CHANGES IN CYTOKINES LEVELS IN ASTHMA PATIENTS WITH FREQUENT EXACERBATIONS

Pham Dac The[1,2], Ta Ba Thang[2], Nguyen Huy Luc[2], Dao Ngoc Bang[2]*

Abstract

Objectives: To evaluate changes in serum levels of some cytokines in asthma patients with frequent exacerbations. ***Methods:*** A prospective, descriptive, longitudinal study on 60 asthma patients with frequent exacerbations and 60 ones with few exacerbations, treated as outpatients at the Asthma Management Department, Hai Phong International Hospital from January 2020 to May 2023. Patients underwent clinical examination, chest X-ray, respiratory function test, and assessment of inflammatory markers and serum cytokine levels at the time of the study. ***Results:*** The asthma group with frequent exacerbations had significantly higher IL-4 and IL-13 concentrations than the asthma group with few exacerbations (21.10 and 9.93 pg/mL vs. 16.48 and 3.95 pg/mL). Concentrations of IFN-α, IFN-γ, IL-17, and inflammatory cytokines (IL-12, TNF-α, IL-1β) were not different between the 2 asthma groups. In the asthma group with frequent exacerbations, most serum cytokine concentrations in male patients were insignificantly higher than in females, except for IL-12. IL-17, IFN-α, IL-4, and IL-12 concentrations in patients aged ≥ 60 years were insignificantly higher than those in the group < 60 years old. ***Conclusion:*** There are significantly higher serum levels of IL-4 and IL-13 in asthma patients with frequent exacerbations compared to those with few exacerbations.

Keywords: Frequent exacerbation asthma; Serum cytokine levels.

INTRODUCTION

Asthma is a heterogeneous disease with the impact of many risk factors and has a very complex pathogenesis. Th2, Th1, Th17, and innate immune cells have been identified to play an important role in determining the pathogenesis of airway inflammation

[1]**Hai Phong International Hospital**

[2]**Military Hospital 103, Vietnam Military Medical University**

[*]**Corresponding author: Pham Dac The (dr.dacthebmh@yahoo.com)**

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in asthma patients. In particular, cytokines are the connecting link and factors that directly affect pathogenesis [1]. The result of the synergistic interaction between cells in the immune response leads to a wide variety of clinical symptoms of the disease, as well as the response to different therapies. Although there have been many advances in the prevention of acute exacerbations, there are 10 - 15% of patients with difficult-to-treat and resistant asthma, despite optimal treatment according to GINA guidelines, leading to asthma phenotype with frequent exacerbations. This phenotype is often associated with persistent symptoms and reduced lung function, causing medical and economic burden [2]. To optimally treat this phenotype, it is necessary to understand the pathogenesis of inflammation and immune response to properly evaluate the endogenous phenotype of asthma to help target treatment. There have been studies on changes in cytokine concentrations to determine endogenous phenotypes worldwide, but they are still limited in focusing on the Th2 phenotype group. In Vietnam, there have been no studies on immune characteristics in asthma patients with frequent exacerbations. Therefore, this study was conducted: *To evaluate changes in serum levels of some cytokines in asthma patients with frequent exacerbations.*

MATERIALS AND METHODS

1. Subjects

120 outpatients with stable asthma treated at the Outpatient Asthma Management Department, Hai Phong International Hospital from January 2020 to May 2023, divided into 2 groups:

- Group 1 (research group): Including 60 asthma patients with frequent exacerbations.

- Group 2 (control group): Including 60 asthma patients with few exacerbations.

* *Inclusion criteria:* Patients have a confirmed diagnosis of asthma according to GINA standards (2019), receive outpatient control and management treatment at the Outpatient Asthma Management Department, and are currently out of exacerbation; criteria for frequent exacerbations are determined according to GINA standards (2019) [3]; criteria for patients with few exacerbations are having less than 2 exacerbations in the last 1 year.

* *Exclusion criteria:* Patients with exacerbation; patients who do not comply with treatment, or drop out of treatment within one year of the study.

Healthy group (group 3): This group includes 30 healthy people who currently have no acute or chronic diseases, have a history of allergies, or are receiving immunosuppressive therapy.

