A PREDICTIVE AND PROGNOSTIC MARKER IN COVID-19 PATIENTS: EXHALED NITRIC OXIDE (FENO)

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ABSTRACT

Introduction: *It is known that nitric oxide (NO) is actively involved against microorganisms due to its antiviral and bacteriostatic activity in the upper respiratory tract and rises in the blood and exhaled air during this process. Therefore, considering that COVID-19 can damage various tissues and cells in the body, it is predicted that Covid-19 may be an early biomarker in the diagnosis of measurement of Nitric oxide (FENO) in Exhaled air.*

Materials and methods*: 102 COVID-19 patients with different clinical findings, whose diagnoses were made by polymerase chain reaction (PCR) and whose treatment was started, were included in the study. A control group was formed from healthy volunteers. Demographic features, clinical findings and the amount of nitric oxide (FeNO) measured in exhaled air were recorded.*

Results*: In terms of exhaled nitric oxide (FeNO) values, the exhaled nitric oxide value in COVID-19 patients was 31.73 ± 14.80 mmol / lt, while it was 15.48 ± 6.64 mmol / lt in the control group and was statistically significant. In the Roc graph, FeNO threshold value was measured as 24.5 Mmol level and its sensitivity was 62.1% and specificity was 96.0% in diagnosing Covid-19.*

Conclusion*: Non-invasive method, FeNO measurement, can be used in an easy and outpatient environment and is reproducible. This shows that it is a candidate to be among the diagnostic methods in the future.*

Keywords: *COVID-19, SARS-CoV-2, Nitric oxide, Pneumonia, Exhaled Nitric Oxide (FENO) Measurement.*

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Introduction

The new type of coronavirus disease (COVID-19), which also occurs in China, has adversely affected all areas of life[(1)]. The outbreak was first reported in the city of Wuhan, Hubei Province, China, in late December 2019 as cases of pneumonia of unknown etiology[(2)]. The disease, which spread to 34 regions of China until January 30, 2020, was declared by the World Health Organization (WHO) as a public health epidemic and international emergency[(3)].

The COVID-19 pandemic initially increased at a dramatic rate with its characteristic contagiousness, and COVID-19 cases can occur in 3 different clinics, ranging from asymptomatic (mild), moderate to life-threatening pneumonia[(4)]. It is observed that COVID-19 disease is transmitted especially during the incubation period when most of the patients are asymptomatic or have very mild non-specific symptoms(5). Common symptoms associated with this disease are high fever, cough, shortness of breath, and lesions in the lungs seen on computed tomography; In the worst cases, it can cause severe pneumonia, acute respiratory distress syndrome (ARDS) and mortality[(6)]. The World Health Organization (WHO) has recommended several guidelines for collecting samples from affected or suspected COVID-19 patients. Affected or suspected persons are required to cooperate with local health units for other procedures, such as collecting, storing and properly sending samples.

According to the WHO recommended guidelines, the sample will be isolated from two main sources, the lower respiratory tract and the upper respiratory tract[7].

Currently, various diagnostic tools have been approved by WHO recommending the collection of upper respiratory tract samples (sputum, endotracheal aspirate, bronchoalveolar lavage), blood, stool and lung autopsy materials[8]. Recently, WHO has approved the immediate use of qSARS-CoV-2 IgG / IgM rapid serological tests where fingertip blood can be used. This test identifies the IgM and IgG antibodies produced by the patient in response to the SARS-CoV-2 infection[9]. Various blood and biochemical abnormalities have been observed in COVID-19 patients. The most common irregularities include leukocytosis, leukopenia, lymphopenia, increased C-reactive protein(CRP), normal procalcitonin, lactate dehydrogenase (LDH) and erythrocyte sedimentation rate (ESR)[10]. When the COVID-19 virus enters the body, the virus is contaminated to the oropharyngeal region, causing the first symptoms of COVID-19, then descending into the lower respiratory tract, causing inflammation in the lungs, then reaching the bloodstream[11].

