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Research Article

Nitric Oxide, Procalcitonin and Oxidative Stress Index Levels in Acute Bronchitis Patients

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Abstract:

Background: Acute bronchitis, an inflammation of the lower respiratory tract characterized by an acute cough, is a prevalent clinical illness that leads patients to seek out primary healthcare services. About 5 percent of adults in the United States report having acute bronchitis annually, with 90 percent of those affected seeking medical attention.

Objectives: The study aimed to determine Nitric oxide, Procalcitonin (PCT), WBCs, neutrophils, lymphocytes, and Oxidative stress index (OSI) levels in acute bronchitis patients.

Methods: The study involved 120 volunteers aged 20–50 years old in Al-Zahra Teaching Hospital, Wasit City. 80 patients with acute bronchitis were conducted between (10 November 2022 to 20 March 2023). 40 people were used as a control group. Blood samples were collected from patients and controls. Complete blood account CBC was calculated using a blood sample with EDTA. Serum was used to calculate NO, PCT, and OSI. Blood counts were performed using the SYSMEX XP-300. Nitric oxide and Procalcitonin levels were measured using an ELISA kit. OSI was calculated using the equation OSI = Total oxidant status /Total antioxidant status x100.

Results: The current research presents the results of the Procalcitonin, nitric oxide, oxidative stress index, neutrophils, and lymphocytes. Age, BMI, and WBCs in acute bronchitis did not show any significant variances when compared between the two groups. In contrast, nitric oxide, Procalcitonin, oxidative stress, and Neutrophil levels showed a highly significant change among the acute bronchitis patient group compared to the control group.

Conclusion: Procalcitonin and nitric oxide may have a role in the diagnosis of acute bronchitis, in addition to lymphocytes and neutrophils.

Keywords: Acute bronchitis; lymphocytes; Neutrophils; Nitric oxide; Procalcitonin.

Introduction

Acute bronchitis is a common clinical illness resulting in visits to primary care physicians because it causes inflammation of the lower respiratory tract and, consequently, an acute cough. Around five percent of adults in the United States report having acute bronchitis annually, with ninety percent of those affected seeking medical attention. Antibiotics are not effective in treating acute bronchitis since the condition typically gets better on its own within a week or two of its beginning and is caused by a virus in at least 90 percent of the cases (1,2). Nitric oxide (NO) is a crucial signaling molecule and a free radical gas. The Nitric Oxide Synthase (NOS) enzymes, which catalyze the conversion of L-arginine to NO and L-Citrulline, are ubiquitously expressed and their expression is controlled in a cell-type-specific manner, Vasodilation, systemic circulation, hemodynamics, neuronal functions such as neurotransmission, neuroprotection, or memory, and immune response activities such as innate immunity or inflammation are just a few of the many physiological processes in which NO plays a part, immune system macrophages produce NO locally to eliminate dangerous bacteria (3,4). NO is regarded as a pro-inflammatory mediator that induces inflammation in abnormal situations due to excessive production (5). Viral infections, especially respiratory disorders, can cause oxidative stress through a variety of mechanisms, and many studies have proven this phenomenon (6). Chronic oxidative stress impairs immunological function, apoptosis, inflammatory response, and may induce organ and tissue failure in almost all viral infection patients (7). Oxidative stress arises from a state of disequilibrium between the endogenous generation of oxidative stress, antioxidant defenses, and reactive oxygen species (8,9). Procalcitonin is a gene product that is closely linked to calcitonin. It is produced by human epithelial cells in response to bacterial infections, but its expression is lowered during viral infections (10). The production of the biomarker procalcitonin (PCT) occurs in the parenchymal tissues through the mediation of cytokines IL-6, TNF-α, and IL-β. The extent to which PCT levels increase is directly associated with the severity of the infection. PCT synthesis, on the other hand, is inhibited by

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interferon-γ, a cytokine that is mainly secreted in response to viral infection (11,12). White blood cells WBCs play a significant role in the immune response against the invasion of pathogens. Neutrophils, which are the predominant WBCs in the human body, assume a pivotal function in the initiation of acute inflammation caused by pathogens (13). Lymphocytes are integral components of the immune system, providing crucial assistance in combating malignancies, as well as external pathogens such as viruses, bacteria, and parasites (14). The study aims to evaluate the relationship between nitric oxide and procalcitonin levels and oxidative stress, in addition to knowing the change in white blood cells in patients with acute bronchitis.

