JOURNAL OF
CONTEMPORARY MEDICINE

DOI:10.16899/jcm.1107527
J Contemp Med 2022;12(4):574-578

Original Article / Orijinal Araştırma

# Clinical Importance of Serum Prolidase and Carbonic Anhydrase III Levels In Patients with Stable Chronic Obstructive Pulmonary Disease

## Stabil Kronik Obtrüktif Akciğer Hastalığı Hastalarında Serum Prolidaz ve Karbonik Anhidraz III Düzeylerinin Klinik Önemi

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## Abstract

**Aim**: Chronic obstructive pulmonary disease (COPD) is a disease characterized by irreversible airway flow limitation and chronic airway inflammation. We aimed to investigate the clinical importance of serum prolidase enzyme, which is an indicator of collagen degradation, and Carbonic anhydrase (CA) III enzyme, which has an important function in acid-base regulation, in patients with COPD

**Material and Method**: In this study, 56 stable COPD patients and 32 healthy subjects without smoking history and comorbidities were included. Serum CA III and prolidase enzyme levels were compared between the two groups.

Results: The statistical difference was not found between the two **groups** in terms of prolidase enzyme levels (p=0.831). There was a statistically significant increase in CA III levels in the COPD group (p=0.001). There were moderate positively correlation between CAIII with partial pressure of carbon dioxide in blood ($pCO_2$) and negatively correlation between CA III with partial pressure of oxygen in blood ($pO_2$) in COPD patients (r:0.302, p<0.025; r:-0.314, p:0.02).

**Conclusions**: We think that there is an important clinical relationship between CA III and COPD, and therefore, CA III may be a candidate biomarker in the follow-up of COPD.

**Keywords**: COPD, prolidase, carbonic anhydrase III, arterial blood gase

## Öz

**Giriş**: Kronik obstrüktif akciğer hastalığı (KOAH), geri dönüşümsüz hava yolu akış kısıtlaması ve kronik hava yolu iltihabı ile karakterize bir hastalıktır. Kollajen yıkımının bir göstergesi olan serum prolidaz enzimi ile asit-baz regülasyonunda önemli işlevi olan karbonik anhidraz (CA) III enziminin KOAH'lı hastalarda klinik önemini araştırmayı amaçladık.

**Gereç ve Yöntem**: Bu çalışmaya 56 stabil KOAH'lı hasta ile sigara öyküsü ve ek hastalığı olmayan 32 sağlıklı olgu dahil edildi. Her iki grup arasında serum CA III ve prolidaz enzim düzeyleri karşılaştırıldı.

**Bulgular**: Prolidaz enzim düzeyleri açısından iki grup arasında istatistiksel fark bulunmadı (p=0,831). KOAH grubunda CA III düzeylerinde istatistiksel olarak anlamlı bir artış vardı (p=0,001). KOAH hastalarında CA III enzimi düzeyi ile kanda kısmi karbondioksit basıcı ($pCO_2$) arasında orta derecede pozitif, kanda kısmi oksijen basıncı ($pO_2$) arasında ise negatif korelasyon vardı (r:0,302, p<0,025; r:-0,314, p:0,02).

**Sonuçlar**: CA III ile KOAH arasında önemli bir klinik ilişki olduğunu ve bu nedenle CA III'ün KOAH takibinde aday bir biyobelirteç olabileceğini düşünüyoruz.

**Anahtar Kelimeler:** KOAH, prolidaz, karbonik anhidraz III, arter kan gazı

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**Received *(Geliş Tarihi)*:** 22.04.2022 **Accepted *(Kabul Tarihi)*:** 19.05.2022

## INTRODUCTION

The prolidase enzyme is involved in the destruction of proline and hydroxyproline, those of the most important amino acids in the collagen structure.[1] Prolidase located in many tissues such as the kidneys, liver, lungs and heart is considered an indicator for collagen turnover.[2] In many studies, plasma prolidase activity was found to be high in clinical pathologies associated with chronic inflammation and collagen deposition in the tissue.[3-5]

