Is endogenous carboxyhemoglobin level a useful biomarker of clinical course and prognosis in COVID-19 patients?

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Abstract

Objective: SARS-CoV-2 has caused nearly 4 million confirmed cases of COVID-19 worldwide in the approximately 4 months since it emerged in Wuhan, China in December 2019. Comorbidities increase morbidity and mortality in COVID-19, and many laboratory parameters have been associated with mortality. The aim of the present study was to identify the relationship between endogenous carboxyhemoglobin (COHb) level and the clinical course and prognosis of COVID-19. Methods: The study included 48 non-smokers or ex-smokers aged 18 years or older who presented to the emergency department, were diagnosed with COVID-19 by real-time PCR analysis of nasopharyngeal swab sample, and were treated in the pulmonary diseases ward of the Atatürk University hospital after between March 24, 2020 and April 15, 2020. The patients' laboratory parameters and demographic data were analyzed retrospectively. Results: Prothrombin time and C-reactive protein (CRP), troponin-I, and D-dimer levels decreased in COVID-19 patients during follow-up (p=0.024, p=0.001, p=0.001, p=0.001), while PaO2/FiO2 ratio and COHb increased (p=0.002, p=0.001). COHb level at admission was significantly lower in patients who developed macrophage activation syndrome (MAS), acute respiratory distress syndrome (ARDS), and those who died compared to the other patients (p=0.002, p=0.001). COHb level on day 5 of treatment was significantly higher in patients with ARDS and patients who died (p=0.001, p=0.001).Significant correlations were detected between COHb level and CRP (r=-0.425, p=0.001), ferritin (r=-0.395, p=0.001) and PaO2/FiO2 ratio (r=0.431, p=0.001). Conclusions: COHb level may be an easily accessible biomarker that guides early follow-up and treatment planning to avoid ARDS, MAS, and mortality in COVID-19.

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Short title: Carboxyhemoglobin level in COVID-19 patients

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Conclusions: COHb level may be an easily accessible biomarker that guides early follow-up and treatment planning to avoid ARDS, MAS, and mortality in COVID-19.

Keywords: COVID-19, endogenous carboxyhemoglobin, acute respiratory distress

What's already known about this topic?

Carbon monoxide (CO) is naturally synthesized in the body and plays an important role in the regulation of physiological functions such as vasodilation, angiogenesis, vascular remodeling, protection against tissue damage, and modulation of the inflammatory response. Approximately 85% of CO is produced by heme oxygenase and is excreted from the body through the respiratory system. In critical diseases such as acute respiratory failure, chronic obstructive pulmonary disease, acute pulmonary embolism, and acute myocardial infarction, low initial endogenous carboxyhemoglobin (COHb) level has been associated with high mortality and poor prognosis . The leading causes of morbidity and mortality in COVID-19 are acute respiratory failure, microthombi, and cardiac involvement.

What does this article add?

Low COHb level at admission in COVID-19 patients may be an easily accessible biomarker that guides early follow-up and treatment planning to avoid ARDS, MAS, and mortality.

Introduction

After appearing in Wuhan, China in December 2019, coronavirus disease (COVID-19) spread rapidly around the world, with over 3 million confirmed cases by the end of April 2020. In the majority of infected patients, COVID-19 is either asymptomatic or presents with mild symptoms such as loss of taste and smell, sore throat, fatigue, and joint pain. However, it can have a much more severe course in older people, patients with hypertension (HT), and conditions that can impair immunity such as diabetes mellitus (DM), HIV, long-term immunosuppressive therapy, and pregnancy [1].

While many comorbidities have been associated with COVID-19 mortality, there are also laboratory diagnostic tests associated with early poor prognosis. Of these, the most frequently used parameters are D-dimer, ferritin, leukopenia, fibrinogen, prothrombin time, and IL-6 level. These parameters alone are not effective in directing treatment, but evaluation of correlation with clinical condition revealed a relationship with macrophage activation syndrome (MAS), which is among the most important causes of mortality. This led to the investigation of parameters that can be associated with mortality in this emerging disease [2,3].