Patients and methods

The current research was conducted in Iraq to determine NO, PCT, and OSI levels in acute bronchitis patients, This research included 120 volunteers aged 20–50 years old in Al-Zahra Teaching Hospital, Wasit City. Eighty patients with acute bronchitis were conducted between(10 November 2022 to 20 March 2023). Forty people were used as a control group

Blood sample collection: Five ml of blood was taken from every patient and controlled through venipuncture using a 5 ml syringe. One ml of blood was placed into a tube containing ethylene diamine tetra etic acid (EDTA), and this blood was isolated and used to use to calculate the CBC. Serum was used to calculate Nitric oxide (NO), Procalcitonin (PCT), and oxidative stress index (OSI).

Acute bronchitis-related parameters determination: For a complete blood count, the SYSMEX XP-300 from Sysmex Corporation, Japan, was used. An ELISA kit was used to measure nitric oxide (NO) and procalcitonin (PCT) levels according to the manufacturer's instructions (MyBioSource, America). While OSI was calculated using the equation OSI = Total oxidant status (mM H2O2/L) /Total antioxidant status (mM vit.C/L) x 100.

Statistical analysis:

Statistical analysis software (SPSS 25) was used to analyze the findings. The General descriptive statistic was used to explain the primary findings, and an independent *t*-test was used to compare groups. The cutoff values for the parameters were determined using the receiver operating characteristic curve (ROC).

Results:

According to the data in Table 1, acute bronchitis patients were compared to the controls, the levels showed non-significant differences in age and body mass index.

Table 1: The mean ±SD for the age and BMI of patients with acute bronchitis and healthy subjects

Parameter	Mean ±SD		*P-value*
	Control Group (n= 40)	Group1 Acute bronchitis (n=80)	
Age (Yr).	37 ± 8	31 ± 10	0.1
BMI (Kg/m^2)	26.1± 2	25.7 ± 3.8	0.3

According to Table 2, the results of the WBC revealed a mean ±SD for acute bronchitis patients and controls (8.1 ±0.6), (6.9 ± 0.2), respectively. The results indicate a nonsignificant change in the white blood cells in the two groups (P>0.05). The mean and SD of neutrophils compared to control is (66.5 ± 2.3) (60.2 ± 0.8), respectively. The results show a significant change among the two groups in Neutrophils (P<0.05). Lymphocyte results revealed a mean SD for acute bronchitis patients and controls (23.8 ± 2.1) and (30.8 ± 0.8) which shows a significant difference between the two groups regarding lymphocyte number (P< 0.05).

Table2. Distribution of WBC, Lymphocytes, and Neutrophils for patient

parameter	Mean ±Sd		p-value
	Control Group (n= 40)	Group1 Acute bronchitis (n=80)	
WBCs (k/ul)	6.9 ± 0.2	8.1 ±0.6	0.18
Lymphocytes (%)	30.8 ± 0.8	23.8 ± 2.1a	0.007
Neutrophils (%)	62.2 ± 0.8	66.5 ± 2.3a	0.008

*Significant using ONEWAY-ANOVA and at 0.05 level.

a) Indicate a significant difference between control and Group1

Oxidative stress index results show a statistically significant difference between the control and patient groups, as shown in Table 3 patient groups (P<0.05). The results show that there is a clear significant difference in the concentrations of nitric oxide in patients with acute bronchitis compared to the control group (P<0.05), as shown in Table 3. It was found that there are significant differences between patients with acute bronchitis and the controls regarding NO and PCT (P<0.05; Table 3).

Table3. Distribution of Oxidative stress index, Nitric oxide and Procalcitonin for patients and control groups

parameter	Mean ±Sd		p-value
	Control Group (n= 40)	Group1 Acute bronchitis (n=80)	
Oxidative stress index	1.41±0.44	0.59±0.09a	0.02
Nitric oxide (pg/ml)	429.7±30.7	748.3±36.4a	0.00
Procalcitonin (pg/ml)	172.6±4.7	366.3±16.6a	0.00

*Significant using ONEWAY-ANOVA and at 0.05 level.

a) Indicate a significant difference between control and Group1

Receiver Operating Characteristic (ROC)

According to the Receiver Operating Characteristic (ROC) curve for patients with acute bronchitis, the results show sensitivity (86%) for Nitric oxide and specificity of (99%) with a cutoff value (of 605), and procalcitonin shows a sensitivity of (100) and specificity of (100) with a cutoff value (254) as shown in Figure (1)

