*Original Research*

# The role of inflammation and oxidative stress markers in the occurrence and severity of coronary artery disease in young patients with ST-segment elevation myocardial infarction

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**ABSTRACT**

**Background**: Atherosclerosis is modulated by inflammation and oxidative stress, which play pivotal roles in the pathogenesis of coronary artery disease (CAD), especially in young patients without traditional risk factors for atherosclerosis. In this study, we aimed to study the role of some inflammatory and oxidative stress markers in triggering ST segment elevation myocardial infarction in Egyptian young patients and the potential correlation to the severity of coronary artery lesions in the Egyptian population. **Methods**: This case-control study recruited 115 premature STEMI patients (aged <45 years) and 55 age-matched healthy controls. Serum CRP, TNF-$\alpha$, IL-10, and oxidative stress biomarkers [reduced glutathione, total antioxidants, L-ascorbic acid, nitric oxide (NO), and lipid peroxides] were assayed using commercially available kits and correlated with the coronary artery lesion severity, assessed by the SYNTAX score (SS). The ROC curve assessed the biomarker potential of CRP, TNF-$\alpha$, IL-10, and the TNF-$\alpha$/IL-10 ratio to discriminate patients from healthy controls. **Results**: In our study, we found that serum CRP (38.7 ± 1.9 versus 0.8 ± 0.04 mg/L; P < 0.001), TNF-$\alpha$ (68.4 ± 4.7 pg/mL versus 10.8 ± 0.7 pg/mL; P < 0.001), IL-10 (11.9 ± 0.8 pg/mL versus 8.7 ± 0.4 pg/mL; P = 0.001), and TNF-$\alpha$/IL-10 ratio (6.8 ± 0.5 versus 1.7 ± 0.2; P < 0.001) were significantly higher in patients compared to healthy controls. Regarding oxidative stress markers, serum T-AOC levels (25.5 ± 0.9 versus 14.9 ± 0.4 U/mL; P < 0.001), NO (10.5 ± 0.3 versus 8.1 ± 0.3 nmol/mL; P < 0.001), serum LPO levels (15.8 ± 0.4 versus 4.1 ± 0.2 $\mu$mol/L; P < 0.001) and lower GSH levels (3.8 ± 0.1 $\mu$g/mL versus 5.2 ± 0.2; P < 0.001) were significantly different in patients versus controls. CRP, TNF-$\alpha$, and NO levels have been significantly correlated with the severity of CAD as assessed by the SYNTAX score. **Conclusion**: Inflammation and oxidative stress are pathogenically implicated in premature STEMI and correlated with the severity of CAD lesions. The investigated biomarkers can be utilized in risk stratification and are theranostically targetable.

**Key words:** Inflammation, Egyptian Population, Pro-inflammatory Cytokines, ST-Segment Elevation Myocardial Infarction (STEMI), Coronary Artery Disease

## INTRODUCTION

Coronary artery disease (CAD) is a primary cause of death worldwide, with an increasing prevalence[1]. The bigger challenge is the premature CAD affecting young populations, which is an unanticipated risk with a high recurrence rate and socio-economic impact[2]. Premature CAD is more prevalent among men who are obese, smokers, and those with dyslipidemia, a sedentary lifestyle, and drug abuse. Although they have a lower rate of hypertension and diabetes, their traditional cardiovascular risk factors are mostly uncontrolled, owing to their low adherence to risk factor modification measures[3,4].

Atherosclerotic cardiovascular disease is characterized by chronic inflammation, on top of being a lipid metabolic disorder. Inflammation heralds the initiation, progression, instability, and rupture of the atherosclerotic plaque[5,6]. C-reactive protein (CRP), the homopentameric protein, is principally secreted from hepatocytes, macrophages, endothelial cells, lymphocytes, smooth muscle cells, and adipocytes. CRP is an acute systemic inflammatory biomarker that spikes in reaction to infection or inflammatory disorders, like cardiovascular disease, in asymptomatic people. CRP levels above 3 mg/L are pathogenic and associated with the severity and prognosis of atherosclerotic CAD[7,8].

Tumor necrosis factor (TNF)-$\alpha$ is a pro-inflammatory pleiotropic key regulatory cytokine implicated in the pathophysiological mechanisms that govern inflammation[9]. The TNF-$\alpha$ role is paramount in the initiation stage of the formation of the atherosclerotic plaque, as it prevents the clearance

of damaged vascular cells and apoptotic bodies[10]. In contrast, the anti-inflammatory interleukin (IL)-10 is crucial in counteracting inflammation by controlling the homeostasis of inflammatory cells[11]. Moreover, oxidative stress is associated with coronary atherosclerosis and the reduced bioavailability of the angio-protective nitric oxide (NO) due to its conversion into the more damaging peroxynitrite radical[12]. Inflammation, gauged with CRP and governed by the interplay among oxidative stress, TNF-$\alpha$, and IL-10, is implicated in coronary atherosclerosis and is related to its severity. They are being investigated as theranostic targets[13,14]. Uneven or disrupted blood flow can lead to the development of atherosclerotic plaque. Low wall shear stress (WSS), produced by this flow pattern (which is a well-known mechanism of atherosclerosis), can cause arterial inflammation and propel the development of atherosclerosis and plaque[15]. Adhesion proteins and other inflammatory chemicals are produced in greater quantities when WSS is reduced, which attracts leucocytes and guides their migration into the artery wall[15]. This mechanism could elucidate how local inflammation events that are far from atherosclerotic lesions may trigger inflammatory activation and the advancement of coronary plaque[15].

In this study, we planned to investigate how CRP, TNF-$\alpha$, IL-10, and oxidative stress are implicated in the development of premature ST segment elevation myocardial infarction (STEMI), along with the correlation to disease severity in young Egyptian patients, based on their major role as pathogenic mediators and severity biomarkers.

## METHODS

### Setting and participants

This prospective case-control study involved 115 consecutively enrolled and consented young CAD patients who presented with acute STEMI. Inclusion criteria: acute STEMI patients (aged < 45 years); for the disease diagnosis, we employed the European Society of Cardiology guidelines and its $4^{th}$ MI definition[16]. The study was conducted over six months, from April 2022 to September 2022. The study sample size was calculated with a power of 0.95 and a hypothetical effect size of 0.33, using G Power software. Patients were admitted to the Department of Cardiology, Assiut University Hospitals, Assiut, Egypt. As a control group, 55 age-matched and apparently healthy volunteers were recruited. We excluded: STEMI patients over 45 years, those with chronic diseases (diabetes, hepatic or renal failure), COVID-19 patients, and addicts (for drugs and/or alcohol). The patient group is

part of our previous cohort study that investigated the implication of PCSK-9 in premature CAD pathogenesis[17].

