

Article

# Immunological and Molecular Evaluation of miRNA-146a in Asthma Patients

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**Abstract:** Asthma cause airway obstruction and are associated with chronic airway inflammation. Abnormal immune response to environmental stimuli has caused many cells of the innate and adaptive immune system to act. miRNAs play an important role in regulating key pathogenic mechanisms in allergic inflammation, including polarization of adaptive immune responses and activation of T cells. **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 miRNA-146a gene has been decrease significantly in asthma. Regarding the intensity of miRNA-146a 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 miR-146a gene expression has been significantly changed in asthma. In terms of the intensity of miR-146a 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 mir-146a in Asthma.

**Keywords:** miRNA-146a , Asthma, Chronic Obstructive Pulmonary Disease, COPD

## 1. 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 includes 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).

Different roles of microRNAs in epigenetic regulation of disease are as human asthma becomes important as a research field, a better understanding of these roles may identify targets for the development of new treatments for severe asthma (8). Each microRNA can contain several mRNAs. Target In this way, they play a tremendous role in regulating cellular activities (9). It has been found that specific miRNAs play an important role in regulating key pathogenic mechanisms in allergic inflammation, including polarization of adaptive immune responses and activation of T cells (e.g. miR-21, miR-146) from the family members miR-146 containing miR-146a and miR-146b are elevated in a mouse model of asthma. miR-146a and miR-146b are negative regulators of inflammatory gene expression in multiple cell types, fibroblasts, endothelial, and smooth muscle, including lymphocytes of asthma airway (10). Asthma and the expression of miR-146a have increased in is airway involved biopsies in patients with mild asthma. In addition, the expression level of miR-146a is increased in airway smooth muscle following stimulation by inflammatory agents (11).

## 2. Materials 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
- Data analysis in the following

### 2.1. 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.

### 2.2. 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.

### 2.3. 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.

### 2.4. Isolation of RNA for MicroRNA-146

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.

### 2.5. cDNA synthesis for microRNA-146 genes

cDNA synthesis steps were performed according to the DyNAmo-cDNA kit protocol. Real-time PCR for microRNA-146 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.

#### *2.6. 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).

#### *2.7. Ethical improvements*

In this study, we tried to follow the protocol of the code of ethics so that the information in the checklists remained completely protected. Before carrying out the plan, informed consent was obtained from the people included in the plan. In this study, no additional costs were imposed on the patients. This research is carried out and approved under the ethical code In the Faculty of Medicine of the Islamic Azad University .

#### *2.8. Descriptive analysis of data*

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

P value	Tests		Asthma		Controls		Indicator's
	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	

### 2.9. 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%) 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

P value	Tests		Asthma		Controls		Indicators
	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	
P=0.0001	t=-16.67	6.69	18.24	.18	1.03	%Eo	

### 2.10. Distribution of microRNA-146 gene expression

The hypothesis of normality of the data (Shapiro-Wilk test) was rejected ( $P < 0.05$ ). A significant difference (Mann-Whitney test) is observed between the two groups in terms of gene expression intensity ( $P$  Value=0.0001).

**Table 3.** Distribution of microRNA-146 gene expression

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Group
U=9.84	1.220	2.965	5.271	.112	.942	Control
P- Value=0.0001	.409	.721	1.841	.023	.066	Asthma

The hypothesis of the normality of the data (Shapiro-Wilk 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=0.815).

**Table 4.** Relationship between age and microRNA-146 gene expression

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Age
U=-0.238 P-Value=0.815	1.412	1.718	4.515	.115	.002	Less than 50 years
	1.410	1.647	5.271	.023	.000	More than 50 years

### 2.11. Relationship between Gender and expression of microRNA-146 gene expression

The hypothesis of normality of the data (Shapiro-wilk test) was rejected ( $P < 0.05$ ). No significant difference (Mann-Whitney test) was observed between the two groups in terms of gene expression intensity ( $P$  Value=0.435).

**Table 5.** Relationship between Gender and microRNA-146 gene expression

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Gender
U=-0.787	1.473	1.608	5.271	.023	.000	Man
P- Value=0.435	1.327	1.755	4.351	.102	.006	Woman

### 2.12. Correlation between asthma severity and microRNA-146 gene expression

The hypothesis of normality of the data (Shapiro-wilk test) was accepted ( $P < 0.05$ ). There is no significant difference (Fisher's test in the analysis of variance) between the four groups in terms of gene expression intensity ( $P$  Value=0.719).

**Table 6.** Correlation between asthma severity and microRNA-146 gene expression

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Asthma severity
F=0.449 P- Value=0.719	.529	.643	1.544	.023	.634	One
	.493	.825	1.841	.283	.125	Two
	.171	.663	.883	.281	.388	Three
	.403	.688	1.547	.102	.403	Four

### 2.13. Relation between shortness of breath and intensity of MicroRNA gene expression

The hypothesis of normality of the data (Shapiro-Wilk test) was rejected ( $P < 0.05$ ). No significant difference (Mann-Whitney test) was observed between the two groups in terms of gene expression intensity ( $P$  Value=0.137).

**Table 7.** Correlation of shortness of breath with intensity of 146 MicroRNA 1 gene expressions.

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	shortness of breath
U=-1.50 P-Value=0.137	.349	.656	1.547	.102	.043	Two
	.518	.904	1.841	.023	.971	Three

### 2.14. The relationship between the duration of asthma and the intensity of microRNA-146 gene expression:

The hypothesis of normality of the data (Shapiro-Wilk test) was accepted ( $P > 0.05$ ). Significant difference (Student's test): There is no significant difference between the two groups in terms of gene expression intensity ( $P$  Value=0.099).

