Free Radical Research, April 2006; 40(4): 385–391

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Plasma 8-iso-prostaglandin $F_{2\alpha}$, a marker of oxidative stress, is increased in patients with acute myocardial infarction*

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Accepted by Professor B. Halliwell

(Received 19 December 2005)

Abstract

Background: Oxidative stress has been implicated in the pathogenesis of atherogenesis. The aim of our study is to examine whether the plasma 8-iso-prostaglandin $F_{2\alpha}$ level, a marker of oxidative stress, is elevated in patients with acute myocardial infarction.

Methods: Three groups of patients were enrolled: (1) patients with no or minimal coronary artery disease (CAD) ($n = 15$); (2) patients with stable CAD ($n = 31$); (3) patients with acute myocardial infarction ($n = 13$).

Results: Plasma 8-iso-prostaglandin $F_{2\alpha}$ levels were significantly elevated ($p < 0.001$) in patients with acute myocardial infarction (290.7 ± 73.9 pg/ml) as compared to patients with stable CAD ($182.0 + 75.7$ pg/ml) and patients with no significant CAD (118.9 ± 85.5 pg/ml). This remained significant after correcting for coronary atherosclerosis risk factors, age, extent of atherosclerosis, and C-reactive protein (CRP) level.

Conclusion: Plasma 8-iso-prostaglandin $F_{2\alpha}$ levels are elevated in patients with acute myocardial infarction. Endogenous oxidative stress may contribute to the pathogenesis of atherosclerosis and its complications, namely myocardial infarction.

Keywords: *Oxidative stress, myocardial infarction, isoprostanes, artherosclerosis*

Introduction

Cardiovascular disease is the major cause of mortality and morbidity in North America [1]. Traditional risk factors are less than ideal in identifying and explaining the pathogenesis of atherosclerosis. The prevalence of traditional risk factors such as hyperlipidemia, smoking, diabetes and hypertension in patients with documented atherosclerosis is only slightly higher compared to that in elderly patients with no known atherosclerotic disease [2]. Thus, the need for a common mechanism that can better explain the pathogenesis of atherosclerosis and its complications continues to emerge. There is a growing body of evidence to suggest that inflammation is playing a major role in the initiation and progression of atherosclerosis [3–7].

One specific marker of inflammation, C-reactive protein (CRP), has attracted attention with accumulating evidence implicating its role in the pathogenesis of atherosclerosis [8,9]. Interestingly, CRP was found to mediate the production of reactive oxygen species (ROS) by macrophages and smooth muscle cells [10,11], underscoring the important role of oxidative

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*Grant support: NIH K24 HL-69840 and NIH R01 HL-63911.

ISSN 1071-5762 print/ISSN 1029-2470 online © 2006 Taylor & Francis
DOI: 10.1080/10715760500539154

stress in inflammation. In fact, the role of ROS as mediators and markers of inflammation has become recognized with accumulating evidence implicating oxidation in atherogenesis [12].

Under normal conditions, low levels of ROS are generated in response to growth factors and cytokines. Both enzymic and non-enzymic mechanisms operate to protect against ROS toxicity under normal homeostasis. However, under pathophysiological states, ROS production can exceed the scavenging ability of antioxidant systems and result in an oxidative stress that damages DNA, proteins and lipids of the cells.

Recently, isoprostanes have emerged as a reliable measure of oxidant injury *in vivo* [13–16]. Isoprostanes are prostaglandin isomers formed *in situ* in cell membranes through the free radical catalyzed peroxidation of the ubiquitous polyunsaturated fatty acid, arachidonic acid, independently of the activity of the cyclo-oxygenase [16,17]. Potential free radicals that are generated by a number of cellular enzymatic systems (e.g. the mitochondrial electron transport system, the NAD(P)H system, etc.) result in the formation of the isoprostanes [16]. The F_2-isoprostanes are regarded as stable and accurate measure of oxidative stress [13].

Several human studies have demonstrated a relationship between increased circulating or urinary F_2-isoprostanes and many of the risk factors linked to atherosclerosis such as cigarette smoking [18], hyperlipidemia [19], diabetes mellitus [20] and obesity [21,22].

Urine F_2-isoprostanes were found to be significantly elevated in patients with unstable angina compared to patients with stable angina and normal controls [23]. More recently, urine F_2-isoprostanes levels have been found to be elevated in patients with an acute myocardial infarction following thrombolytics and percutaneous balloon angioplasty and oxidative stress has been suggested to increase following reperfusion [24]. However, whether plasma F_2-isoprostanes are elevated in patients with an acute myocardial infarction is not yet known.