Carbonic anhydrase (CA) is a family of zinc metalloenzymes with at least 14 different isoenzymes.[6] CA III is a cytosolic enzyme and it is found especially in the uterus, testis, skeletal muscle, lungs, red blood cells, colon, and kidneys.[7] CA III enzyme participates in the reversible hydration-dehydration reaction of carbon dioxide: $CO_2+H_2O \leftrightarrow HCO_3^- + H^+$. This enzyme plays a role in many physiological processes such as pH regulation and maintenance of ionic balance.[8,9] In many studies, the relationship of CA III enzyme with different diseases has been investigated. Vanaah et al. found that only serum myoglobin increased in patients with infarction, while both serum myoglobin and CA III were significantly increased in patients with neuromuscular disease.[10,11] In the study of Kharbanda et al., it was found that hepatocellular damage due to alcoholism causes low CA III levels in the blood.[12] Therefore, they emphasized that the association of myoglobin and CA III in blood can be used to show skeletal muscle damage.

Chronic obstructive pulmonary disease (COPD) is a disease characterized by irreversible airway flow limitation as a result of exposure to harmful particles and chronic airway inflammation.[13] Due to protease-antiprotease imbalance caused by chronic inflammation, serious effects may occur in the production and destruction cycle of collagen, which is a structural element of the alveolar wall. In addition, airflow limitation and chronic inflammation, gas diffusion ($CO_2$, $O_2$) abnormalities in the ongoing process may develop.[14]

COPD is a systemic disease that affects many organs and tissues due to chronic inflammation. Therefore, we aimed to investigate the clinical importance of serum prolidase enzyme, which is an indicator of collagen cycle, and CA III enzyme, which has an important function in acid-base regulation, in patients with COPD. Our study is the first to investigate the relationship between COPD and CA III enzyme levels in the light of literature data.

## MATERIAL AND METHOD

This single-center, prospective study was conducted at Harran University, Faculty of Medicine, Department of Chest Diseases between Januray 2019 and June 2019. The study included 56 stable COPD patients newly diagnosed in the outpatient clinic and 32 healthy cases no smoking history and without any comorbidities. This study supported by Harran University Scientific Research Projects (Project No:19302, Approval Date:02/12/2019). A written informed consent was obtained from each participant. The study protocol was approved by the local Harran University Faculty of Medicine Ethics Committee (Date: 13.06.2019, Decision No: HRU/19.06.04). The study was conducted in accordance with the principles of the Declaration of Helsinki. Patients over the age of 18, newly diagnosed with COPD and without any additional systemic disease were included in the study. Patients under the age of 18, with additional systemic disease, previously diagnosed with COPD and receiving treatment, and who presented to the outpatient clinic or emergency service due to an attack of COPD were excluded from the study. Smoking history (in packs/year) and demographic data of all cases were recorded. All patients were diagnosed with COPD based on their medical history, physical examination findings, and pulmonary function test results. Pulmonary function tests of the patients were performed with a spirometer in sitting position. The threshold value of FEV1/FVC <0.7 after bronchodilator was used for the diagnosis of COPD.[13] Venous blood for routine biochemistry and hemogram examinations and arterial blood (brachial/radial artery) for blood gas evaluation were taken from all patients and healthy subjects. Arterial blood gas was obtained from all cases in room air. Biochemical analyses for prolidase and CA III were performed according to the Fine test Sandwich ELISA kit protocol. Human carbonic anhydrase 3 muscle specific ELISA kit and human Xaa-pro dipeptidase/prolidase ELISA kit were used and the sensitivity was determined as 0.252 ng/mL and 0.23 ng/mL, respectively. After adding 100 µL serum samples to 96-plate in the kit, they were incubated at 37°C for 90 minutes. After the incubation, the plate was emptied and washed twice with washing solution and dried. 100 µL Biotin-labeled Antibody was added on it and incubated for 42 minutes at 37°C for 60 minutes. After the incubation, the plate was emptied and washed 3 times with washing solution and dried. 100 µL HRP-Streptavidin Conjugate was added and incubated at 37°C for 30 minutes. After the incubation, the plate was emptied and washed 5 times with washing solution and dried. 90 µL of TMB Substrate was incubated at 37°C in dark for 20 minutes. After the color formation was observed, 50 µL of Stop Solution was added. The data were obtained by reading the plates at 450 nm absorbance in a microplate reader (Biotec-Cytation-1).