### Ethical considerations

The study was carried out in compliance with the Helsinki Declaration principles after being approved by the Institutional Review Board, Faculty of Medicine, Assiut University (Approval#: IRB17200689). Volunteering participants signed the informed consent form after being briefed on the nature of the study and their roles and rights.

### Assessment of clinical and laboratory parameters

After recording the clinical data, the socio-demographic and anthropometric characteristics of the participants, the presence of traditional risk factors (family history, smoking, hypertension, dyslipidemia, and body mass index [BMI; kg/m$^2$]), and the relevant routine laboratory findings were retrieved. On admission, resting 12-lead surface ECG recording, echocardiography, and percutaneous coronary intervention (primary PCI) were conducted. All coronary lesions with >50% diameter stenosis in a vessel >1.5 mm in diameter were assessed by interventional cardiologists for each patient to determine the SYNTAX score (SS), based on the SS calculator[18]. According to SS, the disease severity was stratified into three levels: SS $\leq$ 12, >12–$\leq$21.5, and >21.5[19]. Healthy volunteers were subjected to history taking, physical examination, and assessment of their BMI.

### Serum recovery and laboratory biomarker investigations

After admission, 5 mL of peripheral venous blood was collected from each participant in a plain tube, left to clot, centrifuged at 3000 rpm for 10 minutes, and the separated serum was stored at -20 °C. We used commercially available specific quantitative ELISA kits to assess serum protein biomarkers: 1) Cardiac troponin T (cTnT) and creatine phosphokinase-MB (CK-MB) (Cat.# ab223860 and ab193696; Abcam, UK); and, 2) CRP (with 0.44 pg/mL sensitivity), TNF-$\alpha$, and IL-10 (Cat.# EL0036Hu, SL1761Hu, and SL0967Hu, Sunlong Biotech Co., Hangzhou, China), as instructed by the manufacturer. We used quantitative colorimetric assay kits (Spectrum, Cairo, Egypt) to assess lipid profile and relevant organ functions [aspartate aminotransferase (AST; REF:

260-002), alanine aminotransferase (ALT; REF: 264-002), alkaline phosphatase (ALP; REF: 216-001), creatine phosphokinase (CPK; REF: 238-002), creatinine (REF: 235-001), urea/BUN (REF: 319-001), total cholesterol (REF: 230006), high-density lipoprotein cholesterol (HDL-C; REF: 266003), triglycerides (REF: 314005), and bilirubin (total and direct; REF 225-001), as instructed]. We calculated LDL-C to be = Total Cholesterol–Triglycerides/5–HDL-C. Total anti-oxidative capacity (TAC; U/mL) measured by $Fe^3+$-2,3,6-tri (2-pyridyl)-1,3,5-triazine reagent, and lipid peroxidation (LPO; $\mu$mol/L) measured as malondialdehyde (MDA), after the reaction with thiobarbituric acid, were colorimetrically assayed (Cat# AK0455-100T-96S and AK0694-50T-58S, Sunlong Biotech Co., Hangzhou, China). Serum nitric oxide (NO; nmol/mL) was estimated as nitrite using Griess reagent against the sodium nitrite standard [20]. Serum reduced glutathione (GSH; $\mu$g/mL) concentration was estimated by the DTNB reagent [21,22]. Estimation of serum L-ascorbic acid ($\mu$g/mL) was done using Folin phenol reagent vs. L-ascorbic acid standard [23].

### Statistical analysis

The nominal data were expressed as frequency (percentage), and $Chi^2$-test was used to compare them between groups. The continuous data were expressed as mean and standard error (SE), and the Student's t-test or one-way analysis of variance (ANOVA), with Bonferroni post hoc test, was used to compare their means among groups. Receiver Operating Characteristic (ROC) curve analysis was used to determine the area under the curve (AUC) for each of CRP, TNF-$\alpha$, and IL-10 to determine their sensitivity and specificity to discriminate patients from healthy controls at several cutoff points. A probability (P value) of <0.05, at a confidence level of 95%, was considered statistically significant. Data entry, analysis, and presentation were done using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM, Armonk, New York, USA).

### RESULTS

### Baseline characteristics and routine serum biomarkers of the premature STEMI patients and healthy controls

The age comparison of our participants was not significantly different (38 ± 0.3 years for healthy controls versus 38 ± 0.4 years for patients). BMI was significantly higher among patients compared to controls (26 ± 0.4 versus 24 ± 0.2 kg/m$^2$; P > 0.001). While

the majority of our patients were smokers, smokers constituted a minority among controls (80% versus 10.9%; P > 0.001). Hypertensive patients constituted 16.5%, and for family history, 13% had hyperlipidemia and 16.5% had premature CAD in their families. Percutaneous coronary intervention (PCI) was performed on 9.5% of our patients (**Table 1**).

The patient group demonstrated non-significantly higher serum levels of total cholesterol (4.8 ± 0.1 mmol/L) and LDL-C (3.2 ± 0.08 mmol/L) compared to healthy controls (4.5 ± 0.1 mmol/L and 3.1 ± 0.1 mmol/L, respectively). Healthy controls had significantly greater HDL-C levels (1.5 ± 0.03 mmol/L versus 1.1 ± 0.02 mmol/L; P > 0.001) and lower triglyceride levels (3.0 ± 0.2 mmol/L versus 3.5 ± 0.3 mmol/L; P = 0.04) compared to the premature STEMI patients. Significantly high levels of CPK, CK-BM, and cTnT were observed in patients compared to healthy controls (P > 0.001) (**Table 1**).
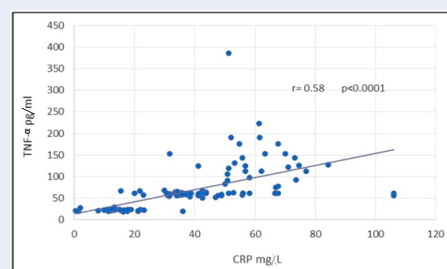


**Figure 1**: **Correlation between C-reactive protein (CRP) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) among patients with premature ST segment elevation myocardial infarction (STEMI)**.

### Changes in serum inflammatory biomarkers in premature STEMI patients versus the control group

Serum CRP levels were significantly higher in premature STEMI patients than healthy controls (38.7 ± 1.9 versus 0.8 ± 0.04 mg/L; P > 0.001). Serum levels of TNF-$\alpha$ (68.4 ± 4.7 pg/mL versus 10.8 ± 0.7 pg/mL; P > 0.001) and IL-10 (11.9 ± 0.8 pg/mL versus 8.7 ± 0.4 pg/mL; P = 0.001) were significantly higher in patients compared to healthy controls. The TNF-$\alpha$/IL-10 ratio was significantly higher in premature STEMI patients than controls (6.8 ± 0.5 versus 1.7 ± 0.2; P > 0.001) (**Table 2**). CRP levels positively correlated with TNF-$\alpha$ levels (r = 0.580 and P > 0.001) (**Figure 1**).

