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Original Article

Predictive effect of exhaled NO and VEGF expression levels on the severity of bronchial asthma and airway inflammation

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Abstract: Objective: To investigate the relationship between exhaled NO and vascular endothelial growth factor (VEGF) expression in bronchial asthma patients with asthma severity and airway inflammation. Methods: From January 2015 to December 2017, 260 patients with bronchial asthma diagnosed in our hospital were enrolled. The expression of VEGF in the induced sputum was measured by enzyme-linked immunosorbent assay (ELISA) in the control (n=260) and asthma groups (n=260) in the same period. The NO concentration was measured on the same day. Results: Intercellular adhesion molecule-1 (ICAM-1), VEGF, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels were significantly elevated in sputum of patients with acute asthma, and the concentration in exhaled NO of all patients was also significantly higher than the control group and the stable phase of asthma. The expression of ICAM-1, VEGF, TNF-α and IL-6 was significantly elevated in patients with severe asthma. There were significant differences in patients with mild to moderate asthma ($P<0.05$). Correlation analysis showed that VEGF, IL-6 and TNF-α were positively correlated with the degree of asthma ($P<0.05$), but negatively correlated with FEV1.0, FEV1.0/FVC% and PEF ($P<0.05$). Conclusion: The expression of VEGF in sputum in patients with bronchial asthma is closely related to the severity of asthma and airway inflammation.

Keywords: Exhaled NO, VEGF, asthma, airway inflammation

Introduction

Bronchial asthma is a group of chronic respiratory diseases characterized by an airway inflammatory response. There is airway hypersensitivity and inflammatory factor activation in bronchial asthma [1, 2]. The expression levels of various proinflammatory factors are closely related to the occurrence and development of airway inflammation, which reflects the pathological characteristics of bronchial asthma to some extent. The severity of airway inflammation greatly affects the prognosis of children [3]. Vascular endothelial growth factor (VEGF) can also be a factor that mediates and binds to specific receptors, and factors that mediate its effects include intercellular adhesion molecule-1 (ICAM-1), which regulates cells together, interacting and participating in vascular proliferation and tissue and organ diseases. Usually, the level of clinical prognosis has a certain relationship with the severity of the disease, reflecting the basic condition of asthma patients. Determination of VEGF expression in patients with sputum may play a role in the diagnosis and treatment of asthma patients [4-6]. In addition, studies have found that the level of exhaled nitric oxide (NO) in asthma attacks is significantly higher than in the control group [7]. This study retrospectively analyzed the expression levels of ICAM-1 and VEGF in 260 induced sputum, as well as the detection of exhaled NO levels, and explored the relationship between VEGF expression levels and adult clinical prognosis and confirmed that exhaled NO can be used to assess airway inflammation. In this paper, the expression of ICAM-1 and VEGF and the level of exhaled NO in 260 cases of induced sputum were detected. The relationship between patient prognosis and VEGF expression levels was studied and confirmed that

Table 1. Comparison of the distribution of factor expression levels in sputum between control group and asthma group

Groups	n	ICAM-1 (μg/L)	VEGF (μg/L)	TNF-α (μg/L)	IL-6 (μg/L)
Control	260	6.35±4.58	10.36±2.67	10.24±2.52	6.15±0.73
Asthma					
Stable period	243	22.63±6.78	25.45±7.48	22.19±2.52	13.53±3.84
Acute period	260	38.34±8.37	37.67±4.38	36.29±4.62	25.46±1.25

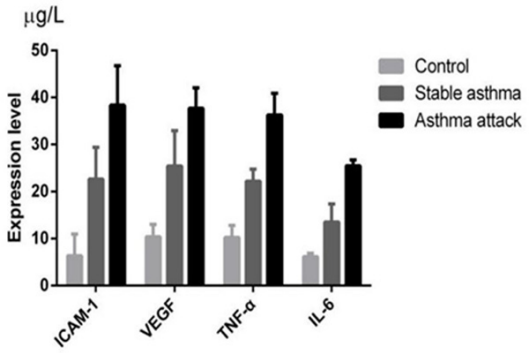

Figure 1. Comparison of the distribution of factor expression levels in sputum between control group and asthma group.

exhaled NO can be used to assess airway inflammation.

Subjects and methods

Subjects

260 children with bronchial asthma who were hospitalized between January 2015 to December 2017 were included in the asthma group. The diagnosis of bronchial asthma is based on the relevant diagnostic criteria of the Chinese Medical Association Pediatrics [8]. The study was approved by the hospital ethical committee and all patients in the study signed the informed consent.