### Statistical Analysis

SPSS for Windows version 22.0 (SPSS Inc., IL, USA) was used for statistical analyses. Kolmogorov-Smirnov test was used for evaluating if the continuous data were distributed normally. Continuous data were expressed as mean±SD or median (25-75 IQR). They were compared using the Student t or Mann-Whitney U tests according to the distribution. Receiver operating characteristics (ROC) curve analysis was performed in order to determine the optimal cut-off value of CA III for predicting COPD. Correlation between

CA III enzyme and parametres of blood gas variables were demonstrated using Spearman's test. A p value of < 0.05 was considered as statistically significant.

## RESULTS

A total of 88 cases, five females (5.6%) and 83 males (94.3%), were included in the study. Demographic and laboratory data of both groups are shown in **Table 1**. While smoking was significantly higher in the patient group, FEV1 and FEV1/FVC values were found to be significantly lower (p<0.001, p<0.001, p<0.001, respectively). Albumin and lymphocyte values were significantly lower in the patient group compared to the control group; C-reactive protein (CRP), white blood cell (WBC), neutrophil, and monocyte ratios were high.

**Table 1. Comparison of the demographic and laboratory data of the patient and control groups.**

| | Patient Group (n=56) | Control Group (n=32) | P |
|---|---|---|---|
| Age, years | 62 (55.5-70.0) | 58 (54.0-63.0) | 0.094 |
| Gender, f/m | 4/52 | 1/31 | 0.466 |
| Cigarette, package/year | 40 (30-55) | 0 | **<0.001** |
| FEV1,l | 38 (26.0-53.5) | 92 (86.0-97.0) | **<0.001** |
| FEV1/FVC, % | 62 (54-68) | 85 (79-91) | **<0.001** |
| Glucose, mg/dL | 99 (90-135) | 94 (90-100) | **0.026** |
| Urea, mg/dL | 29 (23.5-37.5) | 28.0 (25.0-35.0) | 0.746 |
| Creatine, mg/dL | 0.8 (0.7-0.8) | 0.9 (0.7-1.0) | **0.010** |
| AST, U/L | 14 (10.0-19.5) | 15.0 (13.0-22.0) | 0.090 |
| ALT, U/L | 19 (13-26) | 20.0 (15.0-23.0) | 0.678 |
| Albumin, g/dL | 4.1 (3.6-4.6) | 4.4 (4.1-4.5) | 0.219 |
| LDH, U/L | 208.0 (182.5-268.5) | 165.0 (135-210.0) | **0.001** |
| Potassium, mE/dL | 6.8 ±17.8 | 4.2 ±0.7 | 0.416 |
| CRP, mg/dL | 3.2 ±5.2 | 0.21 ±0.15 | **0.002** |
| WBC, $10^3$/mL | 9.3 (7.4-11.3) | 7.4 (6.5-8.6) | **0.001** |
| Lymphocyte, $10^3$/mL | 1.7 (1.1-2.3) | 2.3 (2.0-2.9) | **0.001** |
| Neutrophil, $10^3$/mL | 5.8 (4.3-8.1) | 4.9 (3.8-5.1) | **0.001** |
| Monocyte, $10^3$/mL | 0.7 (0.5-0.9) | 0.3 (0.2-0.5) | **<0.001** |
| Eosinophil, $10^3$/mL | 0.1 (0.0-0.3) | 0.1 (0.1-0.2) | 0.749 |
| MPV, f/L | 7.3 (6.6-8.0) | 8.6 (7.5-9.4) | **<0.001** |
| Platelet, $10^3$/mL | 282 (242-308.5) | 245.0 (223.0-3337.0) | 0.526 |
| RDW, % | 12.5 (11.2-14.1) | 13.0 (11.2-13.8) | 0.986 |

FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactat dehydrogenase; CRP, C-reactive protein; WBC, white blood cell; MPV, mean platelet volume; RDW, red cell distribution width; CA 3, carbonic anhidrase 3.

