 SD), the mean of TAG was 237.7 (± 19.1 SD), among control group, the mean of TC was 141.08 (± 23.6 SD), the mean of LDL was 60.84 (± 6.04 SD), the mean of TAG was 101.1(± 31.03 SD). There was high statistically significant difference between studied groups as regard TC, LDL and TAG.

In contrast to our study **Kals *et al.*** [28] showed that there was no significant difference between the patients and the controls in total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glucose or EIDV.

Wang *et al.* [29] showed that higher TG, LDL-C, and CRP levels as well as lower HDL-C were found in CAD(+) respect to the CAD(−). As total cholesterol (TC), triglycerides (TG), HDLC, and LDL-C concentrations were measured enzymatically (First Chemical Co. Japan) on a specific automated analyzer (Olympus Au2700). CRP was determined using an enzyme-linked immunosorbent assay kit (Quantikine, R&D Systems).

Wolfram *et al.* [30] showed that TC levels were comparable between all groups. As for HDL cholesterol, the highest value was found, as expected, within the healthy control group at 55 F 10, with a significant difference as compared to the CHD and CMP groups (43 F 8, 45 F 8, and 43 F 8, $p < 0.001$, respectively).

Serdar *et al.* [27] showed that LDL-C, triglyceride, apo B and Lp(a) levels were elevated with increased stenosed vessel number, HDL-cholesterol and apoAI levels were decreased. Plasma MDA had a positive correlation with LDL-C, apo B, Lp(a) and had a negative correlation with HDL-C, apo AI, antioxidant enzymes (except GPx) and vitamins. Erythrocyte MDA had a positive correlation with total cholesterol, LDL-C and Lp(a) and had a negative correlation with antioxidant enzymes (except G6PD and arylesterase) and vitamin E and carotenoids. Δ- MDA had a positive

correlation with total cholesterol, LDLC, apo B and Lp(a) and had a negative correlation with HDLC, erythrocyte GPx, PON, vitamin C and carotenoids. Serum protein carbonyls had a positive correlation with LDL-C and triglyceride and had a negative correlation with HDL-C, Apo AI, antioxidant enzymes (except GPx and G6PD) and vitamin E.

This study showed that among patients Group I, the mean of 8-Isoprostane was 490.2 (± 295.5 SD), the mean of Blood vitamin C was 0.7 (± 0.3SD). Among patients Group II, the mean of 8-Isoprostane was 552.6(± 298.0 SD), the mean of blood vitamin C was 0.6 (± 0.2 SD). Among patients Group III, the mean of 8-Isoprostane was 617.2 (± 340.5 SD), the mean of blood vitamin C was 0.5 (± 0.2 SD). Among patients Group IV, the mean of 8-Isoprostane was 693.1 (± 301.3 SD), the mean of blood vitamin C was 1.1 (± 0.9 SD). Among control group, the mean of 8-Isoprostane was 17.7 (± 9.6 SD), the mean of blood vitamin C was 1.3 (± 0.4 SD).

Comparison between the different groups as regard to plasma level of 8-Isoprostane (pg/ml) show high statistically significant difference between those studied groups (p value <0.001). Comparison between the different groups as regard to serum level of vitamin c (mg/dl) show high statistically significant difference between those studied groups (p value <0.001)

Similar results had been conducted by **Wang *et al.*** [(29)] who showed that there is a significant difference between the 8-iso-PGF2α concentrations in the CAD(+) and CAD(−) groups (337.7 ± 80.2 and 263.8 ± 74.2 pg/ml and P < 0.001).

Similar results were reported by **Serdar *et al.*** [(27)] who showed that in patients with CAD, we found significantly lowered levels of vitamin C, vitamin E and carotenoids compared with the controls, whereas there was no significant difference between one, two and three vessel CAD groups. Serum protein carbonyls had a positive correlation with LDL-C and triglyceride and had a negative correlation with HDL-C, Apo AI, antioxidant enzymes (except GPx and G6PD), vitamin C and vitamin E.

In agreement with our results, **Kals *et al.*** [(28)] showed that there was significant difference between the patients and the controls in F2-IsoPs (also in creatinine indexed F2-IsoPs).

This study showed that among patients Group I, according to CAD severity, 9(36.0%) were > 25-50 % reduction, 10(40.0%) were > 50-75 % reduction, 6(24.0%) were > 75-90% reduction, 0(0.0%) were >90%, the mean of CAD severity was 1.9 (± 0.8 SD). Among patients Group II, according to CAD Severity, 8(32.0%) were > 25-50% reduction, 5(20.0%) were > 50-75% reduction, 10(40.0%) were > 75-90% reduction, 2(8.0%) were >90%, the mean of CAD Severity was 2.2 (± 1.0 SD). Among patients Group III, according to CAD severity, 5(20.0%) were > 25-50% reduction, 6(24.0%) were > 50-75 % reduction, 11(44.0%) were > 75-90% reduction, 3(12.0%) were >90%, the mean of CAD severity was 2.5 (± 1.0 SD). Among patients Group IV, according to CAD severity, 0(0.0%) were > 25-50 % reduction, 7(28.0%) were > 50-75% reduction, 11(44.0%) were > 75-90% reduction, 7(28.0%) were >90%, the mean of CAD severity was 3.0 (± 0.8 SD). There was high statistically significant difference between studied groups as regard CAD severity.

Aksoy *et al.* [(33)] showed that all patients underwent coronary angiography. Severe CAD (≥ 70% narrowing in at least one major coronary artery > 2 mm) was observed in all subjects. Twenty five (59.5%) patients underwent percutaneous coronary intervention (PCI), 5 (11.9%) patients received coronary artery bypass surgery, and medical treatment alone was given to 12 (28.5%) subjects. In the medical treatment group, 7 (16.6%) patients had noncritical coronary lesions likely after spontaneous clot lysis and recanalization of the culprit lesion. Five (11.9%) patients had critical coronary artery lesions that were not amenable to either PCI or surgery. The SYNTAX score was calculated for all subjects. Thirty (71.4%) subjects displayed a low SYNTAX score (< 22) and 12 (28.5%) subjects had moderate to high SYNTAX scores (≥ 22).

Limitations of the study: Some limitations of this study also should be considered. The study group consisted of consecutive patients undergoing coronary angiography for suspected CAD, who were divided into two groups with and without CAD. Some patients without CAD have more risk factors and differ in unknown ways from healthy controls. These may have caused the possibility of recruitment bias and may have confounded the results.

CONCLUSION

Oxidative stress may play an important role in the pathogenesis of CAD. A high 8-isoPGF2α is a strong and independent risk factor for presence of CAD. There is significant relation between high levels of Plasma 8-iso-PGF2α (represent oxidative stress) levels and low level of vitamin c (represent antioxidants) with severity of CAD.

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