

Exhaled Nitric Oxide Levels in Patients with Chronic Obstructive Pulmonary Disease

KRONİK OBSTRÜKTİF AKCİĞER HASTALIĞI OLAN HASTALARDA EKSPİRE EDİLEN HAVADAKİ NİTRİK OKSİT DÜZEYİ

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Summary

Endogenous nitric oxide (NO) which plays a significant role in the bronchomotor control of airways and regulation of pulmonary vascular system also, has important functions in pathophysiology of certain pulmonary diseases and physiological control of airways,

We aimed to study the relationship between the exhaled NO levels of subjects with chronic obstructive pulmonary disease (COPD); and cigarette and COPD exacerbation in this study.

Four groups were composed by 11 stable COPD Patients, 14 unstable COPD patients, 50 smokers with no COPD, and 58 healthy non-smokers.

Exhaled NO levels were measured by NO chemiluminescence analyser in these groups in the collection bag.

The exhaled NO level was significantly lower in the smoker group with no COPD when compared to the healthy non-smoker group (4.02 ± 1.51 ppb versus 7.71 ± 1.73 ppb, $p < 0.05$). In the stable COPD group, the levels of the exhaled NO were significantly higher than those of the smokers with no COPD (7.73 ± 1.42 ppb versus 4.02 ± 1.51 ppb, $p < 0.05$).

There was statistically no significant difference when the exhaled NO levels of healthy non-smoker group were compared to the levels of the stable COPD group. (7.71 ± 1.73 ppb versus 7.73 ± 1.42 ppb, $p > 0.05$).

The exhaled NO levels of unstable subjects with COPD were significantly higher than the levels of the stable subjects with COPD (11.43 ± 17.7 ppb versus 7.73 ± 1.42 ppb, $p < 0.05$).

As a result, there was an increase in the exhaled NO level as COPD developed and exacerbation of COPD further increased the exhaled NO level. The exhaled NO measurement may be considered as a non-invasive marker in the development of COPD and activation in cigarette smokers.

Key Words: Nitric Oxide, Chronic obstructive pulmonary disease, Smoking, Exhaled air

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Özet

Havayollarının bronkomotor kontrolünde ve pulmoner vasküler regülasyonunda önemli rol oynayan endojen nitrik oksit (NO); birçok akciğer hastalıklarının patofizyolojisinde ve havayolu fonksiyonlarının fizyolojik kontrolünde görev almaktadır.

Bu çalışmada kronik obstrüktif akciğer hastalığı (KOAH) olan olgularda ekspirasyon NO düzeyini ve bunun sigara ve KOAH egzezarbasyonu ile ilişkisini araştırmayı amaçladık.

Bu amaçla çalışmaya 11 stabil KOAH'lı hasta, 14 unuttabil KOAH'lı hasta, 50 sigara içici ancak KOAH olmayan ve 58 sağlıklı sigara içmeyen olgulardan ibaret 4 grup alındı.

Ekshalasyon NO düzeyleri ehemiluminescence cihazında, NO'yi geçirmeyen polietilen torbalarda toplanarak ölçüldü.

Sigara içen ancak KOAH olmayan grubun ekshalasyon NO düzeyi, sağlıklı kontrol grubundan oldukça düşük bulundu (4.02 ± 1.51 ppb x 7.71 ± 1.73 ppb, $p < 0.05$). Stabil KOAH'lı grubun ekshalasyon NO düzeyi, sigara içen ancak KOAH olmayan gruptan oldukça yüksek bulundu (7.73 ± 1.42 ppb x 4.02 ± 1.51 ppb, $p < 0.05$).

Bununla birlikte stabil KOAH'lı grup ile sağlıklı kontrol grubu arasında ekshalasyon NO düzeyi açısından istatistiksel bir fark bulunamadı (7.73 ± 1.42 ppb x 7.71 ± 1.73 ppb).

Unstable KOAH'lı grubun ekshalasyon NO düzeyi, stabil KOAH'lı gruptan belirgin olarak yüksek bulundu (11.43 ± 17.7 ppb x 7.73 ± 1.42 ppb, $p < 0.05$).

Sonuç olarak, KOAH gelişimi ile ekshalasyon NO düzeyinde bir yükselme olduğu; KOAH egzezarbasyonunun ekshalasyon NO düzeyini daha da artırdığını gözledik. Ekshalasyon NO ölçümü, sigara içenlerde KOAH gelişimi ve hastalığın aktivasyonunda noninvasif bir marker olarak düşünülebilir.