**Table 1**: Baseline characteristics and routine serum laboratory workup of the patients with premature ST segment elevation myocardial infarction (STEMI) patients (n = 115) compared to the healthy control group (n = 55)

| Parameter | Patients (n=115) | Control (n=55) | P-value |
|---|---|---|---|
| Age, year | 38 ± 0.4 | 38 ± 0.3 | 0.63 |
| BMI, kg/m$^2$ | 26 ± 0.4 | 24 ± 0.2 | > 0.001 |
| Sex | Male: 108, (94.7%) Female: 6, (5.3%) | Male: 50, (90.9%) Female: 5, (9.1%) | 0.66 |
| Smoking | Smokers: 92, (80%) | Smokers: 6, (10.9%) | > 0.001 |
| Hypertention | Hypertensive: 20, (17.3%) | Hypertensive ( 0 ) | 0.14 |
| Family history of PCAD | Yes: 19, (16.5%) | Yes: 2, (3.6%) | 0.19 |
| Family history of hyperlipidimia | Yes: 15, (13%) | Yes: 4, (7.2%) | 0.73 |
| History of previous PCI | Yes: 11, (9.5%) | Yes ( 0 ) | 0.20 |
| **Main CBC parameters** | | | |
| WBCs, 10$^9$/μL | 10.2 ± 0.3 | 5.5 ± 0.1 | > 0.001 |
| Platelets, 10$^9$/L | 276.7 ± 7.8 | 380.2 ± 4.0 | > 0.001 |
| Hb, g/L | 144 ± 16 | 142 ± 18 | 0.32 |
| **Liver biomarkers** | | | |
| Total bilirubin, μmol/L | 6.5 ± 0.1 | 6.3 ± 0.2 | 0.56 |
| Direct bilirubin, μmol/L | 2.2 ± 0.07 | 2.1 ± 0.1 | 0.63 |
| AST, μKat/L | 0.31 ± 0.01 | 0.31 ± 0.01 | 0.87 |
| ALT, μKat/L | 0.29 ± 0.01 | 0.28 ± 0.01 | 0.63 |
| ALP, μKat/L | 1.05 ± 0.02 | 1.06 ± 0.04 | 0.84 |
| **Kidney biomarkers** | | | |
| Creatinie, μmol/L | 68.3 ± 1.4 | 55.4 ± 1.3 | 0.41 |
| BUN, mmol/L | 6.0 ± 0.4 | 3.4 ± 0.1 | > 0.001 |
| **Lipid profile** | | | |
| LDL-C, mmol/L | 3.2 ± 0.08 | 3.1 ± 0.1 | 0.44 |
| Total cholesterol, mmol/L | 4.8 ± 0.1 | 4.5 ± 0.1 | 0.14 |
| HDL-C, mmol/L | 1.1 ± 0.02 | 1.5 ± 0.03 | > 0.001 |
| Triglycerides, mmol/L | 3.5 ± 0.3 | 3.0 ± 0.2 | =0.04 |
| **Cardiac biomarkers** | | | |
| c-Tnt, μg/L | 82.5 ± 16.4 | 0.02 ± 0.01 | > 0.001 |
| CK-MB, μg/L | 1118.5 ± 83.3 | 2.1 ± 0.08 | > 0.001 |
| CPK, μKat/L | 21.9 ± 1.7 | 0.9 ± 0.03 | > 0.001 |

Continuous data was expressed in the form of mean ± SE (compared with Student's t test), while nominal data was expressed in form of frequency (percentage) (compared with Chi2 test). P value was significant if ≤ 0.05.

**Table 2: Changes in serum inflammatory biomarkers in the patients with premature ST-segment elevation myocardial infarction (STEMI) compared to the healthy controls**

| Parameter | Patients (n = 115) | Control (n = 55) | P-value |
|---|---|---|---|
| CRP mg/L | $38.7 \pm 1.9$ | $0.8 \pm 0.04$ | > 0.001 |
| TNF-$\alpha$ pg/mL | $68.4 \pm 4.7$ | $10.8 \pm 0.7$ | > 0.001 |
| IL-10 pg/mL | $11.9 \pm 0.8$ | $8.7 \pm 0.4$ | =0.001 |
| TNF-$\alpha$/IL-10 | $6.8 \pm 0.5$ | $1.7 \pm 0.2$ | > 0.001 |

Continuous data was expressed in the form of mean $\pm$ SE (compared with Student's t test). P-value was significant if $\leq$ 0.05

**Table 3: Changes in serum oxidative stress parameters in the patients with premature ST-segment elevation myocardial infarction (STEMI) compared to the healthy controls**

| Parameters | Patients (n = 115) | Control (n = 55) | P-value |
|---|---|---|---|
| T-AOC, U/mL | $25.5 \pm 0.9$ | $14.9 \pm 0.4$ | > 0.001 |
| NO, nmole/mL | $10.5 \pm 0.3$ | $8.1 \pm 0.3$ | > 0.001 |
| GSH, $\mu$g/mL | $3.8 \pm 0.1$ | $5.2 \pm 0.2$ | > 0.001 |
| L-ascorbic acid, $\mu$g/mL | $7.8 \pm 0.3$ | $7.3 \pm 0.3$ | 0.172 |
| LPO, $\mu$mole/L | $15.8 \pm 0.4$ | $4.1 \pm 0.2$ | > 0.001 |

Continuous data was expressed in the form of mean $\pm$ SE (compared with Student's t test). P value was significant if $\leq$ 0.05.

**Table 4: The association between serum inflammatory and oxidative stress biomarkers and the severity of the premature ST-segment elevation myocardial infarction (STEMI) assessed by SYNTAX score (SS)**

| Parameter | SS $\leq$ 12 | 12 < SS $\leq$ 21.5 | SS > 21.5 | p-value |
|---|---|---|---|---|
| CRP (mg/L) | $30.6 \pm 2.5$ | $46.3 \pm 2.9$ | $53.7 \pm 9$ | > 0.001 |
| TNF-$\alpha$ (pg/mL) | $49.3 \pm 4.3$ | $87.3 \pm 8.6$ | $88.6 \pm 17.4$ | > 0.001 |
| IL-10 (pg/mL) | $11.9 \pm 1.5$ | $11.7 \pm 0.5$ | $14.9 \pm 4.4$ | 0.80 |
| TNF-$\alpha$/IL-10 | $5.9 \pm 0.7$ | $7.6 \pm 0.7$ | $6.5 \pm 0.9$ | 0.23 |
| T-AOC (U/mL) | $26.0 \pm 1.2$ | $25.8 \pm 1.5$ | $26.2 \pm 6.3$ | 0.99 |
| NO (nmole/mL) | $9.8 \pm 0.5$ | $11.0 \pm 0.5$ | $13.5 \pm 1.1$ | =0.04 |
| GSH ($\mu$g/mL) | $3.8 \pm 0.2$ | $4.0 \pm 0.2$ | $4.3 \pm 0.7$ | 0.70 |
| L-ascorbic acid ($\mu$g/mL) | $7.3 \pm 0.5$ | $8.8 \pm 0.6$ | $6.2 \pm 1.7$ | 0.09 |
| LPO ($\mu$mole/L) | $15.7 \pm 0.5$ | $16.3 \pm 0.7$ | $16.5 \pm 0.7$ | 0.08 |

Continuous data was expressed in the form of mean $\pm$ SE (compared with ANOVA test). P-value was significant if $\leq$ 0.05.