Of the 260 cases, 115 were men and 145 were women; the mean age was (48.72±4.27) years; 108 were mild asthma, 117 were moderate asthma, and 35 were severe asthma. In addition, 260 healthy subjects with an average age of (49.13±4.65) years old were enrolled in the control group, including 104 males and 156 females. There was no significant difference in the general data between the case group and the control group (P>0.05), which was comparable.

Inclusion criteria: (1) meet the diagnostic criteria for bronchial asthma [8]; (2) informed consent to the study, complete follow-up. Exclusion criteria: (1) respiratory infections occurred within 3 months; (2) glucocorticoids, bronchodilators, leukotriene receptor antagonists were used in the past 3 months; (3) severe asthma combined with apnea, shock, coma, gastrointestinal bleeding, upper respiratory tract obstruction and other serious diseases; (4) combined with bronchiectasis, heart disease, chronic obstructive pulmonary disease and other serious cardiopulmonary diseases [9]; (5) cannot cooperate with the study.

Methods

Evaluation criteria: Acute asthma attacks are characterized by wheezing, shortness of breath, cough, chest tightness, or acute exacerbations, often accompanied by difficulty breathing, characterized by decreased expiratory flow. The stable period means that the asthma attack has been controlled and the condition is stable for more than 2 weeks [8]. Acute exacerbation of bronchial asthma can be divided into mild, moderate, and severe asthma according to the condition. Mild self-conscious symptoms are mild, moderate breathing difficulties, severe weight including severe and critical, extremely difficult breathing, cyanosis and even syncope [8].

Treatment methods

Children with acute bronchial asthma are treated according to the condition, including oxygen, spasm and electrolyte adjustment. The primary treatment for patients with mild and some moderate acute episodes is multiple inhalation of active β2 agonists. For some patients with moderate and severe acute attacks, oxygen therapy should be given immediately, in addition to repeated application offast-acting β2 receptor agonist. It can be supplied by a pressure-quantitative aerosol storage device or a spray nebulizer [8-10].

Observation indicators

Exhaled NO test was performed using a FeNO analyzer (Niox Mino, Sweden, Aerocrine AB). The subject took the seat while measuring, holding the water meter in one hand, holding

Table 2. Expression levels of factors in sputum of patients with different degrees of asthma

Groups	n	ICAM-1 (μg/L)	VEGF (μg/L)	TNF-α (μg/L)	IL-6 (μg/L)
Mild asthma	108	28.57±9.38	23.75±8.38	21.24±2.39	18.15±0.73
Moderate asthma	115	32.78±8.54	31.67±9.46	33.37±4.38	23.46±1.25
Severe asthma	37	42.54±9.37	43.38±11.32	42.43±2.49	35.15±0.73

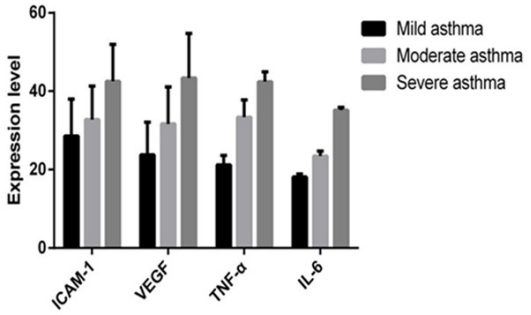

Figure 2. Expression levels of factors in sputum of patients with different degrees of asthma.

the nose in the other hand, exhaling the air in the lungs, then placing the filter tightly in the mouth and inhaling slowly until the total amount of lungs. Through computer graphics observation, when the gas was exhaled from the lungs at a uniform speed of 50 ml/s for 10 s, the reading value was measured after 90 s, which was averaged and detected three times [7].

Patients with chronic obstructive clinical manifestations of pulmonary function were excluded from bronchiectasis or glucocorticoids for more than 24 hours prior to lung function tests. Pulmonary function meter for forced vital capacity (FVC), forced expiratory volume 1 second (FEV1.0), maximum expiratory flow (MMEF), forced exhalation 50% flow (MEF50), and forced exhalation 25% flow (MEF25) was used to calculate the rate of one second (FEV1.0/FVC%). 5 ml of induced sputum was centrifuged at 2000 r/min using a H1850 benchtop high-speed centrifuge with a centrifugation radius of 5 cm [9]. Enzyme-linked immunosorbent assay (ELISA) was used to determine the expression levels of various factors, including ICAM-1, VEGF, IL-6, TNF-α and microplate reader using DR-200Bn enzyme labeling analyzer, the specific method reference kit (USA ABCAM Co. Ltd).