Anahtar Kelimeler: Nitrik oksit, Kronik obstrüktif akciğer hastalığı, Sigara içimi, Ekshalasyon edilen hava

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Nitric Oxide (NO) plays a highly significant role in the regulation of pulmonary functions such as on the vascular system and smooth muscle tonus of the airways. It also functions as a mediator in the inflammatory response. The cellular origin of NO in the expired air is not certain. It is synthesized through the inflammatory cells such as airway epithelium, alveolus or pulmonary macrophage (1,2). There are studies revealing the increase of endogenous NO in pulmonary diseases such as asthma and bronchiectasis (1,3,4). NO is formed from L-arginine by the activity of NO synthase (NOS), of which at least three isoforms have been identified in human airways. Constitutive NO synthase (cNOS) which has two forms is involved in the physiological regulation of airways function. Inducible form of this enzyme (iNOS) is involved in the defense of the host against infections and inflammatory diseases of the airways (5). Nitric oxide is detectable in the exhaled air of animals and normal humans (6).

American Thoracic Society (ATS) defines COPD as a disease characterized by an airway obstruction caused by chronic bronchitis and emphysema accompanied by partially reversible airway hyperactivity, in which the obstruction is usually progressive (7). Cigarette smoking is an important risk factor in the development of COPD and the number of deaths increase day by day due to COPD.

In this study, the effects of NO on subjects with COPD and the relation between cigarette smoking and COPD exacerbation are studied.

Materials and Methods

Subjects

Subjects diagnosed with stable and unstable COPD according to the ATS criteria, who were smokers and non-smokers at the time of the study were admitted to the study. The subjects of the study were divided into four groups (Table 1). 11 stable subjects with COPD constituted the first group and had a mean age of 49.18 ± 4.12 years. All of the subjects used to smoke more than 20 pack-years and at the time they were studied, they had already tried giving up smoking a few times but failed to do so. All of the patients were receiving anticholinergic and B2 agonist when needed.

Except one, all the others were receiving teofilin (400 mg/day).

Second group was composed of the unstable subjects with COPD. Mean age of 14 subjects was 57.43 ± 4.48 years. COPD exacerbation diagnosis of these patients were made upon physical examinations, laboratory finding, and receiving patients' history (5). In addition, all the reasons leading to exacerbation were ruled out, and exacerbation was considered to be secondary to the infection. All the subjects were receiving anticholinergic, B2 agonist and teofilin (400 mg/day). The history of the unstable patients with COPD revealed 17.3 ± 1.7 pack-years consumption of cigarettes. The exhaled NO levels were measured prior to the onset of the treatment. No pulmonary hypertension was detected in any of the patients with COPD.

50 smokers with no COPD who consumed 11.23 ± 8.9 pack-years constituted the 3rd group. None of the subjects was receiving any kind of treatment. The mean age was 31.76 ± 7.5 years.

Non-smoker group (the fourth) consisted of 58 people with a mean age of 42 ± 4.3 years, all of whom were healthy individuals. Pulmonary function test of all the subjects were performed via vitalograph alpha device. The study was approved by the Ethics Committee of our hospital and all subjects gave their informed consent.

Measurement of Exhaled Nitric Oxide

Nitric oxide levels were measured in the ah accumulated by a slow vital capacity maneuver. Subjects were asked to sit up and wear a nose clip. They were then asked to inspire as much air to fill up the total lung capacity and expire it to the level of total residual volume. This has lasted about 5 - 15 seconds. The expired air was accumulated in an NO impermeable polyethylene bag via T-air valve. Exhaled NO measurements were performed using a chemiluminescence analyser (42S; Thermo - Environmental Instruments Inc.) within half an hour following the expiration. No difference of the NO levels was observed in the measurements of the samples performed within 1/2 to 2 hours of the sampling. NO analyser was calibrated using certified NO mixtures (90 ppb) in nitrogen before analysing the samples. The effects of volume of the expired air and expiratory flow rate on the exhaled

Table 1. Subject characteristics

Group	n	Age	Sex M / F	FEV1 (%, predicted)	The mean exhaled NO (ppb)
Stable COPD	11	49.18 ± 4.12	10/1	66.64 ± 3.56	7.73 ± 1.42
Unstable COPD	14	57.43 ± 4.48	10/4	46.64 ± 6.50	11.43 ± 1.70
Smoker	50	34.5 ± 9.3	36/14	85.98 ± 1.0	4.02 ± 1.51
Non-smoker	58	31.76 ± 7.5	37/21	90.5 ± 4.2	7.71 ± 1.73

NO level were studied. There was no significant difference between the expired air of the same subjects within the 5th and 15th seconds of expiration.