Carbon monoxide (CO) is naturally synthesized in the body and plays an important role in the regulation of physiological functions such as vasodilation, angiogenesis, vascular remodeling, protection against tissue damage, and modulation of the inflammatory response. Approximately 85% of CO is produced by heme oxygenase and is excreted from the body through the respiratory system [4,5]. In critical diseases such as acute respiratory failure, chronic obstructive pulmonary disease, acute pulmonary embolism, and acute myocardial infarction, low initial endogenous carboxyhemoglobin (COHb) level has been associated with high mortality and poor prognosis . The leading causes of morbidity and mortality in COVID-19 are acute respiratory failure, microthombi, and cardiac involvement [5-8].

COVID-19 is a new disease that does not have specific laboratory findings as in other known diseases, and as such is the focus of intense research. The aim of the present study was to determine the value of COHb levels measured at admission and follow-up in the prediction of clinical course and prognosis in COVID-19 patients who develop MAS and acute respiratory failure.

Methods

Study population

This retrospective study included never-smokers or ex-smokers aged 18 and over who presented to the Atatürk University Emergency Service with complaints of recent-onset fever, cough, shortness of breath, listlessness, and/or sudden attenuation of taste and smell, and who had returned from travel abroad or had contact with suspected COVID-19 patient within the last 14 days.

As specified in the guidelines, COVID-19 patients with respiratory symptoms underwent posterior–anterior chest radiography, and if any suspicious lesions were detected, a more detailed evaluation was performed using high-resolution chest computed tomography[9]. Nasopharyngeal swab samples were obtained from the patients and COVID-19 diagnosis was established using real-time PCR analysis. A total of 48 patients treated in our pulmonary diseases ward between March 24, 2020 (admission date of the first COVID-19-positive patient to our center) and April 15, 2020 were included in our study. Local ethics committee approval was obtained to use patients' records for our retrospective research study.

Laboratory analysis

Analyses of hematological parameters, biochemical parameters including liver and kidney function tests, coagulation parameters, ferritin, D-dimer, troponin-I, C-reactive protein (CRP), procalcitonin, and arterial blood gas parameters were performed upon admission and repeated daily.

Definitions and Treatment

Fever was defined as an axillary temperature of 37.3°C or higher. Positive endotracheal aspirate or lower respiratory tract sputum culture with signs and symptoms of bacteremia or pneumonia was considered secondary bacterial infection. Treatment of patients diagnosed as having ventilator-associated or hospital-acquired pneumonia was planned based on available guidelines. Diagnosis and grading of acute respiratory distress syndrome (ARDS) was done according to Berlin 2015 diagnostic criteria. If the patients' daily cardiac-specific troponin level was above normal, echocardiography was performed to evaluate for the development of new cardiac pathologies. Coagulopathy was defined as a prothrombin time more than 3 seconds higher than normal and partial thromboplastin time 5 seconds higher than normal. Treatment strategies were implemented according to the Turkish Ministry of Health COVID-19 Adult Diagnosis and Treatment guidelines based on the patients' disease severity. Patients were monitored for MAS in the presence of signs such as persistent fever, persistently high or increasing CRP and ferritin levels, elevated D-dimer levels, lymphopenia/thrombocytopenia, deterioration in liver function tests, hypofibrinogenemia, and increasing triglyceride levels despite treatment. Patients with successive increases in daily measurements of these parameters which could not be explained by secondary bacterial infections were administered 400 mg of tocilizumab for MAS if they had no contraindications. In patients who showed appropriate clinical and laboratory response after 24 hours, treatment was not continued. However, if an appropriate clinical and laboratory response was not observed, treatment was repeated at the same dosage.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY). Between-group comparisons were performed using Pearson's chi-square test for parametric data and Mann–Whitney U test for non-normally distributed numerical data. The groups' demographic data and laboratory parameters were compared using independent-samples t test. Wilcoxon analysis was performed to compare repeated measures for laboratory parameters within groups. Pearson correlation analysis was used to evaluate correlation between COHb and CRP, ferritin, D-dimer level, lymphocyte count, and the ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2). A p-value < 0.05 was considered statistically significant.