Nitric oxide (NO) plays an important role in regulating local blood flow and vasomotor tone in both systemic and pulmonary circulation[12,13]. It has been emphasized that nitric oxide constitutes the primary defense against microorganisms due to its antiviral and bacteriostatic activity in the upper respiratory tract[14]. Measurement of exhaled NO can be applied to detect the activation of iNOS of inflammatory cells from the peripheral airways and alveolar tissue. Excessive cytokine release and hyperinflamation occur in pneumonia. Since NO is a primary marker released during inflammation, NO measured in exhalation is known to increase in patients with pneumonia[15].

Nitric oxide (FENO), measured in exhaled air, with changes in the levels of these components, suggests that COVID-19 can be used to predict and damage damage to various tissues and cells in the body. It is imperative to closely monitor changes in these parameters to reveal early biomarkers of COVID-19. Before the onset of typical COVID-19 symptoms, FENO measurements may be useful in diagnosis and follow-up to observe changes in the levels of molecules and markers of these cells. It will also assist in the development of early treatments for COVID-19 as a result of a better understanding of changes in these parameters.

Therefore, more research is needed in this area to better understand abnormalities. For these reasons, in our study, we aimed to evaluate the role of laboratory values and nitric oxide values measured in exhaled air in patients with PCR + due to COVID-19 in the light of the literature.

Materials and methods

In our study centers in Turkey in an outbreak that COVID-19 patients who underwent routine checks along with follow-up diagnostic and treatment hospital in healthy volunteers were included. 102 COVID-19 patients with different clinical findings whose diagnosis was confirmed by the combined (oropharynx + nasopharyngeal) swab method and whose clinical findings were initiated were included in the study. The Control Group was composed of healthy volunteers without chronic disease and pneumonia. The demographic characteristics, clinical, radiological, laboratory findings and lung computed tomography (CT) findings of the patients and the amount of nitric oxide (FeNO) measured in the exhaled air were recorded.

Exhaled Nitric Oxide (FeNO) Measurement

With the NObreath® (Bedfont Scientific Ltd., UK) device for exhaled nitric oxide, each patient was told by using different disposable mouthpieces, that the patient should first make a deep inhalation and then exhale for 10-12 seconds, and the patient was motivated for continuity during the measurement and measurements were taken. The patient was taken 3 times intermittently and 2 identical measurements were taken as correct and entered into the study data. The patient had different results in 3 measurements, and a total of 5 measurements were performed. The same value was considered correct. Patients whose all measurements differed were re-evaluated for a later date to be evaluated at the clinic where they were treated.

In order for the measurements to give the most accurate results, care was taken to ensure that the patients did not eat or drink anything at least 1 hour before the measurements and rinsed their mouths before the measurement. Since exhaled nitric oxide levels were also affected by smoking, it was especially emphasized that patients were not smoking at least 1 hour before measurement. The device was sterilized in an autoclave after each patient.

Statistical Analysis

SPSS 20.0 (IBM SPSS Statistics , Chicago, USA) program was used for the statistical analysis of the data which were transferred to Microsoft Excel from the Google form format by adhering to the original states of surveys. Data were shown as mean ± standard deviation, number of individuals, and percentage. The suitability of the quantitative data for normal distribution was tested by the Kolmogorov-Smirnov, Shapiro-Wilk test and graphical evaluations. Student's t-test was used for comparison of two groups of quantitative data with normal distribution, and the Mann-Whitney U test was used for comparison of two groups of data with non-normal distribution. Pearson Chi-Square testand Fisher's Exact test were used to compare qualitative data. Significance was set at p<0.05.

Results

The average age of our participants included in our study was 45.14 ± 18.24 years. 79(%) of our participants were male and 73(%) were female. While the population of our study was 102 patients, PCR positive COVID-19 patients were 50 healthy volunteers. Of the COVID-19 patients, 82 had mild, 14 had moderate and 6 had severe clinics. The demographic characteristics of our participants are shown in Table 1.

