ISSN NO: 2770-2936
Date of Publication:11-02-2023

Immunological and Molecular Evaluation of IL-5 in Asthma Patients

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Abstract

Background: Asthma cause airway obstruction and are associated with chronic airway inflammation Interleukin -5 is mainly produced by Th2 cells, innate lymphoid cells group 2 (ILC2), mast cells, natural killer T cells (NKT), and eosinophils themselves. **Material and Method:** A case-control study and the implementation steps are divided into 3 main parts, include: Patient selection and sampling, examining the intensity of gene expression**. Result:** The present study show that the expression of IL-5 gene has increased significantly in asthma. Regarding the intensity of IL-5 gene expression in the patient and control groups, a significant difference can be observed between the two groups in terms of gene expression intensity (P-Value=0.0001). **Conclusion:** The results of the present study show that IL-5 gene expression has been significantly changed in asthma. In terms of the intensity of IL-5 gene expression in the patient and control groups, a significant difference is observed between the two groups (P-Value=0.0001), also the experiment and analysis of the obtained ROC curve shows that a significant change in IL-5 in Asthma.

Keywords: IL-5 -, Asthma, Chronic Obstructive Pulmonary Disease, COPD

Introduction:

Asthma cause airway obstruction and are associated with chronic airway inflammation (1). Abnormal immune response to environmental stimuli has caused many cells of the innate and adaptive immune system to act (2). Bronchospasm is reversible and the most important symptoms of asthma are cough, wheezing and shortness asthma is of a reaction breath (3-4). To inhale In fact, antigens such as Respiratory viruses are airborne pollutants that lead to airway inflammation, airway hyperactivity, and reversible airflow obstruction (5). More than 311 million people around the world have been affected by this disease. It is estimated that 5% to 11% of patients with asthma have severe disease that does not respond to conventional treatments, including corticosteroids (6). The morphological changes characteristic of severe asthma include the increase in the thickness of the airway wall, fibrosis under the basement membrane, the increase of new vessels under the mucosa, the increase in the size of the sub mucosal glands, the metaplasia of the goblet cells of the airway epithelium, and the hypertrophy of the bronchial muscles (7). Asthma is caused by inflammation caused by the infiltration of inflammatory cells and air pollution. Allergens, viruses, and allergens cause inflammation in the airways. The persistence of stimuli and inflammation causes airway changes and causes diseases The main causes of asthma include genetic predisposition and chronic ways of increasing type 1 sensitivity, acute airway inflammation and increased bronchial responsiveness to various stimuli. As mediators in the course of inflammation, many inflammatory cells are involved, but the role of type 2 helper T cells is crucial to Cytokines in the produced pathogenesis of asthma. Cytokines produced by these cells cause asthma symptoms (8).

IL-5 is mainly produced by Th2 cells, innate lymphoid cells group 2 (ILC2), mast cells, natural killer T cells (NKT), and eosinophil's themselves (9-10). Interleukin 5 (IL-5) is among the cytokines that play an important role in the inflammatory process in the airways of patients with asthma and their activation in the asthmatic mucosa. The influx of eosinophils and their activation in the bronchial mucosa are characteristic features of asthma. IL-5 regulates the activity of eosinophils by influencing adhesion processes, expression of membrane receptors, chemotaxis, and the production of multiple mediators. Eosinophilia in the airways in patients with asthma is associated with hyperactivity (11).

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Materials and Methods:

A case-control study and the implementation steps are divided into 3 main parts, include:

- Patient selection and sampling,
- Examining the intensity of gene expression,
- Data analysis in the following

Research Method:

This study was conducted in a case-control method. In this study, the population of patients with asthma has been evaluated. **Subjects**

The subjects in this study included 42 asthmatic patients and 31 controls. The subjects were selected from those who were referred to 22 Bahman Hospital and the emergency department of Imam Reza Hospital in Mashhad in 2022.