2. Methods

* *Study design:* A descriptive, prospective, and longitudinal study. After being diagnosed with asthma, patients were enrolled in the study. These patients were treated to control asthma according to the guidelines of the Ministry of Health (2020) [4] and regularly re-examined monthly. Patients underwent clinical examination, blood count tests, IgE concentrations, chest X-ray, FeNO measurement, respiratory function test, and serum cytokine levels. Determining cytokine concentrations by using the above magnetic particle immunoassay technique Luminex machine system, performed at the Immunology Laboratory, Military Medical and Pharmaceutical Research Institute, Vietnam Military Medical University.

* *Data analysis:* All data were expressed as mean ± standard deviation or median (interquartile range, Q1-Q3) or percentage where available. Differences between groups were examined with either the student's T-test or Mann-Whitney test. A p-value < 0.05 was defined as statistically significant. Data were managed and analyzed with the SPSS version 20.0 (SPSS Inc, Chicago, IL).

3. Ethics

The study was approved by the Hai Phong International Hospital (No. 09/2020/HIH-IRB). The patients signed the consent and commitment forms.

RESULTS

Table 1. Characteristics of the study population.

Characteristics		Group 1 (n = 60)	Group 2 (n = 60)	p
Age ($\overline{X}$ ± SD) (years)		50.73 ± 15.05	50.43 ± 16.56	0.92**
Gender	Female; n (%)	43 (71.7)	34 (56.7)	0.09*
	Male; n (%)	17 (28.3)	26 (43.3)	
Asthma onset	≤ 12 years old; n (%)	34 (56.67)	40 (66.67)	0.26*
	> 12 years old; n (%)	26 (43.33)	20 (33.33)	
Duration of asthma (year) ($\overline{X}$ ± SD)		26.57 ± 18.32	29.55 ± 17.62	0.37**
Allergy history	Family (n = 29); n (%)	14 (23.3)	15 (25.0)	0.83*
	Personal (n = 66); n (%)	44 (73.3)	22 (36.7)	< 0.001*
Step of asthma	Step (2-3); n (%)	8 (13.3)	14 (23.3)	0.16*
	Step (4-5); n (%)	52 (86.7)	46 (76.7)	

(: Chi-Square Test; **: Student's T-test)*

In group 1, the proportion of women was 71.7%, asthma onset after 12 years old was 43.33%; and the average duration of asthma was 26.57 years, insignificantly shorter than that of group 2 ($p > 0.05$). Patients in group 1 had a personal history of allergies of 73.3%, significantly higher than that of group 2 ($p < 0.001$). The proportion of severe asthma (step 4 - 5) in group 1 was higher than that in group 2. There was no difference in the average age between the 2 groups ($p > 0.05$).

Table 2. Medium serum cytokine concentrations in study groups.

Cytokine level Median (p_{25} - p_{75}) (pg/mL)	**Group 1 (n = 60)**	**Group 2 (n = 60)**	**Group 3 (n = 30)**	**p* (1; 3)**	**p* (2; 3)**	**p* (1; 2)**
Non-Th2-dependent inflammatory response cytokines						
IL-17	8.62 (2.88 - 14.92)	4.86 (1.47 - 13.95)	2.78 (1.47 - 9.44)	0.006	0.27	0.11
INF-α	0.57 (0.40 - 1.43)	0.50 (0.40 - 1.43)	0.40 (0.40 - 0.40)	0.028	0.004	0.67
INF-γ	6,54 (5.05 - 11.95)	5.57 (5.05 - 9.96)	5.05 (3.67 - 6.05)	0.005	0.007	0.63
Th2-dependent inflammatory response cytokines						
IL-4	21.10 (15.99 - 33.67)	16.48 (6.75 - 25.54)	10.49 (6.69 - 16.53)	< 0.001	0.06	0.007
IL-13	9,93 (1.73 - 13.83)	3.95 (1.73 - 10.52)	1.73 (1.73 - 10.52)	0.001	0.29	0.01
Cytokines respond to inflammation and inhibit inflammation						
IL-12	55.11 (28.17 - 269.03)	32.24 (17.22 - 271.04)	120.56 (14.88 - 182.16)	0.21	0.83	0.06
TNF-α	10.25 (4.76 - 18.92)	7.30 (3,53 - 17,88)	4.12 (3.53 - 7.98)	0.002	0.08	0.18
IL-1β	1.61 (0.81 - 2.68)	1.39 (0.81 - 3.76)	1.35 (0.81 - 2.15)	0.32	0.23	0.78

(: Manney-White test)*

In group 1, levels of most cytokines were significantly higher than those of group 3 ($p < 0.05$), except for IL-12 concentrations. Besides, levels of IL-4 and IL-13 were significantly higher compared to group 2 ($p < 0.05$).