The aim of this study was to test the hypothesis that plasma F_2-isoprostanes levels are increased in patients with acute myocardial infarction, independent of traditional risk factors of atherosclerosis, CRP and the extent of coronary atherosclerosis.

Methods

Patients and design

Patient recruitment was done between August 1998 and July 1999. Information regarding the patient characteristics was collected from the patients and was supplemented when necessary by review of the medical records. Informed consent was obtained from the patients. The research protocol was approved by the Mayo Clinic Institutional Review Board.

The study included three groups of patients. Group 1 consisted of patients with no significant coronary artery disease (CAD). These were referred for coronary angiogram pre-operatively for a non-cardiac surgery or a cardiac valve surgery, and for atypical symptoms. All patients in group 1 had a normal coronary angiogram or very mild CAD with $<20\%$ luminal diameter narrowing. Patients in group 2 had stable angina or angina equivalent defined as chronic stable effort induced chest pain or shortness of breath relieved by rest or sublingual nitroglycerin. All patients in group 2 had a negative troponin and an angiogram showing at least one coronary artery diameter narrowing of more than 70%. Patients in group 3 had acute myocardial infarction. Acute myocardial infarction was defined as prolonged chest pain accompanied by a troponin T level of more than 0.1 ng/ml. All patients in group 3 underwent a coronary angiogram.

Patients with a complex congenital heart disease or an inflammatory disease (i.e. current infection, vasculitis or rheumatic disease) were excluded from the study.

Coronary angiography was performed according to standard technics using 5- or 6-F Judkins-type catheter via the femoral approach. Blood for laboratory testing was drawn through the femoral sheath before cardiac catheterization.

Definitions

CAD was defined angiographically as the presence of $>70\%$ luminal diameter narrowing. The atherosclerotic burden in the coronaries was assessed by using the modified Jenkins score that included branches in addition to proximal arteries [25].

The definition of hyperlipidemia was consistent with the most recent NCEP report [26]. The body mass index (BMI) was calculated by dividing the patient's weight in kilograms by the square of the patient's height in meters. Patients were considered to be hypertensive if their blood pressure was $>$ 140/90 mm Hg, or if they were being treated with antihypertensive medications.

Plasma protein assays

A highly sensitive latex-particle-enhanced immunoturbidimetric assay (Kamiya Biomedical, Seattle) was used to quantitate the level of CRP. As described in our previous work [27], we followed the methods supplied in the kit provided by Cayman Chemical with a few modifications for the extraction and enzyme immunoassay measurement of 8-iso-prostaglandin $F_{2\alpha}$. Several investigators have validated the good correlation in measurement between the values of

8-iso-prostaglandin $F_{2\alpha}$ obtained by enzyme immunoassay and those obtained by mass spectrometry [28,29]. For storage, serum samples were frozen at $-80°C$. The samples were then thawed on ice within six months of collection to minimize the problem with extended storage periods and the possible peroxidation that might ensue. Plasma isoprostanes circulate either as a free base or esterified to lipoproteins. Esterified isoprostanes were hydrolyzed first to free bases that could then be measured by extraction and enzyme immunoassay. Absolute methanol was first added, followed by mixing and centrifugation. For free isoprostanes, the eluent was poured into a water/buffer solution and kept on ice. The total eluent was poured in a solution of 15% KOH and incubated for 1 h at 38°C. A water/buffer solution was added to the total samples with a pH of 3.1 ± 0.5 in all samples after incubation. Extraction was then performed on a Sep-Pak C 18 column, with washes of hexane and water. A 99/1% ethyl acetate/methanol was then used to elute isoprostanes from the column. Tracer and antibody were then added to standards and samples with incubation overnight at room temperature in plate provided by the kit. The next day, the plate was washed with wash buffer and Ellman's reagent was added. The plate was then read at 405 nm. Though it is known that the free 8-iso-prostaglandin $F_{2\alpha}$ is probably responsible for its postulated biological functions [30], in our study we chose to measure both esterified and free plasma 8-iso-prostaglandin $F_{2\alpha}$ levels in order to obtain the highest potential diagnostic information reflecting the ongoing oxidative stress in the three study groups.