Inclusion Criteria

Patients with asthma infections are included in the study based on the diagnosis of specialist doctors. All patients were selected in the active phase and chronic phase of the disease. Written consent was obtained from all participants in the study.

Experimental Method

The present study was conducted as a case-control study on 42 asthmatic patients and 31 controls referred to 22 Bahman Hospital in Mashhad. To enter the study, written informed consent was obtained from all subjects. After receiving the initial information, blood was collected from all subjects and centrifuged, and the blood serum was separated and the blood serum was kept at -11 degrees Celsius.

Isolation of RNA for interleukin-5 genes:

RNA was extracted using an extraction kit (RNX plus RNA, Sinagen, Iran) and according to the protocol of the kit, and the concentration of RNA was checked with a Nanodrop device.

cDNA synthesis for interleukin-5: cDNA synthesis steps were performed according to the DyNAmo-cDNA kit protocol.

Real-time PCR for interleukin-5 genes

The changes in the gene expression level of the patient group compared to the control group were checked by the "CORBETT-6000" device and Gene runner software.

Exclusion criteria of the study

In this study, people were excluded if they overlapped with other respiratory diseases such as asthma or COPD and autoimmune diseases. Patients under 12 years of age and patients who consume any medication other than the routine medication of the treatment protocol of corona disease will also be excluded from the study. In the control group, history was also taken, and in case of taking any anti-inflammatory drugs, steroids, and cytotoxic drugs or drugs that suppress the immune system, they will be excluded from the study.

Statistical Analysis

In the description of the data, appropriate statistical tables and indicators such as mean and... have been used, and in the data analysis, the normality of the data has been investigated using the Shapiro-Wilk test, which was confirmed by the method Appropriate parametric tests such as Student's test and analysis of variance (Tukey's comparisons) were used, and for non-normal data, the Yeoman-Whitney test (Bonferroni-pairwise comparisons) was used. Pearson's correlation coefficient was used to check the correlation and a multivariable linear model was used to check the results. The software used in this research is SPSS v.26 and Graph Pad Prism 9, and the significance level of the tests is less than (5% in the results).

Ethical approvements

The study was conducted in accordance with the Declaration of Helsinki's ethical principles. Patients' verbal and informed consent was obtained before any samples were taken. The study methodology, subject information, and consent form were reviewed and authorized by the neighborhood Ethics Committee, in accordance with document Data Analysis 1025, dated 4/11/2021.

Descriptive analysis of data

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In this study, in the control group, there were 14(45.2%) men and 17 (54.8%) women, and in the asthma group, there were 26 (61.9 %) men and 16(38.1%) women; according to Chi-square test no statistically significant difference was observed between the two groups (Likelihood Ratio=2.02, P=0.155).
In the table below, it can be seen that for the parameters of age, weight, height, body mass index and body surface index, there was no statistically significant difference (Student's test) between the two groups (P>0.05) in this study based on the body mass index in the control group. 10 people are normal, 14 people are overweight and 7 person is obese, and in the asthma group, 13 people are normal, 15 people were overweight and 14 people were obese.

Table 1- distribution of main variables

Tests		Asthma		Controls		Indicator's
P value	Ready Test	Standard Deviation	Mean	Standard Deviation	Mean	
P=0.130	t=-1.53	13.76	57.17	14.72	52.03	Age
P=0.837	t=-0.206	11.04	73.36	10.03	72.84	Weight
P=0.225	t=1.22	8.04	163.93	9.78	166.48	Height
P=0.323	t=-0.99	4.15	27.40	3.99	26.44	Body Mass Index
P=0.712	t=0.239	.15	1.82	.15	1.83	Body Surface Area Index

Distribution of asthma indicators

In the table below, it can be seen that there is a statistically significant difference (Student's test) between the two groups for all variables P<0.05. Other results in this study show that

- According to the ACT.Score.pre index, in the control group, 31 people (100 percent) were normal, 0 people (0.0 percent) were abnormal, and in the asthma group, 0 people (0.0 percent) were normal, 42 people (100 percent) were abnormal.
- According to the FEV1.pre index, in the control group, 24 people (77.4%) were normal, 7 people (22. 6%) were abnormal, and in the asthma group, 0 people (0.0%) were normal, 42 people (100 0.0%) were abnormal.
- According to the FENO.pre index, in the control group, 31 people (100%) were normal, 0 people (0.0%) were abnormal, and in the asthma group, 2 people (4.8%) were normal, 40 people (95.2%) were abnormal.