**Table 5: Receiver operating characteristic (ROC) analysis of serum levels of the biomarkers**

| Biomarker | Cut off point | Sensitivity | Specificity | AUC |
|---|---|---|---|---|
| CRP | > 1.45pg/mL | 97% | 99.5% | 0.98 |
| TNF-$\alpha$ | > 22.31 pg/mL | 83% | 99.5% | 0.97 |
| IL-10 | > 11.15 pg/mL | 50% | 85% | 0.67 |
| TNF-$\alpha$/IL-10 | > 3.55 | 77% | 93% | 0.91 |
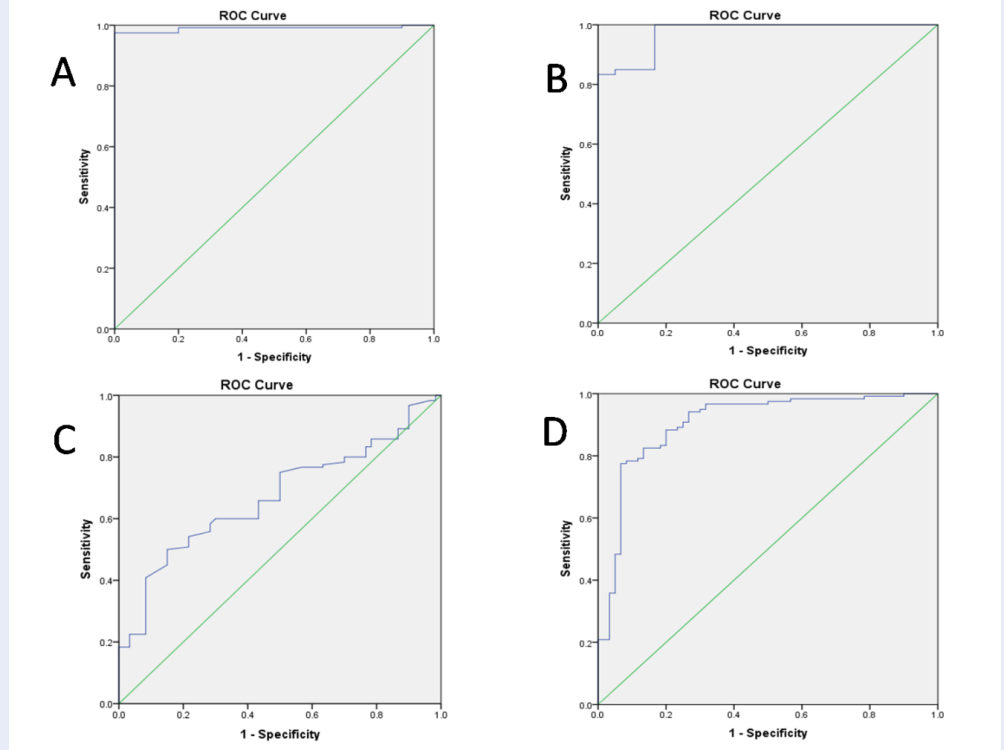
**Figure 2**: ROC assessment of the ability of serum levels of C-reactive protein (CRP) (A), tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (B), interleukin -10 (IL-10) (C), and TNF-$\alpha$/IL-10 ratio (D) to differentiate patients with premature ST-segment elevation myocardial infarction(STEMI)from healthy controls.

## Changes in serum oxidative stress biomarkers in premature STEMI patients versus healthy controls

Serum T-AOC levels were significantly higher in premature STEMI patients than healthy controls (25.5 $\pm$ 0.9 versus 14.9 $\pm$ 0.4 U/mL; P > 0.001). The higher serum levels of NO (10.5 $\pm$ 0.3 versus 8.1 $\pm$ 0.3 nmol/mL; P > 0.001) and the lower GSH levels (3.8 $\pm$ 0.1 $\mu$g/mL versus 5.2 $\pm$ 0.2 $\mu$g/mL; P > 0.001) were significantly different when comparing patients and controls. Serum vitamin C levels were non-significantly higher in patients than controls (7.8 $\pm$ 0.3 versus 7.3 $\pm$ 0.3 $\mu$g/mL; P = 0.17). Serum LPO levels were markedly higher in patients than in the healthy group (15.8 $\pm$ 0.4 versus 4.1 $\pm$ 0.2 $\mu$mol/L, P > 0.001) (**Table 3**).

## The association of the inflammatory and oxidative stress biomarkers with the disease severity assessed by the SYNTAX score

There were significant progressive increases in serum levels of CRP (P > 0.001), TNF-$\alpha$ (P > 0.001), and NO (P = 0.04) with the progress in disease severity assessed by SS (**Table 4**).

## Receiver operating characteristic (ROC) analysis of serum levels of the biomarkers

ROC was utilized to determine the cutoff levels, sensitivity, specificity, and area under the curve (AUC) to investigate the ability of CRP, TNF-$\alpha$, IL-10, and the TNF-$\alpha$/IL-10 ratio to differentiate premature ST-segment elevation myocardial infarction (STEMI) patients from healthy controls. A serum CRP cut-off point >1.45 pg/mL had a sensitivity of 97% and a specificity of 99.5% for the prediction of premature STEMI, with an AUC of 0.980. A serum TNF-$\alpha$ cutoff point >22.31 pg/mL displayed a sensitivity of 83% and a specificity of 99.5% in predicting premature STEMI, with an AUC of 0.970. Serum IL-10 levels >11.15 pg/mL displayed a sensitivity of 50% and a specificity of 85% for the prediction of premature STEMI, with an AUC of 0.670. A serum TNF-$\alpha$/IL-10 ratio cutoff point >3.55 displayed a sensitivity of 77% and a specificity of 93% for the prediction of premature STEMI, with an AUC of 0.910 (**Figure 2**, **Table 5**).