Statistical processing

SPSS 24.0 (IBM, SPSS, Armonk, NY, USA) software was used for data analysis. The count data according to the normal distribution is expressed with % and compared by a chi-square test; the measured data is represented as x±s, and the comparison between groups is performed using an independent sample t test or a paired sample t test. Nonparametric rank sum test was performed on data that does not conform to a normal distribution. Correlation between inflammatory factor levels and lung function in sputum agglutination in children with asthma was studied using Pearson correlation analysis. P<0.05, was considered significant.

Results

Induction of expression levels of ICAM-1, VEGF, TNF-α and IL-6 in sputum

Acute exacerbation is the acute phase of asthma in, and stable after treatment is a stable phase of asthma. The levels of TNF-α, VEGF, ICAM-1 and IL-6 in the sputum of the control group, the acute phase of asthma and the stable phase were measured. The expression levels of the four factors in the asthma group were higher than the stable period. The difference was statistically significant (P<0.05) (**Table 1**; **Figure 1**).

Expression levels of TNF-α, VEGF, ICAM-1 and IL-6 in sputum of patients with mild, moderate and severe asthma

The levels of TNF-α, VEGF, ICAM-1 and IL-6 in sputum of patients with mild, moderate and severe asthma were measured. The results showed that the expression levels of various factors increased significantly with the increase in severity. The differences between the groups were significant (P<0.05) (**Table 2**; **Figure 2**).

Correlation between ICAM-1 and VEGF levels in sputum during acute exacerbation and clinical indicators in patients

Pearson correlation analysis showed that ICAM-1 and VEGF were positively correlated with IL-6, TNF-α and their severity (P<0.05), and negatively correlated with FEV1, FEV1/FVC and PEF (P<0.05). There was no significant correlation with gender and age index (P>0.05) (**Table 3**).

Table 3. Correlation of ICAM-1 and VEGF levels with gender and age index

Item	ICAM-1		VEGF	
	r	P	r	P
Gender	0.562	>0.05	0.594	>0.05
Age	0.423	>0.05	0.564	>0.05
Severity	0.725	<0.05	0.763	<0.05
FEV1	-0.689	<0.05	-0.735	<0.05

Table 4. Exhaled NO levels in the control group and the asthma group (acute phase, stable phase)

Groups	n	NO (ppb)
Control	260	9.17±3.24
Asthma		
Stable period	243	27.25±4.31
Acute period	260	36.65±5.82

Table 5. Comparison of exhaled NO concentrations between mild, moderate, and severe asthma groups

Groups	n	NO (ppb)
Mild asthma	108	16.2±3.53
Moderate asthma	115	26.74±2.37
Severe asthma	37	34.19±2.42

Comparison of exhaled NO concentration in asthmatic group and control group and exhaled NO concentration in patients with different disease severity

The concentration of exhaled NO in stable and acute asthmatic patients was higher than that in the control group, P<0.05, which was statistically significant; however, the stable phase was less meaningful than the acute phase (P>0.05). There was a comparability between exhaled NO concentrations in patients with mild, moderate, and severe asthma (P<0.05), and as the severity increased, the exhaled NO concentration also increased (**Tables 4**, **5**; **Figure 3**).

Discussion

Bronchial asthma is a chronic airway inflammation caused by a variety of inflammatory factors and a variety of sensitizing cells. The main pathologic and physiologic factor of bronchial asthma is airway hyperresponsiveness [11]. The incidence of asthma in adults is high, and the bronchospasm of patients is mainly caused by the hypersensitivity of the airway and the interaction of various inflammatory factors in the airway. The level of airway inflammatory factors has a good correlation with the condition of patients. Early diagnosis and treatment of bronchial asthma can greatly improve the prognosis of patients and improve the quality of life of patients [12, 13].

ICAM-1 is an important adhesion factor that mediates the adhesion reaction. Under normal conditions, its expression level in vascular endothelial cells is low. When its expression level is increased, it indicates that the interaction between cells is enhanced, including white blood cells. As well as enhancing migration of endothelial cells, this mechanism is significantly associated with damage to the bronchial endothelium and smooth muscle cells [14-16]. VEGF is a specific heparin-binding growth factor for vascular endothelial cells and an important substance regulating angiogenesis. It binds to the corresponding receptors to promote the proliferation of endothelial cells, leading to dysfunction of the bronchial structure, further adversely affecting the development of bronchial function and disease in asthma patients [17, 18]. Tumor necrosis factor-α is an important cellular inflammatory factor. Induction of TNF-α in sputum can stimulate the activation of a large number of inflammatory factors in the bronchus, leading to the secretion of various cellular inflammatory factors by lymphocytes [19]. TNF-α and IL-6 are important clinical indicators of the severity of childhood bronchial asthma [20], which can help predict the child's condition and clinical outcome.