Statistical analysis

Continuous variables were presented as mean ($\pm$ standard deviation) for generally symmetric distributions, and as median (with inter-quartile range) for skewed distributions. Discrete variables were summarized as frequencies and percentages. One-way analysis of variance and Pearson's chi-squared test were used to test for differences between the three groups. Skewed variables were log-transformed for one-way analysis of variance. Pairwise comparisons were adjusted with Tukey's method for multiple comparisons.

Linear models were used to estimate the CRP and 8-iso-prostaglandin $F_{2\alpha}$ associations with the three groups after covariance adjustment for clinically relevant variables. Both models included age, extent of atherosclerosis, diabetes, hypertension, hyperlipidemia, smoking history and gender as covariates. The model for 8-iso-prostaglandin $F_{2\alpha}$ also included CRP and the model for CRP included also 8-iso-prostaglandin $F_{2\alpha}$. CRP was log-transformed for both models. The normality assumptions were checked with residual diagnostic plots.

Results

Patient characteristics

There were 15 patients with no significant CAD (group 1), 31 patients with stable CAD (group 2), and 13 patients presenting with an acute myocardial infarction (group 3) enrolled in the study.

Of the 13 patients with an acute myocardial infarction, 7 patients had a non-ST elevation myocardial infarction and 6 patients had an ST elevation myocardial infarction. Ten patients subsequently had myocardial revascularization with percutaneous interventions (7 patients) or coronary bypass surgery (3 patients). Out of the 6 patients with ST elevation MI, 4 had thrombolytic therapy before blood draw for protein assays. Thrombolysis was successful in all 4 patients.

The table summarizes the characteristics of the 59 patients included in the study. Patients with stable CAD were significantly older than patients with no CAD and patients presenting with an acute myocardial infarction. There was no difference in the total coronary atherosclerotic burden between patients with an acute myocardial infarction and patients with stable CAD. There were no statistically significant differences in sex, BMI, hypertension, hyperlipidemia, smoking status, use of oral anti-oxidants (i.e. vitamin C and E) and creatinine level between the three groups.

Plasma 8-iso-prostaglandin $F_{2\alpha}$ level

Plasma 8-iso-prostaglandin $F_{2\alpha}$ level (both in esterified and free form) was collected at the time of cardiac catheterization. Two patients with acute myocardial infarction (15%) were on intravenous nitrates and 5 patients with stable CAD (16%) were on oral long acting nitrates at the time of blood sampling. The blood samples in patients with myocardial infarction were collected at a median time of 40 h (range, 6–144 h) after the start of chest pain. The 4 patients who received thrombolytics had their blood samples drawn 49 ± 19 h after the start of intravenous thrombolytics. In patients with stable CAD, blood was collected at a median time of 72 h (range, 8–960 h) after the start of chest pain.

Plasma 8-iso-prostaglandin $F_{2\alpha}$ level was significantly elevated in patients with an acute myocardial infarction compared to patients with stable CAD and patients with no significant CAD (Table I, Figure 1). In a model that included age, extent of coronary atherosclerosis as assessed by the modified Jenkins score, diabetes mellitus, hyperlipidemia, hypertension, smoking history, gender and CRP level as covariates, plasma 8-iso-prostaglandin $F_{2\alpha}$ level remained significantly elevated in patients presenting with an acute myocardial infarction compared to patients with stable CAD ($p = 0.001$) and patients

Table I. The clinical and laboratory characteristics of the patient population

Variable	Clinical characteristics			
	No CAD (*N*=15)	Stable CAD (*N*=31)	Acute MI (*N*=13)	*P*-value
Age	53.4 ± 14.3	69.3 ± 10.7	61.5 ± 11.4	<0.001
Male gender, No. (%)	7 (47%)	22 (71%)	8 (62%)	0.28
Body mass index	29.8 ± 8.9	29.0 ± 5.1	29.7 ± 4.1	0.89
Hypertension, No. (%)	4 (27%)	15 (48%)	5 (38%)	0.37
Diabetes mellitus, No. (%)	1 (7%)	6 (19%)	2 (15%)	0.53
Hyperlipidemia, No. (%)	9 (60%)	27 (87%)	10 (77%)	0.11
Tobacco, No. (%)				0.06
Never\Ex-smoker	13 (87%)	28 (90%)	8 (62%)	
Current	2 (13%)	3 (10%)	5 (38%)	
Use of oral anti-oxidants⋆	3 (20%)	5 (16%)	2 (15%)	0.93
CAD, No. (%)	0 (0%)	31 (100%)	13 (100%)	<0.001
Coronary atherosclerosis†	0.8 ± 1.6	14.0 ± 5.8	13.1 ± 6.3	<0.001
Creatinine, Median (Q1, Q3) [mg\dl]	1.1 (0.9, 1.1)	1.2 (1.0, 1.4)	1.1 (1.1, 1.2)	0.17
CRP, Median (Q1, Q3) [mg\l]	0.3 (0.1, 0.4)	0.2 (0.1, 0.7)	1.1 (0.6, 3.2)	<0.001
Total 8-iso-prostaglandin $F_{2\alpha}$ [pg\ml]‡	118.9 ± 85.5	182.0 ± 75.7	290.7 ± 73.9	<0.001