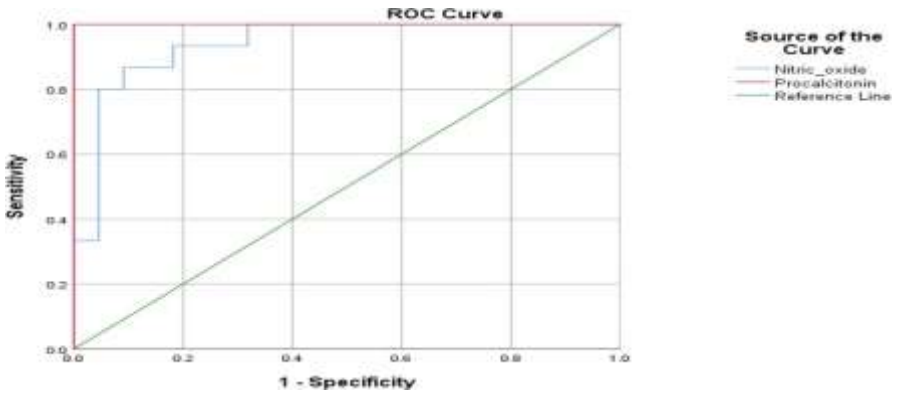

Figure 1. ROC curve for Nitric Oxide and Procalcitonin in acute bronchitis patients.

Discussion:

In this study, it was found that age does not have a significant effect, since (P> 0.05). While in a previous study, there were a total of 99 males (or 77.95%) and 28 females (22.05%). The incidence of acute bronchitis was highest in people between the ages of one and sixty years old, a group of people aged 11-20 years old (20.47%), than those aged 21-30 years old (27.56%) (15). Likewise, for the body mass index (BMI), It was found that it had no significant effect. While a previous study indicated that bronchitis incidence is more likely to occur in adolescents whose body mass index is in the higher percentiles, who are overweight, or who are obese (16), obesity and underweight have been observed in multiple studies to increase the risk of infection in adults in a U-shaped pattern, suggesting that normal weight is associated with a lower risk of infection in the majority of participants (17).

The clinical diagnosis of infection frequently involves a routine blood WBC count (18). Our study indicated there is no change in the white blood cell count (WBC) in acute bronchitis was found to be similar in smokers and non-smokers, but higher in people with a history of bronchitis (19). The research results showed a decrease in the percentage of lymphocytes in patients with acute bronchitis. A 2020 meta-analysis showed that lymphopenia was associated with worse outcomes in individuals infected with COVID-19 (20). Lymphocytes are the primary effector cells of the immune system. Lymphocytes count is inversely related to inflammation and positively related to immunity and defense against harmful germs (21). The results indicated a high percentage of neutrophils in patients with acute bronchitis. As previous studies indicated, the inflammatory response in both the upper and lower airways during viral-induced respiratory disease is characterized and dominated by airway neutrophilia (22). The body's usual response to infection or inflammation is a slight or temporary increase in neutrophils (23). Immune cells tend to react rapidly near the site of infection when harmful microorganisms penetrate the body. These immune cells serve the role of host defense as well as immune control (24). The results show a clear decrease in the levels of OSI in the patients compared to the control group. Many studies have been published demonstrating a relationship between oxidative stress and human health and disease. Oxidative stress can induce inflammation, mucus hypersecretion, airway remodeling, and fibrosis in the bronchial tubes, leading to chronic obstructive pulmonary disease (COPD) (25). OSI studies have proven to be dependable, practical, and clinically helpful (26). Oxidative stress is generated by a variety of viral diseases and is associated with the severity of infections and their ability to predict, including HIV-1, viral hepatitis B, C, and D viruses, herpesviruses, and respiratory viruses such as coronaviruses (27,28). Nitric oxide (NO) exhibits various antiviral mechanisms in host defense. These mechanisms include the nitrosylation of cysteine residues, resulting in the deactivation of viral enzymes. Additionally, NO contributes to the generation of reactive nitrogen species, such as peroxynitrite, which induces breaks in DNA strands. Furthermore, NO suppresses viral transcription factors, thereby inhibiting viral replication and the propagation of disease states (29,30). Nitric oxide (NO) is recognized as a pro-inflammatory mediator that can induce inflammation when produced excessively in abnormal circumstances (31). To the best of our knowledge, this study is the first of its kind, linking acute bronchitis and procalcitonin The results of our

research showed a direct relationship between acute bronchitis and high procalcitonin levels. Procalcitonin exhibits a direct correlation with the severity of sickness in cases of pure viral infection, and its levels remain unaffected by interferon signaling. This proposition posits that procalcitonin has superior efficacy as an indication of illness severity in comparison to bacterial coinfection in the context of viral respiratory infections (32.33)