When arterial blood gas parameters were compared between the two groups, while partial pressure of oxygen in blood ($pO_2$) and oxygen saturation in the blood ($SO_2$) values were found to be significantly lower in the patient group, partial pressure of carbon dioxide in blood ($pCO_2$) and bicarbonat ions in the blood ($HCO_3$) were significantly higher, there was no significant difference between pH levels (**Table 2**).

**Table 2. Comparison of the arterial blood gas of the patient and control groups**

| | Patient Group (n=56) | Control Group (n=32) | p |
|---|---|---|---|
| pH | 7.38 (7.36-7.41) | 7.40 (7.38-7.41) | 0.232 |
| $pO_2$, mmHg | 59.1 (51.3-69.1) | 86 (84.6-88.1) | **<0.001** |
| $pCO_2$, mmHg | 49.9 (45.14-57.4) | 36.2 (35-38) | **<0.001** |
| $HCO_3$, mEq/L | 27.2 (25.3-30.1) | 24 (23.4-25) | **<0.001** |
| $SO_2$, % | 90.6 (85.2-94.9) | 97 (96-99) | **<0.001** |
| Lactate, mmol/L | 1.8 (1.2-2.1) | 1.1 (1.0-1.2) | **<0.001** |

$pO_2$ = partial pressure of oxygen in blood; $pCO_2$ = partial pressure of carbon dioxide in blood; $HCO_3$= bicorbonat ions in the blood; $SO_2$ = oxygen saturation in the blood.

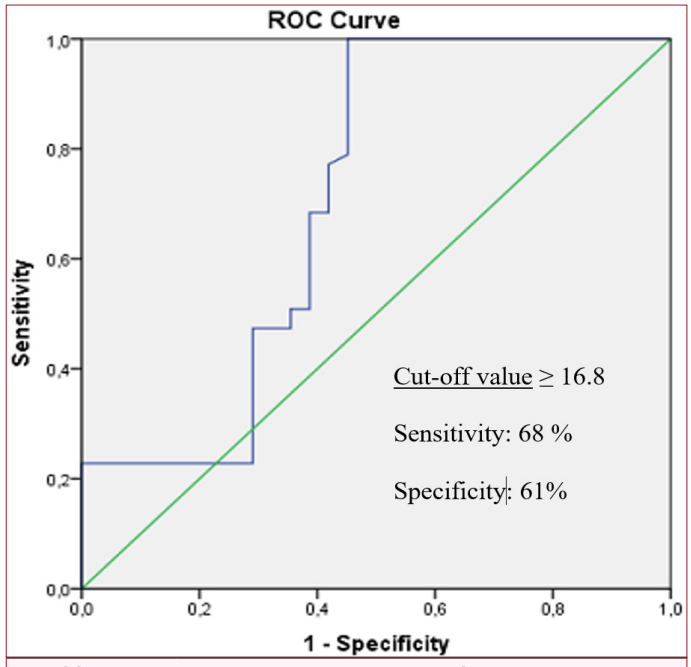
CA III and Prolidase enzyme levels were compared between the two groups. While no statistical difference was found between the two groups in terms of prolidase enzyme levels, there was a statistically significant increase in CA III enzyme levels in the patient group (respectively, p=0.831, p=0.001) (**Table 3**).

**Table 3. Comparison of CA III and Prolidase enzyme levels between patient and control groups**

| | Patient Group (n=56) | Control Group (n=32) | P |
|---|---|---|---|
| Prolidase, U/L | 13.9 (2.8-40.7) | 11.8 (2.6-53.3) | 0.831 |
| CA3, units | 20 (14.9-39.2) | 7.4 (3.8-44.7) | 0.001 |

Correlation between variables was demonstrated using Spearman's test. The serum CA III value was negatively correlated with $pO_2$ value and positively correlated with $pCO_2$ value (r:-0.314, p:0.02; r:0.302, p<0.025).