In this study, 260 asthmatic patients were tested for expression levels of various factors in sputum, including ICAM-1, VEGF, TNF-α, and IL-6. The study found that the expression levels of four factors in patients with asthma were higher than those in the control group, reflecting the pathological characteristics of patients with asthma, and physiological changes may lead to changes in ICAM-1 and VEGF. This study further analyzed the levels of ICAM-1 and VEGF in patients with different levels of asthma. The severity of the disease in asthma patients is positively correlated with the levels of ICAM-1 and VEGF. Therefore, overexpression of ICAM-1 and VEGF is considered to be closely related to the structural function of the bronchi. In addition, further correlation analysis found that both ICAM-1 and VEGF were positively correlated with IL-6, TNF-α and disease severity, and

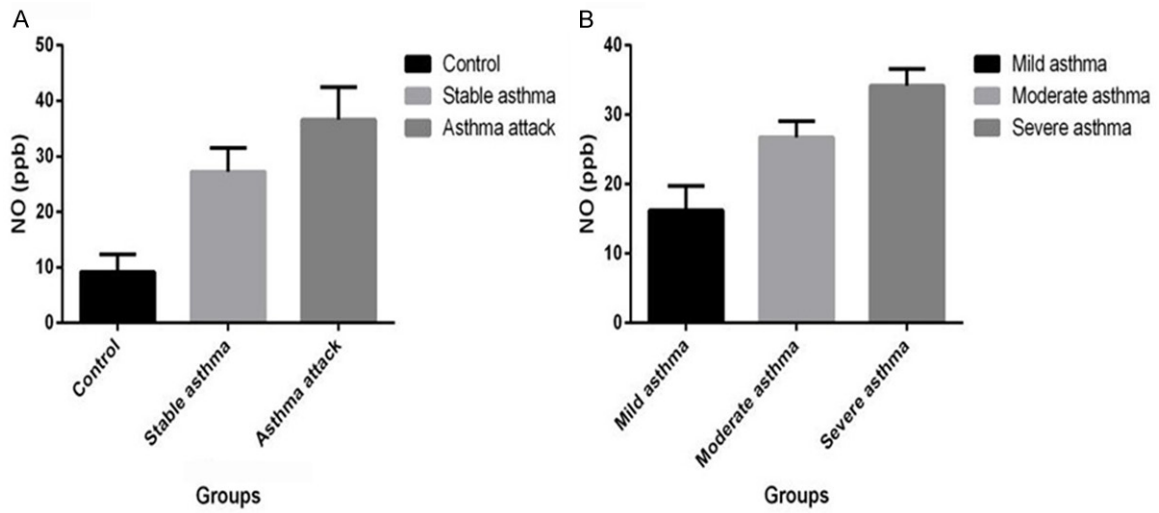

Figure 3. A: Comparison of exhaled NO levels between control group and asthma group (acute phase, stable phase); B: Comparison of exhaled NO concentrations between mild, moderate, and severe asthma groups.

negatively correlated with FEV1.0, FEV1.0/FVC% and PEF, indicating ICAM-1 VEGF has a good correlation with airway inflammation and disease in patients with asthma and is involved in the pathophysiology of asthma.

In addition, when airway epithelial cells and macrophages are stimulated by inflammatory factors and the like, the formation of NO synthase is promoted, resulting in an increase in exhaled NO. In this study, the exhaled NO level in patients with acute and stable phase was higher than that of the control group, which provided a good reference for the diagnosis and treatment of clinical acute asthma.

Conclusion

In summary, asthma is a common clinical condition. Bronchial inflammation is the main pathologic factor leading to asthma. The levels of ICAM-1 and VEGF in induced sputum have a good correlation with the severity of airway inflammation and bronchial asthma in patients. The detection of exhaled NO can provide a reference for clinical diagnosis and treatment. Close monitoring of the levels of ICAM and VEGF in the induced sputum and exhaled NO may help to improve the child's condition and deserve clinical attention.

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Disclosure of conflict of interest

None.

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