Table 3. Medium serum cytokine concentrations in the asthma group with frequent exacerbations by gender

Cytokine level Median (p_{25} - p_{75}) (pg/mL)	**Male (n = 17)**	**Female (n = 43)**	**p***
Non-Th2-dependent inflammatory response cytokines			
IL-17	10.67 (5.92 - 17.38)	8.19 (1.47 - 13.07)	0.23
IFN-α	0.68 (0.40 - 1.85)	0.57 (0.40 - 1.22)	0.32
IFN-γ	8.98 (5.05 - 12.43)	6.05 (5.05 - 10.95)	0.37
Th2-dependent inflammatory response cytokines			
IL-4	21.10 (14.98 - 33.52)	21.10 (16.53 - 34.23)	0.85
IL-13	10.52 (3.48 - 22.52)	9.60 (1.73 - 11.49)	0.46
Inflammation and inhibited inflammation cytokines			
IL-12	45.09 (31.53 - 258.22)	78.19 (27.68 - 271.83)	0.90
TNF-α	13.16 (8.62 - 22.80)	8.88 (3.53 - 17.19)	0.05
IL-1β	1.64 (0.81 - 2.68)	1.61 (0.81 - 2.68)	0.26

(: Manney-White test)*

In the asthma group with frequent exacerbations, the medium serum concentrations of most cytokines in male patients were higher than those in the female group, except for the IL-12 concentration, but the difference was not statistically significant ($p > 0.05$).

Table 4. Medium serum cytokine concentrations in the asthma group with frequent exacerbations by age group

Cytokine level Median (p25 - p75) (pg/mL)	< 60 years old (n = 36)	≥ 60 years old (n = 24)	p*
Non-Th2-dependent inflammatory response cytokines			
IL-17	8.16 (2.13 - 13.07)	10.35 (4.65 - 17.54)	0.30
IFN-α	0.53 (0.37 - 1.31)	0.68 (0.40 - 1.47)	0.31
IFN-γ	7.02 (5.05 - 19.14)	5.57 (5.05 - 10.49)	0.22
Th2-dependent inflammatory response cytokines			
IL-4	21.10 (11.82 - 34.02)	21.84 (16.53 - 33.27)	0.76
IL-13	10.52 (2.96 - 14.94)	9.27 (1.73 - 10.70)	0.29
Inflammation and inhibited inflammation cytokines			
IL-12	45.67 (29.61 - 257.82)	155.36 (28.17 - 323.91)	0.55
TNF-α	10.93 (3.53 - 19.05)	10.23 (5.99 - 17.99)	0.86
IL-1β	1.61 (0.81 - 2.68)	1.61 (0.81 - 2.68)	0.65

(: Manney-White test)*

In the asthma patients with frequent exacerbations, medium serum concentrations of IL-17, IFN-α, IL-4, and IL-12 in the group ≥ 60 years old were higher than those of the group < 60 years old, but the difference was not statistically significant (p > 0.05).

DISCUSSION

1. Characteristics of asthma patients with frequent exacerbations

The study results showed that the asthma patients with frequent exacerbations had insignificantly higher proportions of female and disease onset after age 12, and a shorter average duration of the disease than those of the group with few exacerbations. Asthma patients with frequent exacerbations had a significantly higher personal history of allergies than those of the group with few exacerbations. There was no difference

in age or asthma grade between the 2 groups. Similar results have been proved in many previous studies. In the research by Ten Brinke A (2005), risk factors for exacerbations in patients with difficult-to-treat asthma were evaluated, the results of a younger patient group (the average age was 38 compared to 47 years old) and shorter duration of disease (12 vs. 24.5 years) had a higher risk of exacerbation. In addition, asthma patients with many exacerbations often have a family history of asthma and allergies. Patients with allergic conditions are 10.7 times more likely to have an exacerbation than those without allergic conditions. In particular, patients with increased IgE specific to house dust have a 6.9-fold increased risk of exacerbation [6]. In a cluster study by Kim M (2017), the results showed that the group of patients with non-allergic asthma, late-onset, and poor respiratory function, and the one with allergic asthma, early onset, and poor respiratory function had a higher risk of asthma exacerbation than the one with allergic asthma, early onset, and preserved respiratory function and the one with non-allergic asthma, late-onset, and preserved lung function [6]. With the results of previous studies, it can be seen that most of the results showed that being female, having a history of allergies, having many symptoms, and exhibiting poor respiratory function are risk factors for frequent exacerbations.

