Original Article

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TANAFFOS

Inflammatory Markers in the Serum and Bronchoalveolar Lavage in Children with Non-Cystic Fibrosis Bronchiectasis

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Background: It is known that inflammatory responses occur in the airways of patients with non-cystic fibrosis bronchiectasis during respiratory exacerbations but the role of these cytokines is not clear in this condition. Herein we evaluated the levels of interleukin-1β (IL-1β), IL-8 and tumor necrosis factor α (TNF-α) in the serum and bronchoalveolar lavage among children with non-cystic fibrosis bronchiectasis.

Materials and Methods: This cross-sectional study was performed on all children with non-cystic fibrosis bronchiectasis who were admitted with respiratory exacerbation in the pediatric pulmonology ward of Masih Daneshvari Hospital, Tehran-Iran. All patients underwent fiberoptic bronchoscopy and spirometry before and after the bronchoscopy. IL-1β, IL-8, and TNF-α levels were measured in the serum and bronchoalveolar lavage.

Results: Patients included 10 (59%) female and 7 (41%) male subjects with mean age of 13.8 years (range, 5-18). Mean values for forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were below the normal range before and after bronchoscopy. Mean value for FVC (from 55% to 63%, P= 0.01) and FEV1 (from 60% to 64%, P= 0.26) increased after bronchoscopy compared to before that. IL-1β and IL-8 levels were increased and TNF-α level was decreased in the serum and bronchoalveolar lavage but no significant correlation was found between spirometry and these cytokines levels.

Conclusion: Changes in inflammatory cytokines levels in serum and bronchoalveolar lavage during respiratory exacerbation in patients with non-cystic fibrosis bronchiectasis have no significant correlation with spirometry and cannot be used in clinical practice.

Key words: Non-cystic fibrosis bronchiectasis; Inflammatory cytokine; Bronchoalveolar lavage; Serum

INTRODUCTION

Bronchiectasis is characterized by dilated bronchi and episodes of acute infection and inflammation together with purulent sputum production. Several etiologic factors are involved in the development and progression of bronchiectasis including immune system dysfunction, primary ciliary dyskinesia, and severe respiratory infections. Cystic fibrosis (CF) is the most common cause of bronchiectasis (1). There are many cases of non-CF bronchiectasis (NCFB) with increasing prevalence (2). NCFB is a chronic inflammatory lung disease with little study devoted to it (3). The incidence and severity of NCFB are increased with the increase of age and have an association with some underlying diseases such as inflammatory conditions and chronic obstructive pulmonary disease (4, 5).

Lower respiratory tract infection with gram negative organisms such as Pseudomonas aeruginosa (P. aeruginosa) is one of the most important factors of morbidity and mortality among patients with CF bronchiectasis (CFB) and NCFB (6, 7). Neutrophil replication and infiltration in the airways of patients with NCFB result in the release of neutrophilic elastase into the airway which cause pulmonary tissue destruction (8, 9). Studies on bronchiectasis in children showed that neutrophilic inflammation of the airways and increased levels of proinflammatory cytokines such as interleukin-8 (IL-8), IL-1β, IL-6, and tumor necrosis factor α (TNF-α) are involved in the inflammatory response to the bacterial insult. Neutrophil counts and the above-mentioned cytokines are increased during infective exacerbations and continue to persist during clinically stable periods as well (10).

Inflammatory responses in the airways are correlated with interactions between proinflammatory factors such as IL-8, IL-1β, and TNF-α at one side and anti-inflammatory cytokines on the other side. The level of IL-8 and TNF-α is significantly increased in patients with NCFB (11-13). However, the role of increased level of these inflammatory factors within the serum and airway of patients with NCFB in worsening of their symptoms is not well-defined. Therefore, we evaluated the level of three main inflammatory cytokines including IL-1β, IL-8, and TNF-α in the serum and bronchoalveolar lavage fluid (BALF) of children with NCFB during respiratory exacerbation in order to evaluate their effect on pulmonary function in this period.

MATERIALS AND METHODS

This cross-sectional study was conducted on all children with NCFB who were admitted with pulmonary exacerbation in the pediatric pulmonary ward of the Masih Daneshvari Hospital. The study was approved by The Medical Ethics Committee of Shahid Beheshti University of Medical Sciences (ethical code IR.SBMU.NRITLD.REC.1395.271). The study was funded by the National Research Institute of Tuberculosis and Lung Diseases (NRITLD) and no additional costs were charged to the patients for the study investigations.

After obtaining informed consent from guardians of the patients who had an indication for fiberoptic bronchoscopy, the patient was included in the study. Indications for bronchoscopy were the presence of pulmonary collapse/atelectasis or consolidation based on the high resolution CT (HRCT) scanning of the lung which was performed at the same admission. Diagnosis of bronchiectasis was also made based on the HRCT findings. Exclusion criteria were as follows: history of heart failure or neuromuscular disorders, forced expiratory volume in one second (FEV1) < 30%, or inability to perform bronchoscopy for any reason.