Statistical Analyses

All data were expressed as means ± standard deviation. Student's t - test, linear variation analysis (ANOVA), and Student - Newman - Keul's tests were employed for statistical analyses.

Results

The mean exhaled NO level was found to be the highest in the unstable subjects group with COPD (11.43 ± 1.70 ppb) (Tablo 1). The mean level of the exhaled NO was significantly higher than both the mean level of the stable group with COPD and the mean levels of smokers with no COPD and non-smoker group (p<0.001).

While the mean exhaled NO level of the stable group with COPD was 7.73±1.42 ppb, It was 4.02 ± 1.51 ppb for the smoker group with no COPD. The difference was statistically significant (p<0.05).

The mean exhaled NO level of the healthy smoker group was 4.02±1.51 ppb, It was significantly lower than that of the healthy non-smoker group (7.71±1.73 ppb, p<0.05).

There was no statistical difference between the levels of the stable group with COPD and the healthy non-smoker group (7.73±1.42 ppb versus 7.71±1.73 ppb, p>0.05).

Discussion

COPD is closely associated with cigarette smoking. Therefore, 90% of the patients have a history of smoking. In smokers, initially a mononu-

clear cellular inflammation of respiratory bronchus is observed. Thousands of toxic substances are held responsible for these changes. Another other respiratory tract factor being affected by cigarette smoking is NO. Many studies prove that cigarette smoking is a factor decreasing the exhaled NO level (2,9,11).

It has been claimed that NO and CO in the cigarette smoke down regulate the nitric oxide synthase enzyme. This, therefore, leads to a decrease in the level of the exhaled NO. With such decrease in NO synthesis not only bactericidal activity of phagocytes and mucociliar clearance are affected but also bronchodilatory effect of bronchodilator nerves are decreased due to their being endogenous transmitters. Thus, it leads to creation of a medium available for the development of COPD (12-14). Similarly, in our study the exhaled NO levels of the smoker group with no COPD were lower than those of the healthy non-smoker group. The exhaled NO levels of the stable COPD subjects, on the other hand, were significantly higher than those of the smokers with no COPD.

Robbins and collègues, in then study, found that the exhaled NO levels of the stable group with COPD Were similar to the levels of non-smoker control group (15). In another study by Rutgers and collègues, a meaningful NO increase in the group with COPD, when stable group with COPD was compared to 16 - subject healthy control group, which was composed of 8 smokers and 8 non - smokers was found (16).

Corradi and collègues studied on the ex - smoker group with stable COPD and a healthy control group composed of smokers and non-smokers, and their study revealed that there was a significant increase in the levels of NO with COPD formation (17).

Maziak and collègues, found in the same way that the exhaled NO levels were higher in the stable COPD smoker group and ex-smoker group with stable COPD. They claimed that when a subject with COPD gave up smoking, the exhaled NO level is further increased. In the studies on this subject, it has not been explained why the NO level is higher in the smoking COPD group than that of smoking without COPD group (18).

There are no common opinions on the correlation of NO levels and COPD formation. That people give up smoking when COPD develops is considered to be a factor (18). However, the increase in the exhaled NO level needs to be accounted for with other mechanisms. In our study, the higher exhaled NO levels of stable group with COPD compared to the smoker with no COPD groups' levels is considered to be a finding of the onset of the inflammatory process in the airways due to COPD. The existence of an inflammatory process in the subjects with COPD is emphasized although not studied as much as the asthma cases. In the subjects with COPD, an increase of neutrophil and IL - 8 in mucus is noted and neutrophilic inflammation is claimed to be an important component of COPD obstructions (19,20).

Another point we would like to emphasize in this study is that when compared to the non-smoker healthy group, the exhaled NO level decreasing with smoking increases with COPD formation and reaches to the level of exhaled NO level of healthy non-smoking group almost completely. This time this increase does not indicate good - health but an inflammation due to COPD.

In this study, the exhaled NO level in subjects with exacerbated COPD was higher than that of the stable group with COPD. Maziak and collègues reached similar results. It is also claimed that inflammation increases with further exacerbation (18).

Kharitonov and collègues claim that in respiratory tract infections, NOS are stimulated leading to an increase of the exhaled NO level (21). They also showed that there is a correlation between the exhaled NO level and exacerbation intensity. Therefore; when exacerbation is of more intense nature, the exhaled NO level further increases (18).

As a result, as well as cigarette smoking, NO which is affected by cigarette smoking place an important role in COPD formation and the exhaled NO level increases both by COPD formation and exacerbation. We believe further studies on the exhaled NO levels of subjects with COPD will bring up new approaches to the COPD pathogenesis.

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