Conclusions:
procalcitonin and nitric oxide may have a role in the diagnosis of acute bronchitis, in addition to lymphocytes and neutrophils

Authors' declaration:-
We hereby affirm that all the Figures and Tables included in the manuscript are the original work of the authors. Authors sign on ethical consideration's approval-Ethical Clearance: This research was approved by the Committee of the University of Baghdad, College of Science for Women, Department of Chemistry with session 10, number 6364/22 on 5/12/2022.
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Author contributions:
Study conception & design: (Ekhlass M. Taha). Literature search: (Huda A. Abdulsada). Data acquisition: (Huda A. Abdulsada). Data analysis & interpretation: (Huda A. Abdulsada). Manuscript preparation: (Huda A. Abdulsada). Manuscript editing & review: (Ekhlass M. Taha).

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مستويات أكسيد النيتريك و بروكالسيتونين مؤشر الإجهاد التأكسدي في مرضى التهاب القصبات الهوائية الحاد

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الخلاصة:
خلفية البحث: التهاب الشعب الهوائية الحاد، وهو التهاب في الجهاز التنفسي السفلي يتسم بسعال حاد، مرض سريري شائع يدفع المرضى إلى البحث عن خدمات الرعاية الصحية الأولية. تقريبا 5 في المئة من البالغين في الولايات المتحدة يبلغون عن تعرضهم لالتهاب الشعب الهوائية الحاد سنويا، ومعظمهم (90 في المئة) يلجأون إلى الرعاية الطبية.
الاهداف : هدفت الدراسة إلى تحديد مستويات أكسيد النيتريك (NO) وبروكالسيتونين (PCT) وخلايا الدم البيضاء (WBCs) وخلايا العدلات والخلايا اللمفاوية ومؤشر الاجهاد التأكسدي (OSI) في مرضى التهاب الشعب الهوائية الحاد.
المواد وطرق العمل: شملت الدراسة 120 متطوعا تتراوح أعمارهم بين 20-50 سنة في مستشفى الزهراء التعليمي في مدينة واسط. أجريت الدراسة على 80 مريضا مصابا بالتهاب الشعب الهوائية الحاد في الفترة ما بين (10 نوفمبر 2022 إلى 20 مارس 2023). تم استخدام 40 شخصا كمجموعة سيطرة(ضابطة). تم جمع عينات الدم من المرضى ومجموعة السيطرة. تم حساب العد الكامل للدم (CBC) باستخدام عينة دم تحتوي على EDTA. تم استخدام المصل لحساب NO و PCT و OSI. تم إجراء عد الدم باستخدام جهاز SYSMEX XP-300. تم قياس مستويات أكسيد النيتريك وبروكالسيتونين باستخدام مجموعة اختبار ELISA. تم حساب مؤشر الاجهاد التأكسدي (OSI) باستخدام معادلة OSI = إجمالي حالة الأكسدة / إجمالي حالة مضادات الأكسدة x 100.
النتائج: تقدم الدراسة نتائج مستوى البروكالسيتونين وأكسيد النيتريك ومؤشر التوتر الأكسدي ونسبة العدلات و الخلايا اللمفاوية. لم يظهر العمر ومؤشر كتلة الجسم (BMI) وخلايا الدم البيضاء أي اختلاف ذو دلالة إحصائية عند المقارنة بين المجموعتين. على النقيض من ذلك، أظهر أكسيد النيتريك وبروكالسيتونين ومستويات التوتر الأكسدي والعدلات تغييرا ذو دلالة عالية بين مجموعة مرضى التهاب الشعب الهوائية الحاد مقارنة بالمجموعة السيطرة.
الاستنتاجات: يمكن أن يكون للبروكالسيتونين وأكسيد النيتريك دور في تشخيص التهاب الشعب الهوائية الحاد، بالإضافة إلى الخلايا اللمفاوية والعدلات.
الكلمات المفتاحية: التهاب الشعب الهوائية الحاد، بروكالسيتونين، أكسيد النيتريك، العدلات، خلايا لمفاوية.