	Covid-19	Control	p
	N=102	N=50	
Age	44,40±19,49	46,66±15,45	0,475
Length(cm)	167,78±14,19	170,02±9,02	0,311
Weight (kg)	72,53±10,96	77,04±12,16	**0,023**
BMI (kg/cm²	25,83±3,56	26,71±4,36	0,189
Gender			
Female, N (%)	50 (%49,01)	23 (%46)	0,726
Male, N (%)	52 (%50,09)	27 (%54)	
Smoking status	41(%40,19)	22(%44)	0,655
Comorbid disease, N (%)	35(%34,31)	0	**<0,001**
Cardiovascular Diseases	16 (%15.68)	0	
Diabetes Mellitus	18 (%17.64)	0	
COPD	7 (%6.86)	0	
Neurological Diseases	4 (%4)	0	
Severity of the disease			
Mild	82(%80,39)	0	
Moderate	14(%13,72)	0	
Severe	6(%5,88)	0	

Table 1: Demographic features of patients.

Considering the laboratory values of our participants in terms of white cell cells (WBC), the WBC value of COVID-19 patients was 6.89 ± 3.27 10^3 / ul in the control group, this value was 8.33 ± 2.55 10^3 / ul and two There was a significant difference between the groups in terms of WBC values. (P = 0.007)

Lymphocyte values were 2.15 (0.21-90) 10^3 / ul in COVID-19 patients and 2.56 (0 -6.62) 10^3 / ul in the control group. Neutrophil values were 3.34 (1.02-21.23) 10^3 / ul in Covid-19 patients, while 4.32 (2.32-9.56) 10^3 / ul in the control group and statistical values in both values (p = 0.048, p = 0.001, respectively) In terms of exhaled nitric oxide (FeNO) values, the exhaled nitric oxide value in COVID-19 patients was 31.73 ± 14.80 mmol / lt in the control group and 15.48 ± 6 in the control group. Was 64 mmol / l and was statistically significant (Table 2) (Figure 1).

Laboratory values	COVID-19 (N=152)	Control (N=50)	p
FeNO (ppb)	31,73±14,80	15,48±6,64	**<0.001**
WBC (10^3/ul)	6,89±3,27	8,33±2,55	**0.007**
Hgb(g/dl)	13,47±1,71	13,84±1,68	0.210
RDW(%)	13,76±4,10	13,74±1,53	0.970
Platelet(10^3/ul)	249,48±103,34	250,44±70,40	0.953
Prokalsitonin(ng/ml)	0,24±0,08	0,24±0,06	0.780
Hematocrit (%)	40,85(30,1-409,0)	42,6(23,6-386)	**0.038**
Neutrophil (10^3/ul)	3,34(1,02-21,23)	4,32(2,32-9,56)	**0.001**
Lymphocytes (10^3/ul)	2,15(0,21-90)	2,56(0 -6,62)	**0.048**
CRP(mg/l)	3.35 (0.01-43.80)	1.03 (0.01-5.9)	**<0.001**

Table 2: Laboratory values of the study group and control group patients.

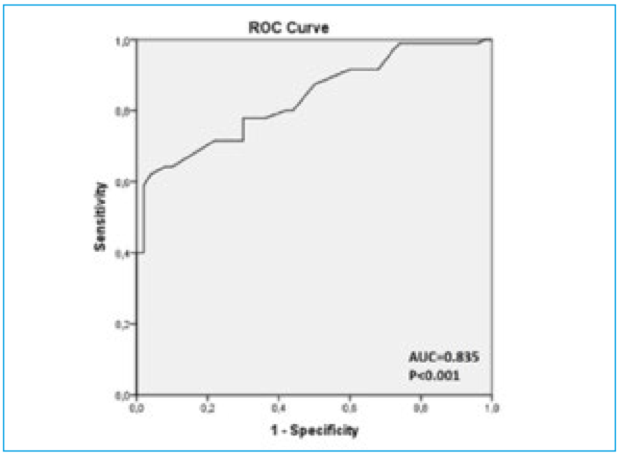

Fig. 1: FENO findings for patients with COVİD-19

When COVID-19 patients were evaluated, 4 out of 102 patients had intensive care hospitalization and the rest were service patients. Among the 102 patients, 3 patients were ex-treated despite the treatments. 90,19% of the patients were combined with Enfluvir (Figure 2).