⋆Oral anti-oxidants included use of vitamins C and E.†Coronary atherosclerosis as assessed by the modified Jenkins score.‡Total 8-iso-prostaglandin $F_{2\alpha}$ includes both esterified and free 8-iso-prostaglandin $F_{2\alpha}$. CAD: coronary artery disease. MI: myocardial infarction. CRP: C-reactive protein.

with no significant CAD ($p < 0.001$). Plasma 8-iso-prostaglandin $F_{2\alpha}$ level remained significantly elevated in the 9 patients with myocardial infarction and receiving no thrombolytics compared to patients with stable CAD ($p = 0.002$) and patients with no significant CAD ($p = 0.002$) even after excluding the 4 patients who received thrombolytic therapy, and after correcting for baseline clinical characteristics, the extent of coronary atherosclerosis and CRP level.

Plasma 8-iso-prostaglandin $F_{2\alpha}$ level was also significantly elevated in patients with stable CAD compared to patients with no significant CAD (Table I, Figure 1). In a model that included age, diabetes mellitus, hyperlipidemia, hypertension, smoking history, gender and CRP level as covariates, plasma 8-iso-prostaglandin $F_{2\alpha}$ level remained significantly elevated in patients with stable CAD compared to patients with no significant CAD ($p = 0.045$).

Plasma CRP level

CRP level was significantly elevated in patients with an acute myocardial infarction compared to patients with stable CAD and patients with no significant CAD; the difference in CRP level was not statistically significant between patients with no CAD and patients presenting with stable CAD, (Table I, Figure 2). In a model

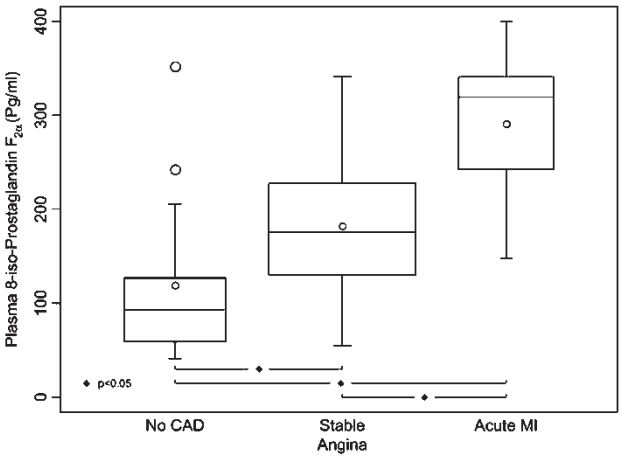

Figure 1. Total 8-iso-prostaglandin $F_{2\alpha}$ boxplot distribution according to clinical presentation. The box indicates the interquartile range and median; the circle within the box represents the group mean. The circles outside the box represent two outliers. (⋆) For 0.05 significance level between the groups using Turkeys method of multiple comparisons.

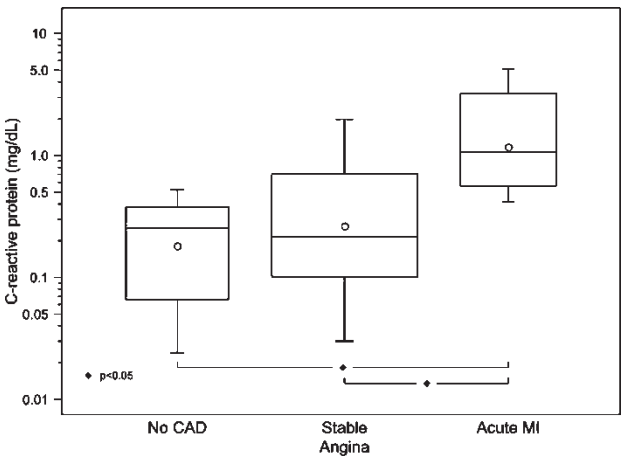

Figure 2. CRP boxplot distribution according to clinical presentation. The box indicates the interquartile range and median; the circle within the box represents the group mean. (⋆) For 0.05 significance level between the groups using Turkeys method of multiple comparisons.