2. Changes in serum cytokine levels in asthma patients with frequent exacerbations

* *Serum cytokine concentrations according to the study patient group:*

The results of this study showed that asthma patients had most of the average concentrations of cytokines in serum significantly increased compared to that of the normal ones, except for the concentration of IL-12. In the asthma group with frequent exacerbations, IL-4 and IL-13 concentrations were significantly higher than in the one with few exacerbations. To compare with other previous studies, we realized similarities in the assessment of the role of Th2-dependent cytokines in exacerbations; however, there are differences in the assessment of the role of Th1- and Th17-dependent cytokines in asthma exacerbations. In the research by Wenzel S (2013) on moderate to severe asthma patients with blood eosinophilia, using ICS in combination with LABA, the results showed that Dupilumab (anti-IL-4 receptor alpha) reduced the number of asthma exacerbations (although the patient had previously stopped ICS + LABA), improved respiratory function

and reduced markers of Th2-dependent inflammation [7]. There was a difference in the study by Djukanovic R (2014) to evaluate the effectiveness of IFN-β on patients with asthma exacerbation due to viral infection, with the results showing that inhalation of IFN-β helps reduce the need for additional drug treatment in the management of exacerbation [8]. It can be seen that the results of those studies were not consistent, so identifying the endogenous phenotype that plays a key role in asthma with frequent exacerbations is still challenging.

** Serum cytokine concentrations according to gender characteristics:*

The results of this research showed that men had higher average serum concentrations of most cytokines than women in the asthma group with frequent exacerbations, except for IL-12 concentration which was insignificant. Some previous studies have proved sex hormones relate to the inflammatory status of the airway and treatment response. In the research by Han Y (2020), testosterone was a protective factor for asthma in women and effective against airway inflammation [9]. An analysing study by Yung J (2018) showed that estrogen promotes, while androgen slightly reduces type 2 inflammatory response [10]. Many studies have shown that cytokine production in the lung parenchyma in women is higher than in men. This is associated with increased responsiveness and airway remodeling. In the experimental model, ILC2s in female mice produced significantly higher levels of IL-5 and IL-13 than in male mice. Increased expression of type 2 inflammatory response genes in female mice with ILC2 activated by IL-33 compared to male mice. The research by Newcomb D (2015) showed that patients with severe asthma have increased IL-17A levels in women when compared to men [11]. It can be seen that there have been studies on the impact of gender on the concentrations of cytokines involved in the pathogenesis of asthma. However, the limitation is that most of the studies were carried out on experimental animals, or sex hormones only played a small role in the results of research. More researches are needed to evaluate the impact of sex hormones on asthma patients with frequent exacerbations.

** Serum cytokine concentrations by age group:*

The results of this study showed that patients in the group with frequent exacerbations who are older (≥ 60 years) have higher average concentrations

of IL-17, IFN-α, IL-4, and IL-12 than the younger ones (< 60 years old) insignificantly. Poor prognosis partly comes from underdiagnosis and undertreatment. Changes in the immune system, structural composition, and function of the airways are the main causes of this consequence. When patients become older, there will be a change in the response of the innate immune system and inflammatory response with an increase in IL-1β, IL-6, and TNF-α. Neutrophilic airway inflammation is also a feature of this patient group. In animal experimental models, this group showed the increased expression of IL-8 and Th17-dependent cytokines in the airways. In the research by Ulambayar B (2018), IL-33 and IL-31 concentrations were significantly lower in elderly asthma patients (≥ 60 years old), while there was no difference in IL-8 concentrations, eotaxin-2, TGF-β1, and periostin between young and elderly asthma ones [12]. In conclusion, elderly asthma patients have special physiological characteristics, with many overlapping factors affecting asthma control (comorbidities, the role of sex hormones, changes in the immune system...); it is necessary to have studies with strict selection criteria to homogenize research subjects to help more accurately evaluate these effects.

CONCLUSION

Evaluating changes in the concentrations of some serum cytokines in patients with frequent asthma exacerbations, we initially draw the following conclusion: There are significantly higher serum levels of IL-4 and IL-13 in asthma patients with frequent exacerbations than in those with few exacerbations.

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