Table 2- distribution of Asthma Indicators in the patient and control group

Tests		**Asthma**		**Controls**		**Indicators**
P value	**Ready test**	**Standard Deviation**	**Mean**	**Standard Deviation**	**Mean**	
P=0.0001	**t=24.23**	**2.37**	**11.55**	**1.40**	**22.29**	**ACT.Score.pre**
P=0.0001	**t=12.08**	**14.12**	**52.76**	**9.15**	**86.03**	**FEV1.pre**
P=0.0001	**t=9.58**	**24.52**	**44.64**	**4.12**	**7.71**	**FENO.pre**
P=0.0001	**t=-5.02**	**8.66**	**20.90**	**3.92**	**13.32**	**%Lym**
P=0.0001	**t=45.90**	**5.70**	**22.95**	**5.00**	**81.77**	**%mQ**
P=0.0001	**t=-21.12**	**9.88**	**36.57**	**1.69**	**3.74**	**%neu**

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P=0.0001	t=-16.67	6.69	18.24	.18	1.03	%Eo

Distribution of IL-5 gene expression:

The hypothesis of data normality (Shapiro-wilk test) was rejected (P<0.05). A significant difference (Mann-Whitney U test) is observed between the two groups in terms of gene expression intensity (=P Value=0.0001).T he expression of IL-5 gene has increased significantly in asthma.

Table 3- Distribution of IL-5 gene expression intensity in the patient and control group

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Group
U=-6.54 P-Value=0.0001	.411	1.134	1.972	.452	.303	Control
	2.447	4.332	8.669	1.036	.001	Asthma

Relationship between age and intensity of IL-5 gene expression

The hypothesis of data normality (Shapirowilk test) was rejected (P<0.05). There is no significant difference (Mann-Whitney test) between the two groups in terms of gene expression intensity (P Value=0.265).

Table 4- Relationship between age and intensity of IL-5 gene expression

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Age
U=-1.12 P-Value=0.265	2.704	3.250	8.669	.458	.000	Less than 50 years
	2.297	2.802	8.452	.452	.000	More than 50 years

Relationship between Gender and IL-15 gene expression

The hypothesis of normality of the data (Shapiro-wilk test) was rejected (P<0.05). No significant difference (Mann-Whitney U test) was observed between the two groups in terms of gene expression intensity (PValue=0.106).

Table 5- Relationship between Gender and IL-15 gene expression

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Gender
U=-0.998 P-Value=0.322	2.472	3.126	8.452	.452	.000	Man
	2.454	2.790	8.669	.458	.000	Woman

Correlation between asthma severity and IL-5 gene expression intensity

The hypothesis of normality of the data (Shapiro-wilk test) was rejected (P<0.05). A significant difference (Kruskal-Wallis test) was observed between the four groups in terms of gene expression intensity (P Value=0.0001).

Table 6- Correlation between asthma severity and IL-5 gene expression intensity

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Asthma severity
K=30.48	.507	1.756	2.509	1.147	.804	one

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P-Value=0.0001	**.668**	**2.211**	**3.412**	**1.036**	**.596**	**two**
	1.515	**6.328**	**8.669**	**2.898**	**.195**	**three**
	1.421	**6.432**	**8.452**	**4.354**	**.348**	**four**

The results of the Bonferroni test

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is 0.05. a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Investigating the effect of different variables on IL-5 Gene Expression

A linear model was used to investigate the effect of quantitative variables on IL-5 gene expression.