When the radiological findings of COVID-19 patients were evaluated, 2 patients (1.3%) had pathological findings on the posteroanterior radiograph (PAAC) of 2 patients. When the findings of Bt were evaluated, 87 (57.23%) patients had atypical findings including pneumonia in thorax bt. Of these, 28 (18.4%) were typical and 23 (15.1%) were compliant with Covid-19 pneumonia of 51 (33.55%) in total (Figure 3).

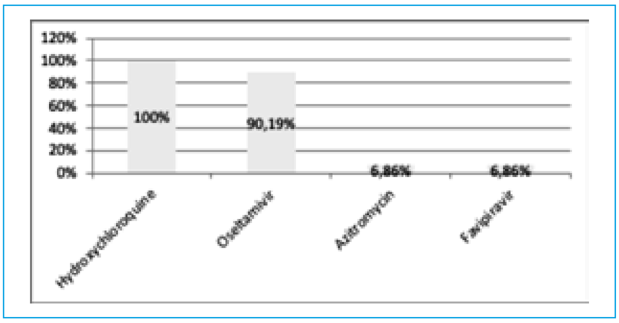

Fig. 2: Medications that were used for treatment of patients.

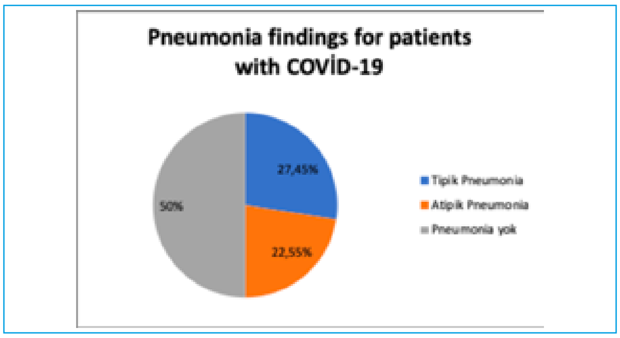

Fig. 3: Pneumonia findings for patients with COVİD-19 (102 patients)

When radiological pneumonia findings were evaluated within themselves, the patient with 51 thorax CT findings had right lung lower lobe involvement in 40 (78.43%) and left lung lower lobe involvement in 35 (68.62%). Bilateral lower lobe involvement was statistically significant compared to other lobe involvement (P <0.001) (Figure 4).

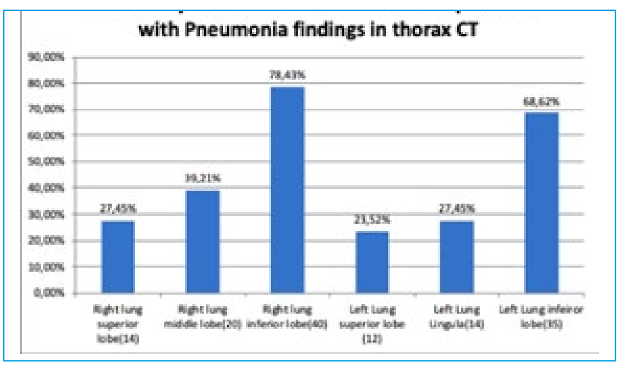

Fig. 4: Pulmonary lobe involvement rates of 51 patients with Pneumonia findings in thorax CT.