ROC curve analysis was performed to determine the cut-off value of the CA III enzyme in predicting COPD. With 69% sensitivity and 62% specificity, the cut-off value of CA III enzyme was ≥16.8 (AUC:0.709, P=0.001) (**Figure 1**).




| Variable | AUC | 95 % CI | P |
|---|---|---|---|
| CA III | 0.709 | 0.579-0.838 | 0.001 |

**Figure 1**. ROC curve of CA3 enzyme level for predicting COPD

## DISCUSSION

The main finding of our study is that there is a positive correlation between COPD and serum CA III level due to physiopathologies such as gas diffusion disorder, but contrary to the current literature, no significant result was obtained between serum prolidase and COPD.

COPD is a disease characterized by airflow limitation and chronic inflammation. Currently the most important risk factor is known as smoking. The pathological changes observed in COPD include chronic inflammation with increased numbers of specific inflammatory cell types (macrophage, neutrophils) in different parts of the lung and structural changes resulting from repeated injury and repair. In addition, the increase in circulating cytokines such as CRP, IL-8, TNF, IL-6 and neutrophils are important laboratory parameters observed.[15] In our study, all patients were active smokers and the neutrophil ratio and CRP level of the patient group was statistically significantly higher compared to the control group.

Chronic inflammation in the airways contributes to COPD pathogenesis by disrupting the protease/antiprotease and oxidant/antioxidant balance. Increased levels of proteases, derived from inflammatory cells and epithelial cells, have been observed in COPD patients. Disruption in the protease-antiprotease mechanism increases the destruction of connective tissue components such as collagen.[16] Significant results have been determined with serum prolidase activity in many different pathologies such as cardiac, gynecological, collagen tissue diseases and it has been emphasized to be an important biomarker in these pathologies.[17-19] Therefore, recently, there are different studies investigating the relation of prolidase enzyme, which has a great role in collagen synthesis, with COPD.[20-23] In our study, there was no statistically significant difference between the COPD group and the healthy group in terms of prolidase levels. This result may be due to the low number of COPD patients and the fact that all COPD patients are in a stable period.

Carbonic anhydrase in the red blood cell and in the pulmonary endothelium facilitates the elimination of $CO_2$ in the lungs. In general, gas transfer for oxygen and carbon dioxide worsens as the disease progresses in the COPD. Reduced ventilation may be also be due to reduced ventilatory drive or increased dead space ventilation. This may lead to $CO_2$ retention and hypoxemia.[24] Mondrup et al. found that the content of CA isoenzyme B in erythrocytes in chronic obstructive pulmonary disease was significantly higher in hypercapnic patients than in normocapnic patients.[25] According to the literature data, there are no clinical studies conducted between CA III and COPD. For the first time, the relationship between CA III enzyme and COPD was compared in our study. In our study, CA III activity was significantly higher in the COPD group than in the control group. Also, by ROC curve analysis, CA III ≥ 16.8 value predicted COPD with 69% sensitivity and 62% specificity. In addition, we found that the CA III enzyme showed a correlation negative with $pO_2$ and a correlation positive with $pCO_2$. The high level of CA III enzyme activity in the COPD group is an indication of the deterioration of acid-base balance in this pathological process and hypoxia-$CO_2$ retention in erythrocytes. The presence of statistically significant low $O_2$ and high $CO_2$ values in arterial blood gases of COPD patients in our study supports this hypothesis.

The main limitations of this study can be listed as follows; 1- low number of patients, 2- being a single center 3- all patients in stable phase, no patients with COPD attack, 4- They are not long-term follow-up.

## CONCLUSION

Although the relationship between COPD and serum prolidase level is not consistent with the literature in this study, we can say that CA III enzyme is associated with gas exchange abnormality in COPD. CA III may be a candidate biomarker to be used in COPD patient follow-up and to provide information about the patient's clinic. We believe that it can lead to studies to be conducted in a larger population.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** The study protocol was approved by the Harran University Faculty of Medicine Local Ethics Committee (Date: 13.06.2019, Decision No: HRU/19.06.04).

**Informed Consent:** All patients signed the free and informed consent form.

**Referee Evaluation Process:** Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

**Financial Disclosure:** This study supported by Harran University Scientific Research Projects (Project No:19302, Approval Date:02/12/2019).

**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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