that included age, extent of coronary atherosclerosis as assessed by the modified Jenkins score, diabetes mellitus, hyperlipidemia, hypertension, smoking history, gender and plasma 8-iso-prostaglandin $F_{2\alpha}$ level as covariates, CRP level remained significantly elevated in patients presenting with an acute myocardial infarction compared to patients with stable CAD ($p = 0.002$) and patients with no significant CAD ($p = 0.003$).

A weak but significant correlation was found between the plasma levels of 8-iso-prostaglandin $F_{2\alpha}$ and CRP (Spearman correlation coefficient = 0.30; $p = 0.024$).

Discussion

Our study demonstrates that plasma 8-iso-prostaglandin $F_{2\alpha}$ levels, a parameter of systemic oxidative stress, are increased in patients with an acute myocardial infarction compared to patients with stable CAD and patients with no significant CAD. This increase is independent of risk factors such as age, gender, diabetes mellitus, hyperlipidemia, hypertension, smoking history and extent of coronary atherosclerosis, and CRP level. Plasma 8-iso-prostaglandin $F_{2\alpha}$ levels are also increased in patients with stable CAD as compared to patients with no significant CAD independent of traditional risk factors for CAD and CRP level. This study supports the concept that endogenous oxidative stress plays a role in coronary atherosclerosis and its complication, namely myocardial infarction.

It is known that the plasma malondialdehyde levels, a parameter of oxidative stress, are elevated in patients with acute myocardial infarction compared to normal subjects [31,32]. Delanty et al. [24] observed that urinary 8-iso-prostaglandin $F_{2\alpha}$ levels in 12 patients with acute myocardial infarction treated with thrombolysis were elevated compared to normal subjects and stable patients with CAD. They concluded that oxidative stress is associated with reperfusion of an ischemic myocardium. However, in this study, the urinary 8-iso-prostaglandin $F_{2\alpha}$ levels were already elevated before thrombolysis in some patients. This evidently raises the possibility that urinary 8-iso-prostaglandin $F_{2\alpha}$ were in fact elevated in the patients with myocardial infarction before reperfusion of their ischemic myocardium, and consequently that oxidative stress was already significant before reperfusion. Moreover, the source of the urinary 8-iso-prostaglandin $F_{2\alpha}$ may be in part produced by the kidneys and may not represent the systemic increase in oxidative stress.

The current study extends these previous observations and demonstrates that plasma 8-iso-prostaglandin $F_{2\alpha}$ levels are elevated in patients with myocardial infarction independent of treatment with thrombolytics. Accordingly, our study suggests that oxidative stress, as assessed by the degree of lipid peroxidation is associated with myocardial ischemia and infarction *per se*, rather than only with the reperfusion of the ischemic myocardium. The rise seen in urinary 8-iso-prostaglandin $F_{2\alpha}$ levels following thrombolytics might hence reflect a late effect of a wash out phase accelerated by reperfusion. Moreover, the rapid decline in urinary 8-iso-prostaglandin $F_{2\alpha}$ within 24 h following treatment might again reflect a successful reperfusion with oxidation abating quicker than in patients not reperfused. More recently, Berg et al. [33] showed in a nicely designed study, a significant rise of 8-iso-prostaglandin $F_{2\alpha}$ in patients with acute myocardial infarction before reperfusion and in a state of reduced coronary blood flow. This adds further support to our work that ischemia must be contributing to oxidative stress before any reperfusion occurs.

Furthermore, F_2-isoprostanes might be not only just biomarkers of atherosclerosis, but may also contribute to its pathogenesis and its complications. We have previously demonstrated that F_2-isoprostanes induce vasoconstriction in the coronary, renal and peripheral arteries of hypercholesterolemic pigs [27]. Others have demonstrated that F_2-isoprostanes induce vasoconstriction in the rat renal [34] and cerebral arteries [35], as well as platelet aggregation in rats [17] and in humans [36]. Thus, it may be speculated that F_2-isoprostanes might contribute to the propagation of myocardial infarction by enhancing platelet aggregation and coronary vasoconstriction.