All patients underwent the following examinations on admission: complete blood count, white blood cell count with differentiation (WBC-diff), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) together with sputum smear and culture in those with sputum production. Eligible patients underwent spirometry before bronchoscopy and in cases with FEV1> 30% a venous blood sample (5 ml) was obtained for cytokine measurement. The blood sample underwent centrifugation (3000 cycle/min for 10 minutes) and its supernatant was reserved at -80 °C for cytokine measurement at the final step of the study.

All eligible patients with FEV1> 30% underwent fiberoptic bronchoscopy and BALF sampling. The day after bronchoscopy all patients underwent spirometry again. BALF samples were examined for cell count and culture and one sample underwent centrifugation (3000 cycle/min for 10 minutes) and its supernatant was preserved at -80 °C for cytokine measurement at the final step of the study. Finally levels of the cytokines IL-1β, IL-8, and TNF-α were measured in the serum and BALF samples using enzyme-linked immunosorbent assay.

Data analysis was performed using SPSS software version 20. Statistical tests including student *t*-test, Pearson correlation test, and one-way analysis of variants

(ANOVA) were used accordingly. *P*-value <0.05 was considered statistically significant.

RESULTS

The study was performed on 17 patients with NCFB with mean age of 13.8 ± 3.9 years (range: 5-18) including 10 (59%) female and 7 (41%) male subjects. No underlying disease was found in 12 (70.6%) patients and 5 (29.4%) children had a known etiology for NCFB including primary ciliary dyskinesia/immunodeficiency, Kartagener syndrome, hyper-IgM syndrome, hyper-IgE syndrome, and common variable immunodeficiency.

WBC was <10,000/microliter (normal) in 9 (53%) patients and >10,000/microliter (leukocytosis) in 8 (47%) patients. Nine (53%) patients had ESR >20/hr and 11 (65%) cases had CRP >10 mg/L. Sputum cell counts showed a WBC of more than 15/mm^3 in 75% of cases. Sputum culture revealed upper respiratory flora (URF) in 11 (65%) patients. In three cases Staphylococcus aureus, P. aeruginosa, and Escherichia coli (E, coli) were isolated from sputum culture. WBC was <8/mm^3 in BALF in 14 (82%) patients. BALF culture showed isolation of URF in 14 (82%) cases, P. aeruginosa in two samples, and combined P. aeruginosa and E. coli in one case.

Table 1 shows the levels of cytokines in the BALF and serum samples. Pearson correlation test showed no significant correlation between serum and BALF in terms of cytokines level.

Table 2 summarizes the spirometry findings of the patients before bronchoscopy. Mean values of the lung volumes were below normal and more than 75% of the children had FEV1 <80%. There was no significant correlation between FEV1 value before bronchoscopy and the inflammatory markers including ESR, CRP, IL-1β, IL-8, and TNF-α (Table 3).

Table 1. Mean values for measured cytokines levels (pg/ml)

Cytokines	Mean ± Standard Deviation		r (Pearson test)	*P*-value
	BALF	Serum		
Interleukin-1β	112.63±165.89	15.57±37.31	-0.17	0.52
Interleukin-8	694.04±671.21	37.64±77.03	0.06	0.8
Tumor necrosis factor α	9.73±12.96	46.0±54.02	-0.07	0.7

BALF, Bronchoalveolar lavage fluid

Table 2. Spirometry values before bronchoscopy

Spirometry Value	Mean	Standard deviation	Minimum	Maximum
FVC	55%	18%	29%	89%
FEV1	60%	25%	32%	110%
MEF_{25-75}	60%	30%	15%	124%
FEV1/FVC	98%	11%	78%	116%

FVC, Forced vital capacity; FEV1, Forced expiratory volume in one second, MEF25-75, Maximal mean expiratory flow between 25% and 75% of FVC

Table 3. Correlation between FEV1 value and inflammatory markers

	ESR	CRP	IL-1β		IL-8		TNF-α	
			Serum	BALF	Serum	BALF	Serum	BALF
r*	-0.295	-0.360	0.311	-0.387	0.110	0.361	0.154	-0.049
P-value	0.211	0.156	0.242	0.138	0.685	0.170	0.570	0.851

*Pearson correlation test; FEV1, Forced expiratory volume in one second; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; IL, Interleukin; TNF, Tumor necrosis factor; BALF, Bronchoalveolar lavage fluid

The day after the bronchoscopy mean values of pulmonary volumes were still below normal but 5 (29.4%) patients had FEV1 of ≥80%. Comparing the spirometry values before and after bronchoscopy revealed that mean value of FVC improved significantly but the improvement in FEV1 was not statistically significant (Figure 1).