## DISCUSSION

Hypertension, dyslipidemia, diabetes mellitus, and smoking are the standard modifiable cardiovascular risk factors. Although 12.9% of patients with STEMI have been demonstrated to be risk factor-free, they exhibit more aggressive disease and adverse outcomes[24]. Currently, atherosclerosis, as a chronic inflammatory process, incorporates inherited and environmental factors, metabolic disorders, and infections. These risk factors initiate and promote lesion development to the extent of acute thrombotic complications and serious clinical events[25,26]. Since inflammation and oxidative burden are at the core of the clinical manifestations and complications of CAD, recent preventive and therapeutic trials have demonstrated the effectiveness of anti-inflammatory treatment for cardiovascular outcomes in patients with CAD. These interventions include changes in the serum CRP levels used as the primary endpoint, cytokine inhibitors and receptor antagonists, potentiating IL-10, and scavenging oxygen-free radicals[27-29]. Therefore, we planned this study to assess the role of CRP, TNF-$\alpha$, IL-10, and oxidative stress in premature STEMI and its correlation to disease severity in Egyptian patients.

The systemic inflammatory biomarker, CRP, is extensively used as the primary endpoint and severity outcomes gold standard biomarker in the reported CAD studies[28,30]. CRP binds the membrane of ischemic/hypoxic cells and activates their phagocytosis, without giving room for their recovery and regeneration[31]. In our patients, CRP revealed significantly higher levels in young STEMI patients than in controls and efficiently predicted premature STEMI at levels >1.45 pg/mL with 97% sensitivity, 99.5% specificity, and an AUC of 0.98. In accordance with our results, the studies of Han *et al.* and Goswami *et al.* reported significantly elevated levels of CRP in CAD patients compared to healthy controls[32,33]. Furthermore, our results presented an association between the severity of coronary artery lesions assessed by the SYNTAX score and the circulating levels of CRP, where the patient group with SS > 21.5 had the highest levels. Consistently, Liu *et al.* suggested that hs-CRP levels can indicate the severity of CAD assessed by SS and aid in CAD risk stratification and prognosis[1]. In the presence of classic risk factors, hs-CRP levels > 6 mg/L were independently associated with the risk of cardiovascular events in aged CAD patients presented with confirmed atherosclerotic occlusive disease. Additionally, a great reduction in the 2- and 5-year event-free survival was observed for patients with

risk factors but lower hs-CRP levels[34]. Serum levels of hs-CRP in CAD patients independently predict the risk of rapid angiographic stenotic progression[35].

Serum levels of TNF-$\alpha$, a major pro-inflammatory cytokine, were found to be significantly and markedly elevated in our premature STEMI patients compared to healthy controls. TNF-$\alpha$ efficiently predicted premature STEMI at a cutoff level >22.31 pg/mL and displayed 99.5% specificity, 83% sensitivity, and an AUC of 0.970. Similarly, Kumari et al. and Goswami et al. reported significantly elevated levels of TNF-$\alpha$ in CAD patients compared to healthy controls[32,36]. Inhibition of TNF-$\alpha$ signaling is one of the investigated new approaches for preventing CAD[29,34]. Levels of TNF-$\alpha$ > 6 pg/mL were independently associated with cardiovascular event risk in aged CAD patients with confirmed atherosclerotic occlusive disease, greatly reducing 2- and 5-year event-free survival[34]. Our results also exhibited a significant association between the severity of coronary artery lesions assessed by the SYNTAX score and the circulating levels of TNF-$\alpha$. TNF-$\alpha$ and CRP are major pathogenic players in STEMI patients, and their levels correlate positively with each other, but nonsignificantly negatively with IL-10. TNF-$\alpha$ levels increase with the increase in stent numbers[30,37]. Patients with angiography-confirmed CAD presented significantly higher serum TNF-$\alpha$ levels compared to healthy controls[38]. Serum levels of TNF-$\alpha$ in CAD patients independently predict the risk of rapid angiographic stenotic progression[35].

IL-10, a cytokine with anti-inflammatory properties, presented higher serum levels in our premature STEMI patients compared to the volunteers, with a cut-off point of >11.15 pg/mL, 50% sensitivity, 85% specificity, and an AUC of 0.67. Consistently, the study by Goswami *et al.* exhibited higher levels of IL-10 in MI patients than controls[32]. In contrast, Kumari *et al.* presented lower levels of IL-10 in CAD patients than the controls[36]. Several previous studies on ACS patients demonstrated a significant association between elevated IL-10 levels and adverse cardiovascular events[39]. Furthermore, we observed a higher TNF-$\alpha$/IL-10 level in premature STEMI patients than controls, which is supported by the studies of Kumari *et al.* and Goswami *et al.*[32,36]. The findings of Karu *et al.* indicated that the serum levels of IL-10 were significantly lower in CAD patients of both sexes compared to healthy controls[40]. The study by Tajfard e*t al.* revealed a significant reduction in the levels of IL-10 between angiographically-confirmed CAD patients and healthy controls[41]. The

serum levels of TNF-$\alpha$ and IL-10, among other cytokines, significantly positively correlated with the severity of CAD assessed by the Gensini score[42]. The cardioprotective effect of paeoniflorin glycoside was correlated with significant increases in IL-10 secretion by ox-LDL-treated Human coronary artery endothelial cells through inhibiting the Wnt/$\beta$-catenin pathway[43]. MicroRNA-34a is implicated in atherosclerosis by increasing macrophage cholesterol, blood lipid, inflammatory cytokines, and cell adhesion molecules. Its plasma levels correlate positively with inflammatory cytokines such as TNF-$\alpha$, but negatively with IL-10[44].

The higher levels of antioxidant biomarkers observed in our patients compared to healthy controls are not surprising in light of previous publications with a high range of variance. Oxidative stress biomarkers have been extensively investigated in atherosclerosis and CAD, as oxidative stress and inflammation are pathogenically implicated in the disease and its risk factors. Oxidative stress is instrumental in the initiation with the deformed ox-LDL and endothelial dysfunction, progression, and complications of CAD, and may predict outcomes independently of traditional risk factors. Quenching reactive oxygen species (ROS) is among the new approaches to combating the disease[45]. Although a long list of oxidative stress biomarkers has been developed and used, they lack standardization and specificity for CAD, with significant limitations that hinder their integration into clinical practice[45]. In our patients, NO showed elevated levels in premature STEMI patients compared to healthy controls and exhibited increased levels with increasing SS, implying a correlation with disease severity. The study by Cheraghi *et al.* supports our findings, presenting increased levels of NO in CAD patients compared to healthy controls[46]. NO has complex involvement in myocardial infarction and CAD. It initially increases in response to ischemia and inflammation, but its bioavailability diminishes due to endothelial dysfunction and oxidative stress, leading to vascular dysfunction[26]. Moreover, serum levels of T-AOC and LPO were elevated, while GSH was reduced in our premature STEMI patients compared to the healthy controls. This is consistent with the findings of Cheraghi *et al.*, where levels of GSH in healthy individuals were higher than in CAD patients[46]. Lower GSH levels may contribute to the development or progression of CAD, as reduced antioxidant capacity may lead to increased oxidative stress and endothelial dysfunction, both of which are implicated in CAD pathogenesis[26,46]. Variations in levels of the oxidative stress biomarkers used

