# Relationship between Oxidative Stress Markers and Presence of Chronic Total Occlusion in Coronary Artery Disease

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## ABSTRACT

**Objective:** To investigate the relationship between chronic total occlusion (CTO) development and oxidative stress markers in stable coronary artery patients.

**Study Design:** A cohort study.

**Place and Duration of Study:** Cardiology Clinic, Kahramanmaras Sutcu Imam University Medical Faculty, between January 2018 and December 2019.

**Methodology:** Patients, who underwent coronary angiography for stable chest pain, were consecutively included. The study group consisted of those with CTO and the control group from those without CTO. Serum total oxidant/anti-oxidant, dynamic thiol/disulfide, antioxidant (ascorbate, alfa-tocopherol, beta-carotene) vitamin levels, and routine biochemistry tests of the patients were compared.

**Results:** The study group (24 men, 5 women, mean age 63.79 ± 9.21 years) and control group (23 men, 6 women, mean age 61.38 ± 8.20 years) consisted of 29 patients each. The oxidative stress markers (total thiol, native thiol, disulfide, reduced thiol ratio, oxidized thiol ratio, thiol oxidation-reduction ratio, total antioxidant status, total oxidant status, and vitamin E) were found to have similar values between the groups. However, of the anti-oxidative vitamins, vitamin C, vitamin A and vitamin C/vitamin E ratio were significantly lower in the CTO group and predicted a CTO lesion (AUC: 0.084, p<0.001, 95% CI: 0.007-0.162; AUC: 0.285, p=0.005, 95% CI: 0.154-0.416 and AUC: 0.181, p <0.001, 95% CI: 0.062-0.299, respectively).

**Conclusion:** The lower serum vitamin C and vitamin A levels and low vitamin C/vitamin E ratio may be useful in predicting the risk of CTO in stable patients with non-critical stenosis in coronary angiography.

**Key Words:** *Chronic total occlusion, Oxidative balance, Stable coronary artery disease, Vitamin A, Vitamin C, Vitamin C / vitamin A ratio*.

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## INTRODUCTION

Chronic total occlusion (CTO) is defined as the complete blockage of the coronary artery characterised by a TIMI 0 flow, which is absence of any antegrade flow beyond the coronary occlusion, present for at least three months.[1] The presence of chronic occlusion of coronary arteries is closely related to poor prognosis.[2] It is seen in approximately one-third of coronary angiographies performed in patients with stable angina pectoris.[3] In this patient group, it may be important to distinguish those with a high risk of developing CTO in order to reduce mortality and morbidity rates.

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The oxidative stress status may be one of the markers that plays a role in and/or show the development of CTO. Reactive oxygen products, which increase by the deterioration of the oxidative balance, can cause damage to tissues and organs, their negative effects on DNA, protein and lipid structures.[4] The oxidative balance has been reported to be associated with the progression, erosion, and instability of atherosclerotic plaques in coronary arteries. However, this relationship has generally been studied in patients with acute coronary syndrome.[5,6] To the best of the authors' knowledge, the relationship between chronic total occlusion development and oxidative stress status in stable coronary artery disease has not been studied.

The aim of this study was to investigate the relationships in the oxidative stress status evaluated over the total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), thiol/disulfide homeostasis, and antioxidative vitamin levels and possible differences in patients with non-critical coronary artery disease and those with CTO.

## METHODOLOGY

Between January 2018 and December 2019, the patients admitted in the Department of Cardiology, Medical Faculty Hospital of Kahramanmaras Sutcu İmam University, Turkey, were consecutively evaluated. The patients with a CTO lesion in at least one major coronary artery but no critical lesion (≥50% stenosis) in other coronary arteries constituted the study group. The patients with similar demographic characteristics and without any critical or CTO lesions on coronary angiogram formed the control group. Being under the age of 18 years, unstable angina pectoris, left ventricular systolic dysfunction (ejection fraction <50%), severe anemia, pregnancy, history of congenital heart disease, thyroid disorders, sepsis, malignancy, chronic hematological disease, malnutrition, collagen tissue disease, obesity (defined as the body mass index *i.e*. BMI ≥30 kg/m$^2$), moderate or advanced liver deficiency, renal deficiency (glomerular filtration rate <60 ml/min/1.73 m$^2$), severe heart valve disease, electrolyte disorders and receiving multivitamin and antioxidant supplementation treatment were determined as the exclusion criteria. BMI was calculated by dividing the body weight (Kg) by the body height in meters squared.