Results

The mean age of the 48 patients included in the study was 57.6±17.6 years. Twenty-two of the patients were women (mean age 55.8±18.3 years) and 26 were men (mean age 59.1±17.3 years). Twenty-eight of the patients had HT, 12 had DM, 4 had asthma, and 1 had epilepsy. Body mass index (BMI) was > 30 kg/m^2 in 15 of the patients. Of 26 patients who developed ARDS, 20 had HT, 8 had DM, 1 had epilepsy, and 6 had BMI > 30 kg/m^2. Of the patients who developed MAS, 10 had HT, 4 had DM, and 1 had epilepsy.

In the physical examination performed at hospital admission, mean respiratory rate (breaths/minute) was 21.4 ± 12.3 among patients who later developed MAS, 24.6 ± 13.4 among patients who developed ARDS, and 15.4 ± 4.8 in patients without ARDS and MAS. Comparison of patients with and without ARDS and MAS showed that respiratory rate was statistically significantly higher in the ARDS and MAS groups (p=0.001, p=0.001). On day 5 of treatment, the mean respiratory rate was 18.4 ± 4.5 in the MAS group, 20.1 ± 5.6 in the ARDS group, and 14.4 ± 3.6 in the group of patients without MAS or ARDS.

A comparative analysis of the patients' laboratory parameters at hospital admission and on day 5 of treatment is shown in Table 1. When patients with and without MAS were compared in terms of their COHb levels at admission and on day 5 of treatment, it was found that patients who developed MAS had lower COHb level at admission (p=0.002). Comparison of the changes in COHb levels over the course of 5 days showed a statistically significant increase in patients who developed MAS (Figure 1) (p=0.001). When patients with and without ARDS were compared in terms of COHb levels at admission, it was found that COHb level was significantly lower at admission but higher on day 5 (p=0.001, p=0.001). As in the patients who developed MAS, COHb levels increased further over the course of 5 days in patients who developed ARDS (Figure 1) (p=0.001). Changes in other laboratory parameters in patients who developed MAS and ARDS are shown in

Tables 2 and 3. Comparison of COHb levels between deceased (n=4) and surviving patients (n=44) showed that in deceased patients, COHb levels were significantly lower at admission and higher on day 5 of treatment (p=0.04, p=0.001).

In the correlation analysis between COHb level and laboratory parameters and demographic data, moderate negative correlations were detected between COHb level and CRP (r=-0.425, p=0.001), ferritin (r=-0.395, p=0.001) (Figure 2), and age (r=-0.314, p=0.001). Moderate positive correlations were detected between COHb and lymphocyte count (r=0.43, p=0.001) (Figure 2) and PaO_2/FiO_2(r=0.431, p=0.001). There was also a weak negative correlation between COHb and troponin-I level (r=-0.287, p=0.05).

In correlation analysis between COHb level on day 5 of treatment and laboratory parameters, a moderate positive correlation was detected between COHb level and CRP (r=0.55, p=0.001), prothrombin time (r=0.387, p=0.001), and creatinine (p=0.408, p=0.001), but no significant difference was detected in other parameters at time of admission.

Discussion

In the present study, we determined that the COHb levels of COVID-19 patients treated in our center were low at admission and increased with treatment. In particular, we observed that low COHb level is an important risk factor for the development of MAS and ARDS. In our study, nonsurviving COVID-19 patients had lower COHb levels than surviving patients. COHb levels increased significantly during 5-day follow-up in patients who developed ARDS and MAS compared to patients who did not develop ARDS and MAS. Correlation analysis between COHb levels and parameters associated with mortality in the literature revealed negative correlations between COHb levels and CRP, ferritin, and troponin and positive correlations between COHb levels and lymphocyte and PaO_2/FiO_2 ratio.

The novel coronavirus detected in Wuhan, China at the end of 2019 was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses because it is closely related to SARS-CoV and MERS-CoV, viruses responsible for past epidemics that caused high morbidity and mortality. Due to the rapid spread of COVID-19, the epidemic in China quickly escalated to a global pandemic, with the number of confirmed COVID-19 cases worldwide approaching 4 million at the time of this writing [10].