Discussion

In our study, it was aimed to investigate the effects of the patients diagnosed and prognosed by conducting FeNO measurements together with hemogram values of patients who were followed up and treated for COVID-19. Similar to our study, different studies have been conducted on hemogram values in Sars-COV, Mers COV and Sars COV-2 patients before, but as a result of our research, we have not observed that FeNO measurements were not performed in patients during the epidemic periods of these diseases. For this purpose, we planned to investigate the location of FeNO measurement values along with hemogram and acute phase reactants in Covid 19 progression and diagnosis in COVID-19 patients. As a result, we showed that Covid 19 patients had NO significantly higher levels of exhalation than healthy individuals. Also, defining FeNO's threshold values can help distinguish COVID-19 patients and healthy individuals.

In the study conducted by Zheng Y. et al. On the hemogram values of COVID-19 patients, neutrophil, lymphocyte, WBC and platelet counts were shown to be independent risk factors in predicting serious disease development[16]. Lymphopenia is a distinctive feature of serious COVID-19 patients because the target of SARS-CoV-2 viral particles is to damage the cytoplasmic component of the lymphocyte and lead to cell destruction[17].

In a study by Qin et al. In 450 COVID-19 positive patients, they reported that severe cases had lower lymphocyte, higher leukocyte (WBC) and higher NLR (Neutrophil) counts compared to mild cases and healthy individuals[18]. These observations suggest that lymphopenia may be related to the severity of infection, and may also indicate disease progression at the level of neutrophils and WBC. In our study, similar to the literature, lymphocyte ratios were found to be lower compared to the healthy control group, neutrophil and WBC values were higher than healthy individuals and were statistically significant (p = 0.048, p: 0.001, p: 0.007, respectively).

CRP is used as an indicator of inflammation in inflammation, infection and tissue damage, and is a positive systemic marker of the acute phase response[13]. In a study by Chen et al. In COVID-19 patients, although there was no statistically significant difference in CRP level between mild and severe COVID-19 patients, the mean CRP level was found to be higher in the severe group compared to the nonsevere group[19]. While procalcitonin (PCT) synthesis is increased in bacterial infections, cytokines such as interferon (INF) -as released following viral infection remain normal or down-regulated due to the down regulation of PCT[20].

Therefore, PCT values are more specific than WBC count and CRP in differentiating a bacterial infection from another inflammatory process[21] The PCT value remains within the reference ranges in patients with uncomplicated SARS-CoV-2 infection; any significant increase reflects bacterial coinfection and the development of a serious form of disease and a more complex clinical picture[22]. Considering our study in terms of CRP values, CRP values were observed to be high in accordance with the literature in people with the disease.

Nitric oxide (FeNO) measured in exhaled air can be used clinically as a noninvasive biomarker of respiratory inflammation, but measurements are affected by asthma patients and smoking[23]. The fact that this technique is noninvasive means that it is easy and convenient due to the fact that it can be useful to determine the effectiveness of modified therapies based on the disease, and serial measurements can be taken repeatedly[24]. In one study, the increase in FeNO levels revealed the usefulness of potential inflammatory and infectious diseases in the respiratory tract in the profiling of treatments, and this prompted us to think about the use of noninvasive biomarkers in this study[23]. In the diagnosis of Covid-19 for the 24.5 Mmol level of the threshold, its sensitivity was 62.1% and the specificity was 96.0%. Based on this, low FeNO values rule out the diagnosis of Covid -19.

Conclusion

As a result; In addition to the laboratory parameters discussed above used in clinical practice, potentially useful new biomarkers for screening, clinical management and prevention of serious complications are still being investigated. These are used to illuminate the clinical values that are predicted as severe and prognostic indicators of the disease. In our study, we thought that FeNO measurement, which is an easy-to-use and non-invasive method, can be used in non-invasive, easy and outpatient settings, and can be used in the asymptomatic period before the discrimination of the disease and PCR, thereby leading to a reduction in costs and success in clinical diagnosis. However, it is beneficial to conduct research in larger samples and patient populations

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