Antecubital venous blood samples of all the patients were taken between 08:00 and 09:30 after 12 hours of fasting. In the obtained serum samples, TAS, TOS, native thiol and total thiol were determined colorimetrically by using a commercial kit and a spectrophotometer (Shimadzu UV-VIS 1800), and vitamin A, C and E levels were determined by an elisa (enzyme-linked immunosorbent assay) kit and device (thermo scientific model: multiskan FC, Finland) collectively.

The coronary artery stenosis levels of <50% in the patients were considered as noncritical stenosis, while lesions with TIMI 0 flow in the lesion region were accepted as chronic total occlusion. Two interventional cardiologists, blinded to the study, made the angiographic assessments and calculated the Gensini score of each patient.[7]

Standard transthoracic echocardiographies of the patients, taken by using the Vivid 7® cardiac ultrasonography device with a 2.5-5-MHz probe, were obtained. All echocardiographic assessments were made in line with the recommendations of the guidelines by the American Heart Association.[8]

Shapiro-Wilk test and histograms were used to test whether the continuous variables satisfied normal distribution. The normally distributed continuous variables are presented as mean ± standard deviation, while the non-normally distributed ones are presented as median (25th percentile-75th percentile). In the comparisons of the groups, Student's t-test for the normally distributed parameters; and Mann-Whitney U-test for the non-normally distributed ones were utilised. Chi-squared test or Fisher's Exact test was used in the comparisons of the categorical variables. To measure the power of the oxidative stress markers to predict chronic total occlusion, receiver operating curve (ROC) calculations were utilised; p <0.05 was accepted as statistically significant. All statistical calculations were made by using the Statistical Package for the Social Sciences version 23.

## RESULTS

The demographic data, comorbidities, and the drugs they used were similar between the groups (Table I). The comparisons showing the relationships between the oxidative stress markers and CTO are given as a summary in Table II. Vitamin E and other oxidative stress markers were similar between groups. Vitamin A, vitamin C, and vitamin C/vitamin E ratio were statistically significantly lower in the CTO group (p = 0.005, p <0.001, p <0.001, respectively).

**Table I: Comparison of demographic data between the groups with and without chronic total occlusion.**

| Variables | non-CTO (n=29) | CTO (n=29) | p |
|---|---|---|---|
| Females, n% | 6 (20.7) | 5 (17.2) | 0.738 |
| Age | 61.38 ± 8.20 | 63.79 ± 9.21 | 0.296 |
| Sistolic BP, mmHg | 135.22 ± 7.25 | 135.42±10.72 | 0.935 |
| Diastolic BP, mmHg | 79.21 ± 5. 77 | 80.59 ± 4.28 | 0.306 |
| GENSINI score | 5.24 ± 2.05 | 39.31 ± 7.58 | **<0.001** |
| Ejection Fraction, % | 60.55 ± 5.05 | 58.21 ±4.80 | 0.075 |
| Comorbidities | | | |
| Smoking, n% | 8 (27.6) | 6 (20.7) | 0.539 |
| Diabetes mellitus, n% | 10 (34.5) | 12 (41.4) | 0.588 |
| Hypertension, n % | 17 (58.6) | 19 (65.5) | 0.588 |
| Hyperlipidemia, n% | 8 (27.6) | 6 (20.7) | 0.539 |
| COPD, n% | 5(17.2) | 5 (17.2) | >0.999 |
| Drug use | | | |
| ACE/ARB inhibitor, n % | 14 (48.3) | 16 (55.2) | 0.599 |
| Beta blocker, n % | 9 (31) | 6 (20.7) | 0.368 |
| CCB, n % | 13 (44.8) | 12 (41.4) | 0.791 |
| Hydrochlorothiazide, n % | 12 (41.4) | 15 (51.7) | 0.43 |
| ASA, n % | 9 (31) | 12 (41.4) | 0.412 |
| Statin, n % | 8 (27.6) | 9 (31) | 0.773 |
| OAD, n % | 10 (34.5) | 12 (41.4) | 0.588 |

*COPD; Chronıc obstrıctıve pulmonary disease, ACE: Angiotensin converting enzyme, ARB: Angiotensin receptor blocker, CCB: Calcium channel blocker, ASA: Acetyl salicylic acid, OAD: Oral anti-diabetic.*