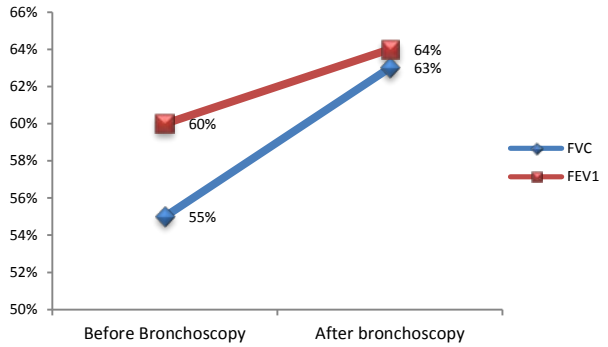

Figure 1. FVC and FEV1 values before and after bronchoscopy
FVC, forced vital capacity; FEV1, forced expiratory volume in one second

DISCUSSION

We evaluated the serum and BALF levels of IL-1β, IL-8, and TNF-α in children with NCFB during pulmonary exacerbation. Mean level of IL-1β was 15.57±37.31 pg/ml in serum and 112.63±165.89 pg/ml in BALF in our study which were higher than normal (14). No significant correlation was found between the serum and BALF levels of this cytokine. IL-1β is one of the proinflammatory cytokines which results in the attraction of neutrophils into the airways. IL-1β level in BALF of patients with stable bronchiectasis was below normal in the study of Angrill et al. (15) but the difference was not significant. The level of IL-1β in the sputum of patients with stable bronchiectasis was not significantly different between patients with and without *P. aeruginosa* infection (9). Our previous study on children with CFB showed that IL-1β level was higher than normal in 83% of sputum samples and 81% of BALF samples but only in 29% of serum samples (14). As it is seen, there is no consistency between different studies in terms of IL-1β level in serum or pulmonary secretions of patients with bronchiectasis and also there is no significant correlation between its levels in different samples.

Mean levels of IL-8 in our patients were 37.64±77.03 pg/ml in serum and 694.04±671.21 pg/ml in BALF which were higher than normal (14) but no significant correlation was found between them. Tsang et al. (9) found no significant difference between patients with stable bronchiectasis and normal subjects regarding sputum level of IL-8. Reeves et al. (12) found an increased level of IL-8 in BALF of patients with CFB. IL-8 level of BALF has been reported higher than normal in both CFB and NCFB patients in some other studies (11, 12, 16). Ayhan et al. (17) reported that IL-8 level in patients with bronchiectasis was higher than normal in serum samples but lower than normal in BALF samples. In our previous study on children with CFB, IL-8 level was higher than normal in all sputum and BALF samples and in 89% of serum samples (14). Muhlebach et al. (18) evaluated the ratio of IL-8 to bacterial count in BALF samples of patients with CFB and NCFB and found that this ratio was significantly higher in CF patients. As seen in different studies the condition of IL-8 is also different but its level seems to be related to the respiratory tract infection.

TNF-α level in this study was 46.0±54.02 pg/ml in serum and 9.73±12.96 pg/ml in BALF, both of them were lower than normal (14) but no significant correlation was found between them. In a study by Guran et al. (13) on children with stable NCFB, mean value of TNF-α level in sputum was 58 (9.2-302) pg/ml. This evaluation was not performed in our study but comparing to our previous study (14) it seems to be within normal limits. In a study on patients with stable bronchiectasis by Tsang et al. (9) sputum level of TNF-α was not significantly different from normal. Upregulation of TNF-α expression within bronchiectatic airways is correlated with neutrophil density within the airway which has a significant role in the attraction of neutrophils toward airways (19). TNF-α is secreted by active macrophages, mast cells, and neutrophils and causes bronchial hyper-reactivity, IL-8 secretion, and attraction of eosinophils and neutrophils together with increased vascular permeability (20). TNF-α causes the release of matrix metalloproteinase from

neutrophils which can result in intensifying the inflammation and remodeling of airways (21-24). Ayhan et al. (17) reported that serum level of TNF-α in bronchiectatic patients is lower than normal controls but its BALF level is higher than normal. In the study of Angrill et al. (15) TNF-α level of patients with stable bronchiectasis was higher than normal in BALF samples. Our previous study on patients with CFB showed that TNF-α level was higher than normal in 29% of serum samples and only 6% of BALF samples but mean values were below normal for both serum and BALF samples (14). The TNF-α level is also different based on different studies with unknown reason but active infectious processes may have a role.

Before bronchoscopy in our patients mean values for FVC and FEV1 were 55% and 60% respectively and 23.5% of the patients had normal FEV1. The day after bronchoscopy, these values reached 63% (8% improvement; *P*= 0.01) and 64% (4% improvement; *P*=0.26), respectively and 29.4% of patients had normal FEV1. No significant correlation was found between the inflammatory cytokines and FEV1. Our previous study on CFB patients also found no significant correlation between the inflammatory cytokines and FEV1 (14). Guran et al. (13) in a study on children with NCFB found that the severity of their symptoms had a negative correlation with FEV1 (r= -0.49). It seems that the clinical changes in pulmonary function are not significantly correlated with the changes in inflammatory markers.

Limitations

This study suffered from some limitations including the infrequent NCFB cases among our patients together with no indication of bronchoscopy in all admitted patients with NCFB which resulted in a small sample size.

CONCLUSION

Based on our study and reviewing the findings of other studies, despite the changes in the level of IL-1β, IL-8, and TNF-α in serum and pulmonary secretions of patients with NCFB, these changes are not consistent and have no significant correlation with spirometry results, so their usage in clinical practice seems irrational and needs further studies.

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