**Table 8.** Correlation between the duration of asthma and the expression intensity of 146 MicroRNA gene expressions

Statistics Test P value	Standard Deviation	Mean	Maximum	Minimum	Normality	Duration of Asthma
t=-1.69 P-Value=0.099	.347	.612	1.544	.102	.303	Fewer than 10 years

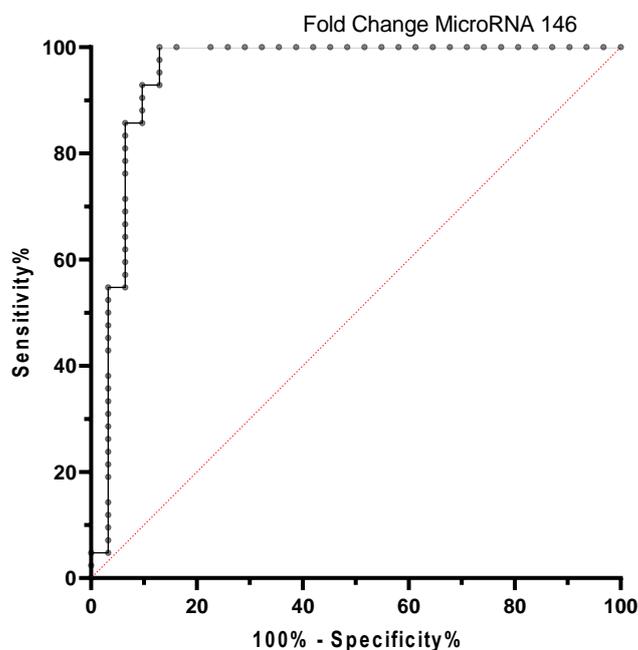
.442	.820	1.841	.023	.433	More than 10 years
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### 2.15. Investigating the effect of different variables on MicroRNA-146 Gene Expression

A linear model was used to investigate the effect of different quantitative variables on MicroRNA 146 gene expression. Natural logarithmic transformation was used to normalize the response variable. The variables of age, body mass index, body surface index, asthma severity, FENO.pre, FEV1.pre, ACT.Score.pre duration of asthma, cough, gender, shortness of breath, neu, mQ and Eo were entered into the model, which this model shows that there is no significant relationship with gene expression ( $P \leq 0.05$ ).

### 2.16. Curve analysis (ROC) of MicroRNA-146 gene expression in asthma patients

The ROC curve of MicroRNA-146 for asthma compared to the control group shows



Area under the ROC curve	
Area	0.9478
Std. Error	0.03360
95% confidence interval	0.8819 to 1.000
P value	<0.0001

## 3. Discussion

Asthma is one of the most common chronic diseases in industrialized countries (12, 13). The study of asthma and allergy genetics was dominated by genome-wide association studies for more than a decade (14). Recently, a lot of attention has been paid to investigating the regulatory functions of microRNA, currently, many different mechanisms involved in the pathogenesis of asthma are known (10) and play an important role in regulating many cellular processes. Undoubtedly, these regulatory molecules are involved in the pathogenesis of asthma, so they can be potential targets for treatment. They are short non-coding RNAs that modulate various biological processes through post-transcriptional regulation of gene expression. Specifically, miRNAs recognize and bind to multiple target mRNA-translated regions (3UTRs) via complement 6. - 8nt long seed regions in minas. When mRNA binds to miRNA, its translation is inhibited or mRNA degradation is initiated by miRNA-binding proteins. As such, miRNAs are also able to regulate

immune responses in various conditions. The miR-146 family consists of miR-146b and miR-146a. miR-146a/b that differ by only two nucleotides in their 3' region, which is less important for their interaction with mRNA targets. Thus, miR-146a/b has been reported to have a similar set of target genes (14). studies have been conducted in this direction, some of which are mentioned to confirm this study. Komar et al. also reported a study titled MicroRNA-146a and microRNA-146b expression and anti-inflammatory function in human airway smooth muscle. miR-146a and miR-146b are negative regulators of inflammation, gene expression in lung fibroblasts, epithelial cells, monocytes, and endothelial cells, the abundance of cyclooxygenase- (COX-2) 2 and IL-1 negatively by family miR-146 is regulated, suggesting that miR-146a and/or miRNA-146b may modulate inflammatory mediators, and the results suggest that miR-146 mimics may be attractive candidates for further preclinical studies as therapeutics to be anti-inflammatory for asthma (10). In a study aimed at investigating the diagnostic, functional, and therapeutic roles of microRNA in allergic diseases and the key role of miRNAs in the regulation of immune homeostatic architecture, Thomas Lu et al. Recent studies have identified miRNA profiles in several allergic inflammatory diseases, including asthma, eosinophilia esophagitis, allergic rhinitis, and atopic dermatitis. Specific miRNAs have been found to play an important role in regulating key pathogenic mechanisms in allergic inflammation, including polarization of adaptive immune responses and activation of T cells (e.g., miR-146) and modulation of IL-13-based epithelial responses. Our understanding of the expression and function of miRNAs in patients with allergic inflammation addresses their role as disease biomarkers (15).

#### 4. Conclusion

The results of the present study show that miR-146a gene expression has been significantly changed in asthma. In terms of the intensity of miR-146a 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 mir-146a in Asthma.

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