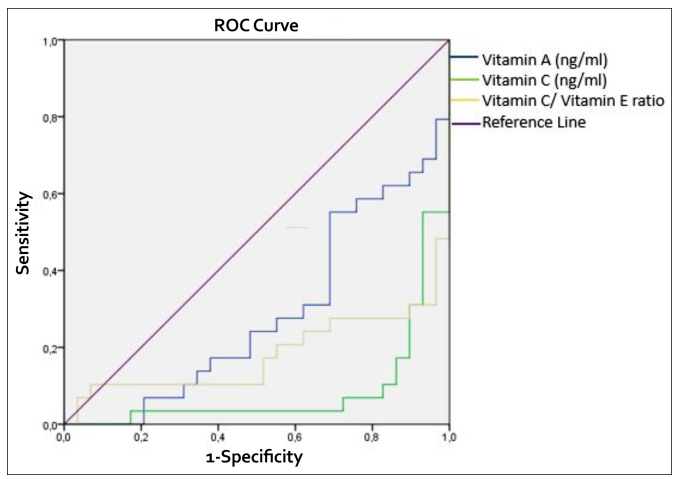


**Figure 1: The relationship of vitamin C, vitamin A and vitamin C/vitamin E ratio with coronary chronic total occlusion.**

In the ROC analyses conducted to reveal the predictability of CTO by the vitamin A and vitamin C levels, it was observed that vitamin A could predict CTO significantly but on a weak level (AUC: 0.285, p=0.005, 95% CI: 0.154-0.416). The predictive power of vitamin C was very weak but similar in significance

(AUC: 0.084, p <0.001, 95% CI: 0.007-0.162). When ROC analysis was carried out, it was seen that the ratio of vitamin C to vitamin E could predict CTO significantly, but on a weak level (AUC: 0.181, p <0.001, 95% CI: 0.062-0.299, Figure 1).

**Table II: Comparison of laboratory data between the groups with and without chronic total occlusion.**

| Variables | non-CTO (n=29) | CTO (n=29) | P |
|---|---|---|---|
| Total cholesterol, mg/dl | 184.07 ± 36.76 | 185.17 ± 31.20 | 0.902 |
| LDL, mg/dl | 117.24 ± 32.70 | 126 ± 18.50 | 0.216 |
| HDL, mg/dl | 40.48 ± 10.11 | 39.24 ± 11.94 | 0.671 |
| TG, mg/dl | 148.41 ± 56.72 | 162.62 ± 72.57 | 0.410 |
| MPV | 10.15 ± 0.79 | 10.57 ± 0.83 | 0.054 |
| Total thiol, µmol / L | 175.52 ± 18.31 | 178.64 ± 19.65 | 0.534 |
| Native thiol, µmol / L | 118.96 ± 17.10 | 122.64 ± 14.86 | 0.385 |
| Disulfide, µmol / L | 28.28 ± 7.86 | 28 ± 9.30 | 0.902 |
| Reduced Thiol Ratio | 67.88 ± 7.70 | 69.05 ± 8.36 | 0.579 |
| Oxidized Thiol Ratio | 16.06 ± 3.85 | 15.47 ±4.18 | 0.579 |
| Thiol Oxidation Reduction Ratio [median(%25-%75)] | 426.93 (352.56-531.03) | 451.26 (363.32-552.45) | 0.529 |
| OSI | 0.40 ± 0.20 | 0.38 ± 0.27 | 0.723 |
| TOS, umol/L | 7.28 ± 3.47 | 7.05 ± 5.08 | 0.843 |
| TAS, mmol/L | 1.86 ± 0.21 | 1.90 ± 0.20 | 0.460 |
| Native thiol/ Total thiol Ratio | 0.68 ± 0.08 | 0.69 ± 0.08 | 0.579 |
| Disulfide/ Native thiol Ratio | 0.24 ± 0.084 | 0.23 ± 0.085 | 0.597 |
| Disulfide/ Total thiol Ratio | 0.16 ± 0.04 | 0.15 ± 0.04 | 0.579 |
| Vitamin A, ng/ml [median(%25-%75)] | 23.65 (8.22-43.79) | 11.96 (5.97-2.26) | 0.005 |
| Vitamin C, ng/ml | 13.32 (9.25-16.77) | 4.08 (2.09-5.21) | <0.001 |
| Vitamin C / Vitamin E Ratio [median(%25-%75)] | 0.84 (0.68-1.34) | 0.27 (0.09-0.72) | <0.001 |
| Vitamin E, nmol/ml [median(%25-%75)] | 14.51 (10.95-8.65) | 13.44 (8.12-6.63) | 0.331 |

*LDL: Low density lipoprotein, HDL: High-density lipoprotein, TG: Triglyceride, MPV: Mean platelet volume, OSI: Oxidative stress index, TOS: Total oxidant status, TAS: Total antioxidant status.*

## DISCUSSION

To the best of the authors' knowledge, this is the first study in the literature which investigated the relationship between chronic total occlusion development and oxidative stress markers in stable coronary artery patients. According to the results of this study, low vitamin A and C levels in those with stable coronary artery disease and those without critical stenosis in their coronary angiography may be associated with CTO development.