in our study compared to previous reports could be attributed to several plausible factors that are mainly dietary, as young CAD patients may be more vigilant about their diet: the prevailing Mediterranean diet, dietary supplements, and the high nitrate/nitrite contents in Egyptian foodstuffs. In middle-aged adults like our patients, the antioxidant capacities would be more vividly induced by reactive oxidative compounds[47,48]. As NO was measured as nitrite, it is possible that it rather reflects peroxynitrite, a pro-oxidant marker, that would correlate with low NO bioavailability. Also, the apparent increase in T-AOC in our patients could be a reaction to the existing oxidative stress, as reflected by the increases in LPO and reduction in GSH.

Preclinical research and extensive epidemiological studies have demonstrated the key role inflammation plays in the pathophysiology of atherosclerosis. There are multiple mediators in the intricate pathophysiological relationship between inflammation and atherosclerosis. Previous studies have demonstrated that anti-inflammatory treatments can lower the risk of cardiovascular disease. However, the results of treatments specifically targeting inflammation remain debatable; further studies are needed to better understand this association in the future[29].

The limitations of our case-control cross-sectional study included the single-center small sample size, without longitudinal patient follow-up and data collection. This limits its generalization and usefulness. Furthermore, as we targeted acute STEMI patients, the biomarkers' characteristics (cut-offs, sensitivity, specificity, and AUC) are neither generalized to non-acute cases nor to the general population. The longitudinal implications of our findings were difficult to assess due to the loss of follow-up for most of our patients.

## CONCLUSIONS

Our findings indicate that the inflammatory markers—CRP, TNF-$\alpha$, IL-10, TNF-$\alpha$/IL-10 ratio, and oxidative stress—contribute to premature STEMI development in the Egyptian population, and they are correlated with the severity of coronary artery lesions. They revealed biomarker potential; strongest for CRP, followed by TNF-$\alpha$, then TNF-$\alpha$/IL-10 ratio, and weakest for IL-10. Future studies may target a deeper understanding of the underlying mechanisms of our observations and investigate other populations.

## ABBREVIATIONS

**ACS** – Acute Coronary Syndrome, **AUC** – Area Under the Curve, **ALP** – Alkaline Phosphatase, **ALT** – Alanine Aminotransferase, **AST** – Aspartate Aminotransferase, **BMI** – Body Mass Index, **BUN** – Blood Urea Nitrogen, **CAD** – Coronary Artery Disease, **CK-MB** – Creatine Kinase-MB, **CRP** – C-reactive Protein, **cTnT** – Cardiac Troponin T, **ECG** – Electrocardiogram, **GSH** – Reduced Glutathione, **HDL-C** – High-Density Lipoprotein Cholesterol, **hs-CRP** – High-sensitivity C-reactive Protein, **IL-10** – Interleukin-10, **LDL-C** – Low-Density Lipoprotein Cholesterol, **LPO** – Lipid Peroxidation, **MDA** – Malondialdehyde, **MI** – Myocardial Infarction, **NO** – Nitric Oxide, **PCI** – Percutaneous Coronary Intervention, **ROC** – Receiver Operating Characteristic, **SPSS** – Statistical Package for the Social Sciences, **STEMI** – ST-segment Elevation Myocardial Infarction, **SYNTAX** – Synergy Between PCI with Taxus and Cardiac Surgery, **TAC** – Total Anti-Oxidative Capacity, **T-AOC** – Total Antioxidant Capacity, **TNF-$\alpha$** – Tumor Necrosis Factor-alpha, **WSS** – Wall Shear Stress

## AUTHOR'S CONTRIBUTIONS

Omnia HM Omar and Marwa A Gaber designed the study protocol development, performed all lab investigations, and writing of the manuscript. Marwan S Mahmoud selected the participants, performed clinical assessments of all patients, interpretation of clinical findings, and participated in revising the manuscript. Abdel-Raheim MA Meki, Ahmed Y Nassar and Ayman KM Hassan revised the manuscript. All authors agreed to be accountable for every aspect of the study and accepted the final version of the article as submitted.

## AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was carried out in compliance with the Helsinki Declaration principles after being approved by the Institutional Review Board, Faculty of Medicine, Assiut University (Approval#: IRB17200689). Volunteering participants signed the informed consent form,after being briefed on the nature of the study.

## CONSENT FOR PUBLICATION

Not applicable.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## REFERENCES

1. Liu Y, Jia SD, Yao Y, Tang XF, Xu N, Jiang L. Impact of high-sensitivity C-reactive protein on coronary artery disease severity and outcomes in patients undergoing percutaneous coronary intervention. Journal of Cardiology. 2020;75(1):60–5. PMID: 31416781. Available from: https://doi.org/10.1016/j.jjcc.2019.06.012.
2. Qu Y, Zhang F, Yang J, Dai Y, Li C, Yang H. Clinical characteristics and outcomes in Asian patients with premature coronary artery disease: insight from the FOCUS registry. Angiology. 2019;70(6):554–60. PMID: 30419756. Available from: https://doi.org/10.1177/0003319718810182.
3. Yanase T, Sakakura K, Taniguchi Y, Yamamoto K, Tsukui T, Seguchi M. Comparison of clinical characteristics of acute myocardial infarction between young (< 55 years) and older (55 to< 70 years) patients. International Heart Journal. 2021;62(1):33–41. PMID: 33518663. Available from: https://doi.org/10.1536/ihj.20-444.
4. Ando H, Yamaji K, Kohsaka S, Ishii H, Sakakura K, Goto R. Clinical Presentation and In-Hospital Outcomes of Acute Myocardial Infarction in Young Patients: Japanese Nationwide Registry. JACC : Asia. 2022;2(5):574–85. PMID: 36518720. Available from: https://doi.org/10.1016/j.jacasi.2022.03.013.
5. Ding Z, Pothineni NV, Goel A, Lüscher TF, Mehta JL. PCSK9 and inflammation: role of shear stress, proinflammatory cytokines, and LOX-1. Cardiovascular Research. 2020;116(5):908–15. PMID: 31746997. Available from: https://doi.org/10.1093/cvr/cvz313.
6. Soehnlein O, Libby P. Targeting inflammation in atherosclerosis - from experimental insights to the clinic. Nature Reviews Drug Discovery. 2021;20(8):589–610. PMID: 33976384. Available from: https://doi.org/10.1038/s41573-021-00198-1.
7. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Frontiers in Immunology. 2018;9:754. PMID: 29706967. Available from: https://doi.org/10.3389/fimmu.2018.00754.