Although most patients with COVID-19 present with fever, coughing, and shortness of breath, a smaller proportion of patients may present to emergency departments with confusion, diarrhea, sore throat, chest pain, nausea, and vomiting. Less common presenting symptoms also include loss or attenuation of smell and taste, and palpitations due to myocardial wall involvement as a complication of viral respiratory tract infections. COVID-19 causes high rates of morbidity and mortality, especially in older patients and those with comorbidities such as HT, DM, immunosuppressive therapy, and obesity. MAS-related multiorgan failure and acute respiratory failure are among the primary causes of mortality in COVID-19 [3,11].

Lymphopenia is the most common abnormality in laboratory tests, which suggests that leukocytes, especially T-lymphocytes, are affected in COVID-19 patients. Viral particles that spread from the respiratory tract and infect other cells create a cytokine storm. In a cytokine storm, myriad proinflammatory cytokines are released, primarily TNF- alpha, IL-1, IL-2, IL-6, and nitric oxide. These cytokines play an important role in increasing vascular permeability, endothelial damage, and microthrombus formation [12]. Progressive increases in CRP, ferritin, D-dimer levels, and if measurable, IL-6 levels in patients' clinical follow-up have become predictive biomarkers of cytokine storm syndrome and MAS for clinicians. However, while there is no definite cut-off value for these parameters, it indicates that clinical follow-up plays an important role in the use of IL-1 antagonist canakinumab and IL-6 antagonist tocilizumab [3,13].

The level of CO naturally synthesized in the body can be measured using COHb, the product of its high-affinity binding to hemoglobin. In addition to its anti-inflammatory activity, it also plays an important role in vascular remodeling and prevention of tissue damage. Endogenous COHb is generated in the body when heme oxygenase-1 (HOX-1) converts heme to biliverdin. COHb released as a result of HOX-1 activation

is eliminated by the respiratory system and can be measured in exhaled breath. HOX-1 has an important role in the reduction of reactive oxygen radicals and induction of enzymes that are cytoprotective for many organ and tissue epithelia, primarily the respiratory tract epithelium[14]. Low COHb level was found to be associated with high mortality in studies of intensive care patients. In addition, low COHb levels were shown to be correlated with poor prognosis in patients presenting with community-acquired pneumonia, myocardial infarction, stroke, and acute pulmonary thromboembolism [5-8,15]. In this study, we observed that COVID-19 patients' COHb levels were low at admission and progressively increased with treatment. Furthermore, COHb levels were even lower in patients who developed MAS and ARDS, for which early treatment is of great importance. In nonsurviving patients who developed ARDS, COHb levels on day 5 of treatment were higher than in surviving patients who did not develop ARDS. Patients who developed MAS exhibited greater changes in COHb on day 5 of treatment relative to patients who did not. This may be related to the higher admitting respiratory rates observed in patients who developed ARDS and MAS compared to those who did not. This higher respiratory rate may have reduced the COHb elimination rate in patients with ARDS and MAS. The lower $PaCO_2$ values in these patients also indicated impaired ventilation capacity at admission, which further suggests impaired CO excretion. The decrease in CO levels in correlation with oxygen saturation in patients with ARDS due to acute pulmonary embolism demonstrates that CO levels decrease with increased respiratory effort, which supports our findings. In addition, although there has been no previous study on this subject in the literature, a decrease in HOX-1 system activation associated with clinical worsening of COVID-19 may also have reduced COHb production. Improved immune response and decreased respiratory workload with treatment may have resulted in an increase in COHb, which is known to have anti-inflammatory activity. CRP, ferritin, troponin, and PaO_2/FiO_2ratio have been shown to be closely associated with MAS and mortality, and the correlations observed between these parameters and COHb support previous studies as well as our present findings.

The main limitation of this study was that the number of nonsurviving patients in our sample was too small to establish an association between COHb level and mortality. However, our finding that COHb levels in patients who developed ARDS and MAS were consistent with those of nonsurviving patients suggests that our results can be generalized.

In conclusion, low COHb level at admission in COVID-19 patients may be an easily accessible biomarker that guides early follow-up and treatment planning to avoid ARDS, MAS, and mortality.

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Figure legends

Figure 1. Comparison of COHb levels at admission and on day 5 of treatment in COVID-19 patients who did and did not develop macrophage activation syndrome (MAS) and acute respiratory distress syndrome (ARDS)

Figure 2. Correlation analysis of COHb level with CRP and PaO_2/FiO_2 levels in COVID-19 patients

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