Plasma 8-iso-prostaglandin $F_{2\alpha}$ in patients with CAD and stable angina were elevated in our study as compared to subjects with no significant coronary atherosclerosis after correcting for coronary atherosclerosis risk factors and CRP. This is consistent with previous studies that showed higher urinary 8-iso-prostaglandin $F_{2\alpha}$ levels in patients with CAD as compared to matched healthy controls [37], and a significant correlation between plasma 8-iso-prostaglandin $F_{2\alpha}$ and the number of diseased coronary arteries [38].

The findings of the current study that plasma 8-iso-Prostaglandin $F_{2\alpha}$ levels were elevated as opposed to plasma CRP levels in patients with stable CAD as compared to patients with no significant CAD, point to the possibility that the role of oxidative stress might not be completely dependent on the presence and extent of inflammation in atherosclerosis. This is further supported by the weak correlation we found between the plasma levels of 8-iso-prostaglandin $F_{2\alpha}$ and CRP.

One possible limitation to the study might reside in not controlling for oral intake of antioxidants and treatment with nitrates. Nonetheless, we found no significant difference between the three studied groups with regards to the intake of vitamin C and E supplementation. On the other hand, the same

percentage of patients with stable CAD and percentage of patients with acute myocardial infarction were treated with nitrates. None of the patients with no or minimal CAD was taking nitrates. Nonetheless, this would have only caused a possible reduction in the levels of 8-iso-prostaglandin $F_{2\alpha}$ in patients with stable CAD and patients with myocardial infarction compared to the patients with no or minimal CAD and would have not changed our conclusion. The difference in age between the 3 groups is another potential limitation to our study. Oxidative stress is thought to be increased with older age [39]. For that reason, age was entered in our multivariate model to try to account for any effect that age could have exerted on plasma 8-iso-prostaglandin $F_{2\alpha}$ levels. To further explore that matter, we measured in a group of 13 patients (which were not part of the current study) with no or minimal coronary artery atherosclerosis with an average age of 62.6 ± 9.4 years the plasma 8-iso-prostaglandin $F_{2\alpha}$ levels. The mean plasma 8-iso-prostaglandin $F_{2\alpha}$ was 126.0 ± 84.3 pg/ml. This was very similar to plasma 8-iso-prostaglandin $F_{2\alpha}$ levels of our group of patients with no or minimal CAD (118.8 ± 85.5 pg/ml) and was significantly lower than the other 2 groups with CAD. Another limitation to our study lies in the non-standardized timing from chest pain to blood draw. However, all patients had their blood sample drawn 6 or more than 6 h since the episode of the chest pain, and accordingly we can potentially assume that the episode of chest pain *per se* is not the major driver for plasma 8-iso-prostaglandin $F_{2\alpha}$ production with its known short half-life (minutes) but probably the atherosclerotic burden and its activity in a particular patient. One more potential limitation could be related to the storage process as studies have shown artifactual formation of plasma 8-iso-prostaglandin $F_{2\alpha}$ if samples are not properly handled and stored [30]. We, however, took great care in collecting and storing the samples at −80°C for a relatively short period of six months before quantitation of plasma 8-iso-prostaglandin $F_{2\alpha}$. No antioxidants were added but in our experience this is not needed with proper handling of samples in accordance with the experience of others [30]. Finally, even though we saw no difference in the level of plasma 8-iso-prostaglandin $F_{2\alpha}$ between the group of patients that received thrombolytics and reperfused before blood draw and the group of patients that did not receive thrombolytics and even though we found no difference in the results of our study whether we included or excluded the former group, our study can not completely rule out the possibility that reperfusion might have occurred spontaneously in patients that did not receive thrombolytics. And though, a previous study by Ulus et al. [40] has shown that reperfusion after cardiopulmonary bypass is associated with increased oxidative stress, this study however differs from our current study in recruiting a different patient population under different clinical conditions. In addition, another study by Berg et al. [33] supports our conclusion that ischemia itself is associated with increased oxidative stress in the absence of significant reperfusion.

To conclude, our study showed that plasma 8-iso-prostaglandin $F_{2\alpha}$ levels are elevated in patients with myocardial infarction independent of reperfusion as compared to patients with stable CAD and patients with no significant CAD. Plasma 8-iso-prostaglandin $F_{2\alpha}$ levels are also elevated in patients with stable CAD as compared to patients with no significant CAD. This study supports the concept that systemic oxidative stress is interrelated with coronary atherosclerosis and myocardial infarction.

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