8. Rupprecht S, Finn S, Hoyer D, Guenther A, Witte OW, Schultze T. Association between systemic inflammation, carotid arteriosclerosis, and autonomic dysfunction. Translational Stroke Research. 2020;11(1):50–9. PMID: 31093927. Available from: https://doi.org/10.1007/s12975-019-00706-x.

9. Mehta AK, Gracias DT, Croft M. TNF activity and T cells. Cytokine. 2018;101:14–8. PMID: 27531077. Available from: https://doi.org/10.1016/j.cyto.2016.08.003.

10. Hachula M, Kosowski M, Ryl S, Basiak M, Okopień B. Impact of Glucagon-Like Peptide 1 Receptor Agonists on Biochemical Markers of the Initiation of Atherosclerotic Process. International Journal of Molecular Sciences. 2024;25(3):1854. PMID: 38339133. Available from: https://doi.org/10.3390/ijms25031854.

11. Carlini V, Noonan DM, Abdalalem E, Goletti D, Sansone C, Calabrone L, et al. The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. Frontiers in Immunology. 2023;14:1161067. PMID: 37359549. Available from: https://doi.org/10.3389/fimmu.2023.1161067.

12. Farah C, Michel LY, Balligand JL. Nitric oxide signalling in cardiovascular health and disease. Nature Reviews Cardiology. 2018;15(5):292–316. PMID: 29388567. Available from: https://doi.org/10.1038/nrcardio.2017.224.

13. Li X, Zhang F, Zhou H, Hu Y, Guo D, Fang X, et al. Interplay of TNF-$\alpha$, soluble TNF receptors and oxidative stress in coronary chronic total occlusion of the oldest patients with coronary heart disease. Cytokine. 2020;125:154836. PMID: 31494339. Available from: https://doi.org/10.1016/j.cyto.2019.154836.

14. Theofilis P, Oikonomou E, Chasikidis C, Tsioufis K, Tousoulis D. Inflammasomes in Atherosclerosis-From Pathophysiology to Treatment. Pharmaceuticals (Basel, Switzerland). 2023;16(9):1211. PMID: 37765019. Available from: https://doi.org/10.3390/ph16091211.

15. Henein MY, Vancheri S, Longo G, Vancheri F. The role of inflammation in cardiovascular disease. International Journal of Molecular Sciences. 2022;23(21):12906. PMID: 36361701. Available from: https://doi.org/10.3390/ijms232112906.

16. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction (2018). Circulation. 2018;138(20):e618–51. PMID: 30571511. Available from: https://doi.org/10.1161/CIR.0000000000000617.

17. Gaber MA, Omar OH, Meki AM, Nassar AY, Hassan AK, Mahmoud MS. The significance of PCSK-9-singles level and polymorphism in premature coronary artery disease: relation to risk and severity. Clinical Biochemistry. 2024;125:110729. PMID: 38342398. Available from: https://doi.org/10.1016/j.clinbiochem.2024.110729.

18. Serruys P, Onuma Y, Garg S, Sarno G, Brand MVD, Kappetein A. Assessment of the SYNTAX score in the Syntax study (EuroIntervention (2009) 5,(50-56)). EuroIntervention. 2009;5:401. Available from: https://doi.org/10.4244/EIJV5I1A9.

19. Gao J, Yang YN, Cui Z, Feng SY, Ma J, Li CP. Pcsk9 is associated with severity of coronary artery lesions in male patients with premature myocardial infarction. Lipids in Health and Disease. 2021;20(1):56. PMID: 34044829. Available from: https://doi.org/10.1186/s12944-021-01478-w.

20. Ding AH, Nathan CF, Stuehr D. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. Journal of immunology (Baltimore, Md: 1950). 1988;141(7):2407–12. Available from: https://doi.org/10.4049/jimmunol.141.7.2407.

21. Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature Protocols. 2006;1(6):3159–65. PMID: 17406579. Available from: https://doi.org/10.1038/nprot.2006.378.

22. Ravi K, Ramachandran B, Subramanian S. Effect of Eugenia Jambolana seed kernel on antioxidant defense system in streptozotocin-induced diabetes in rats. Life Sciences. 2004;75(22):2717–31. PMID: 15369706. Available from: https://doi.org/10.1016/j.lfs.2004.08.005.

23. Jagota SK, Dani HM. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Analytical Biochemistry. 1982;127(1):178–82. PMID: 7165085. Available from: https://doi.org/10.1016/0003-2697(82)90162-2.

24. Kong G, Chin YH, Chong B, Goh RS, Lim OZ, Ng CH. Higher mortality in acute coronary syndrome patients without standard modifiable risk factors: results from a global meta-analysis of 1,285,722 patients. International Journal of Cardiology. 2023;371:432–40. PMID: 36179904. Available from: https://doi.org/10.1016/j.ijcard.2022.09.062.

25. Attiq A, Afzal S, Ahmad W, Kandeel M. Hegemony of inflammation in atherosclerosis and coronary artery disease. European Journal of Pharmacology. 2024;966:176338. PMID: 38242225. Available from: https://doi.org/10.1016/j.ejphar.2024.176338.

26. Medina-Leyte DJ, Zepeda-García O, Domínguez-Pérez M, González-Garrido A, Villarreal-Molina T, Jacobo-Albavera L. Endothelial dysfunction, inflammation and coronary artery disease: potential biomarkers and promising therapeutical approaches. International Journal of Molecular Sciences. 2021;22(8):3850. PMID: 33917744. Available from: https://doi.org/10.3390/ijms22083850.

27. Yu M, Zhu ZF, Yang F, Yuan YF, Liao SD, Liu ML. Different anti-inflammatory drugs on high-sensitivity C-reactive protein in patients after percutaneous coronary intervention: A pilot randomized clinical trial. Journal of Cardiovascular Pharmacology. 2022;10:1097. PMID: 37944130.

28. Zheng S, Ma X, Kong Z, Yang C. Adjunctive Effect and Safety of Chinese Herbal Medicine in Patients with Coronary Artery Disease Undergoing Percutaneous Coronary Intervention: A Systematic Review and Meta-Analysis. Alternative Therapies in Health and Medicine. 2023;30(5):155–161. PMID: 37917887.