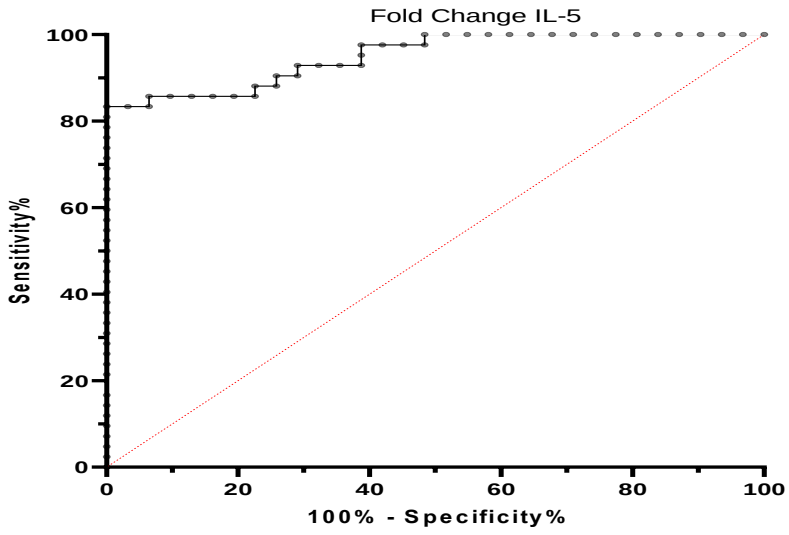
Area under the ROC curve	
Area	**0.9501**
Std. Error	**0.02253**
95% confidence interval	**0.9059 to 0.9942**
P value	**<0.0001**

Natural logarithmic transformation was used to normalize the response variable. The variables of age, body mass index, body surface index, asthma severity, duration of FENO.pre, FEV1.pre, ACT.Score.pre asthma, cough, gender, shortness of breath neu, mQ and Eo were entered into the model, which this model shows It shows that the severity of asthma has a significant relationship with gene expression (P<0.05).

Curve analysis (ROC) of Il-5 gene expression in asthma patients

The ROC curve of IL-5 for asthma compared to the control group shows that gene expression is significantly changed.

Figure-1



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Discussion

In most people with asthma, the immune response is guided by pro-inflammatory cytokines type 2 (interleukin) IL-5, IL-4, and IL-13, which is associated with hypersensitivity to environmental allergens and an increase in the number of eosinophils in the airways and blood (14). In 2011, innate lymphoid cells (ILC2s) were identified as a new type 2 cytokine-producing cell population. They were later found to promote allergen-related immune responses in the airway mucosa, where the alarming cytokine IL-33 is an important trigger. Understanding the molecular mechanisms that cause excessive activation of the immune system, Therefore, one of the important areas of research is asthma. Allergic asthma is the most common asthma phenotype in which chronic airway inflammation is dominated by Th2 (CD4+ T helper 2) cells that produce Th2 cytokines regulated by GATA-3, especially IL-5, IL-4, and IL-13 (12, 15). However, intrinsic asthma is characterized by increased IL-5 and IL-2 (15). The airway epithelium acts as the first line of defense against airborne substances. These cytokines activate innate lymphoid cells (ILC2) group 2, which produce large amounts of IL-13, IL-5, and a small amount of IL-4 without producing specific IgE. These findings may partly explain why patients with severe asthma lack an allergen-induced Th2 response but present with persistent eosinophilia inflammation (16, 17).

Conclusion:

The results of the present study show that IL-5 gene expression has been significantly changed in asthma. In terms of the intensity of IL-5 gene expression in the patient and control groups, a significant difference is observed between the two groups (P-Value=0.0001), also the experiment and analysis of the obtained ROC curve shows that a significant change in IL-5 in Asthma.

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