The oxidative and antioxidative systems are divided into two groups as the enzymatic and non-enzymatic systems. Antioxidative vitamins among the main components of the non-enzymatic system work in synergy with enzyme systems in the case that oxidative stress increases. Vitamins C, A and E are micronutrients with well-known antioxidant activity. While vitamin C is the most important antioxidant of the extracellular fluid, it prevents lipid peroxidation in extracellular tissue with its strong reduction properties.[9] Several studies have demonstrated that vitamin C reduced LDL oxidation, reduced the adhesion of monocytes to the endothelium and slowed down atherosclerotic plaque progression by preventing the apoptosis of smooth muscle cells in atherosclerotic plaques.[9,10] Vitamin A is the most effective quencher of singlet oxygen. It is a strong quencher of oxygen that prevents the peroxidation of membrane lipids. Thus, the progression of atherosclerosis to chronic total occlusion may be significantly related to antioxidant vitamin levels. A meta-analysis investigating the relationship among these vitamins; and cardiovascular mortality showed that increased vitamin C, vitamin E and β-carotene concentrations in circulation were associated with a lower CVD mortality risk.[11-14] On the other hand, there have been other studies reporting that providing high doses of vitamin C, vitamin E and β-carotene with diet supplementation did not reduce cardiovascular risk.[14] It was stated that, not only their concentrations in the diet but also many factors like individuals' activity levels, smoking and drinking statuses and genetic factors play a role in determining the plasma levels of these vitamins. Hence, it was reported that, rather than the amounts of these vitamins in the diet, the presence of their optimal plasma levels may be more important in protection from cardiovascular diseases.[15] The antioxidative property lost by vitamin E by neutralising a free radical is regained by the help of other antioxidants, especially like vitamin C.[12] It was also specified that the optimal vitamin C/vitamin E ratio is important in the maintenance of the antioxidant status.[16] In this study, it was observed in stable coronary artery patients that low plasma vitamin C and vitamin A levels and low vitamin C/vitamin E ratio could be associated with CTO development.

Another parameter showing the oxidant-antioxidant balance in the body is thiol/disulfide homeostasis.[17] The native thiol, total thiol levels, disulfide levels, disulfide/native thiol ratio, disulfide/total thiol ratio and native thiol/total thiol markers allow us to assess the status of the thiol-disulfide homeostasis. The studies showed the role played by an abnormal thiol/disulfide balance in the formation of atherosclerotic coronary artery diseases.[5,6,18] One of the antioxidant systems facilitating the regulation of the oxidation/reduction balance on the cellular level is the thioredoxin system. It was determined that thioredoxin levels increased in myocardial infarction.[19,20] In this study, the thiol balance and thioredoxin system were found to have similar values in stable coronary patients with and without CTO patients.

TAS, TOS, and OSI used in the evaluation of oxidative balance are tests that can evaluate the total oxidant and antioxidant levels cumulatively. In patients with acute coronary syndrome, coronary artery disease was reported to be positively associated with TOS and OSI and negatively associated with TAS values.[21,22] However, in this study, it was determined that there was no significant relationship between TAS, TOS and OSI values and the development of CTO in patients with stable coronary artery disease.

In this study, there was a decrease in vitamin C and vitamin A levels, which are oxidative stress markers, and low levels of vitamin C/vitamin E ratio in chronic total occlusion patients. These results may be related to CTO development. The eating habits of the participants and environmental factors may have

played a confounding role. Even if those with critical coronary stenosis were excluded in the study, the differences in the microvascular bed may have played a confounding role.

## CONCLUSION

Low serum vitamin C and vitamin A levels and a low vitamin C/vitamin E ratio may be an easy-to-use, inexpensive marker that could be used in prediction of the risk of chronic total occlusion development in patients whose coronary angiographies reveal non-critical stenosis. However, to support this finding and determine the limit value, large-volume prospective studies are needed.

**ETHICAL APPROVAL:**
The study protocol, which was compatible with the Declaration of Helsinki, was approved by the Ethics Committee of Clinical Research of the Kahramanmaras Sutcu Imam University Medical Faculty (Approval Date: 14.10.2020; Protocol No. 2020/19/16).

**PATIENTS' CONSENT:**
Because this study was retrospective, the patients' consents were waived.

**CONFLICT OF INTEREST:**
The authors declared no conflict of interest.

**AUTHORS' CONTRIBUTION:**
EA: Conception and design of the study, interpretation of the analysed data and writing of the manuscript, and final approval of the study.
EC, MD, FIT: Patient selection, data acquisition, data analysis, and final approval of the study.
ASB: Critical review, statistical analysis, and final approval of the study.

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