29. Dimitroglou Y, Aggeli C, Theofilis P, Tsioufis P, Oikonomou E, Chasikidis C. Novel Anti-Inflammatory Therapies in Coronary Artery Disease and Acute Coronary Syndromes. Life (Basel, Switzerland). 2023;13(8):1669. PMID: 37629526. Available from: https://doi.org/10.3390/life13081669.

30. Wan M, Hu K, Lu Y, Wang C, Mao B, Yang Q. Co-release of cytokines after drug-eluting stent implantation in acute myocardial infarction patients with PCI. Scientific Reports. 2024;14(1):1236. PMID: 38216681. Available from: https://doi.org/10.1038/s41598-024-51496-8.

31. Sheriff A, Kunze R, Brunner P, Vogt B. Being Eaten Alive: How Energy-Deprived Cells Are Disposed of, Mediated by C-Reactive Protein-Including a Treatment Option. Biomedicines. 2023;11(8):2279. PMID: 37626775. Available from: https://doi.org/10.3390/biomedicines11082279.

32. Goswami B, Rajappa M, Mallika V, Shukla DK, Kumar S. TNF-$\alpha$/IL-10 ratio and C-reactive protein as markers of the inflammatory response in CAD-prone North Indian patients with acute myocardial infarction. Clinica Chimica Acta. 2009;408(1-2):14–8. PMID: 19576194. Available from: https://doi.org/10.1016/j.cca.2009.06.029.

33. Han K, Lu Q, Zhu WJ, Wang TZ, Du Y, Bai L. Correlations of degree of coronary artery stenosis with blood lipid, CRP, Hcy, GGT, SCD36 and fibrinogen levels in elderly patients with coronary heart disease. European Review for Medical and Pharmacological Sciences. 2019;23(21):9582–9. PMID: 31773710.

34. Kablak-Ziembicka A, Przewlocki T, Sokolowski A, Tracz W, Podolec P. Carotid intima-media thickness, hs-CRP and TNF-$\alpha$ are independently associated with cardiovascular event risk in patients with atherosclerotic occlusive disease. Atherosclerosis. 2011;214(1):185–90. PMID: 21067752. Available from: https://doi.org/10.1016/j.atherosclerosis.2010.10.017.

35. Sun J, Yu H, Liu H, Pu D, Gao J, Jin X, et al. Correlation of preoperative circulating inflammatory cytokines with restenosis and rapid angiographic stenotic progression risk in coronary artery disease patients underwent percutaneous coronary in-

tervention with drug-eluting stents. Journal of Clinical Laboratory Analysis. 2020;34(3):e23108. PMID: 31729103. Available from: https://doi.org/10.1002/jcla.23108.

36. Kumari R, Kumar S, Ahmad MK, Singh R, Pradhan A, Chandra S, et al. TNF-a/IL-10 ratio: An independent predictor for coronary artery disease in North Indian population. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2017;12(3):221–5. Available from: https://doi.org/10.1016/j.dsx.2017.09.006.

37. Kozel M, KoV, Lisa L, Budšínský T, Toušek P. Immune-inflammatory response after bioresorbable vascular scaffold implantation in patients with acute myocardial infarction with ST elevation in a long-term perspective. Heart and Vessels. 2019;34(4):557–63. PMID: 30315494. Available from: https://doi.org/10.1007/s00380-018-1281-7.

38. Jaafar AF, Afrisham R, Fadaei R, Farrokhi V, Moradi N, Abbasi A. CCN3/NOV serum levels in coronary artery disease (CAD) patients and its correlation with TNF-$\alpha$ and IL-6. BMC Research Notes. 2023;16(1):306. PMID: 37919772. Available from: https://doi.org/10.1186/s13104-023-06590-x.

39. Roumeliotis S, Veljkovic A, Georgianos PI, Lazarevic G, Perisic Z, Hadzi-Djokic J, et al. Association between biomarkers of oxidative stress and inflammation with cardiac necrosis and heart failure in non-ST segment elevation myocardial infarction patients and various degrees of kidney function. Oxidative Medicine and Cellular Longevity. 2021;2021(1):3090120. Available from: https://doi.org/10.1155/2021/3090120.

40. Karu I, Starkopf J, Zilmer K, Zilmer M. Growth factors serum levels in coronary artery disease patients scheduled for bypass surgery: perioperative dynamics and comparisons with healthy volunteers. BioMed research international. 2013;2013(1):985404. Available from: https://doi.org/10.1155/2013/985404.

41. Tajfard M, Latiff LA, Rahimi HR, Mouhebati M, Esmaeily H, Taghipour A, et al. Serum inflammatory cytokines and depression in coronary artery disease. Iranian Red Crescent Medical Journal. 2014;16(7):e17111. PMID: 25237578. Available from: https://doi.org/10.5812/ircmj.17111.

42. Min X, Lu M, Tu S, Wang X, Zhou C, Wang S, et al. Serum cytokine profile in relation to the severity of coronary artery disease. BioMed research international. 2017;2017(1):4013685. Available from: https://doi.org/10.1155/2017/4013685.

43. Liu S, Li Y, Wu C. Paeoniflorin suppresses the apoptosis and inflammation of human coronary artery endothelial cells induced by oxidized low-density lipoprotein by regulating the Wnt/$\beta$-catenin pathway. Pharmaceutical Biology. 2023;61(1):1454–61. PMID: 37674320. Available from: https://doi.org/10.1080/13880209.2023.2220360.

44. Li H, Chen M, Feng Q, Zhu L, Bai Z, Wang B, et al. MicroRNA-34a in coronary heart disease: correlation with disease risk, blood lipid, stenosis degree, inflammatory cytokines, and cell adhesion molecules. Journal of Clinical Laboratory Analysis. 2022;36(1):e24138. PMID: 34861059. Available from: https://doi.org/10.1002/jcla.24138.

45. Simantiris S, Papastamos C, Antonopoulos AS, Theofilis P, Sagris M, Bounta M. Oxidative Stress Biomarkers in Coronary Artery Disease. Current Topics in Medicinal Chemistry. 2023;23(22):2158–71. PMID: 37138428. Available from: https://doi.org/10.2174/1568026623666230502140614.

46. Cheraghi M, Ahmadvand H, Maleki A, Babaeenezhad E, Shakiba S, Hassanzadeh F. Oxidative stress status and liver markers in coronary heart disease. Reports of Biochemistry & Molecular Biology. 2019;8(1):49–55. PMID: 31334288.

47. Bubb KJ, Drummond GR, Figtree GA. New opportunities for targeting redox dysregulation in cardiovascular disease. Cardiovascular Research. 2020;116(3):532–44. PMID: 31297507. Available from: https://doi.org/10.1093/cvr/cvz183.

48. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nature Reviews Drug Discovery. 2021;20(9):689–709. PMID: 34194012. Available from: https://doi.org/10.1038/s41573-021-00233-1.