http://pmjournal.ir Original Article

Spring 2024, Volume 9, Issue 33 (47- 54)

Evaluation of Severity Persistent Asthma with Hemophilus Influenza Infection in Asthmatic Patients

Emal Zoweiar Alsheihani[1], Ali Neamati[2*], Mohammad Reza Khakzad[3]

[1]MSc of Cellular & Molecular Biology, Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran.
[2]Associate Professor of Physiology, Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad Iran.
[3]Assistant of Immunology, Innovative Medical Research Center, Department of Immunology, Mashhad Branch, Islamic Azad University, Mashhad, Iran.

Corresponding author: Ali Neamati, Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad IranEmail: neamati.ali@gmail.com.

DOI: 10.22034/pmj.2024.713768

Submitted: 2024-02-24
Accepted: 2024-05-20

Keywords:
Asthma
Haemophilus influenza
Real-time PCR

How to Cite this Article:
E.M, Alsheihani. A, Neamati. M.R, Khakzad. "Evaluation of Severity Persistent Asthma with Hemophilus Influenza Infection in Asthmatic Patients" Personalized Medicine Journal, Vol. 9, no. 33, pp. 47- 54.

Abstract:
Asthma is one of the most common non-communicable diseases characterized by reversible obstruction of airflow. It poses many problems for all age groups from infancy to old age. Various studies have shown that the occurrence of viral infections is associated with the severity of asthma symptoms so it can be prevented by controlling viral agents. In this study, the severity of the symptoms of persistent severe asthma with Haemophilus influenza infection was investigated. 31 patients with asthma with different degrees of disease were studied in this study. The results showed that in patients with asthma, the percentage of people with Haemophilus influenza was 71% and in 29% of other asthma cases, Haemophilus influenza virus was not observed. The relationship between asthma, cough and dyspnea with Haemophilus influenza infection showed that with increasing asthma symptoms, the severity of infection increases, and no significant association was observed between cough and dyspnea with Haemophilus influenza. Therefore, the results of this study clearly show that Haemophilus influenzae virus causes asthma symptoms to worsen in patients.

INTRODUCTION

The lung is the central organ of the respiratory system. On the other hand, in addition to receiving oxygen, the body must expel toxic gases from the body, this task is the responsibility of the lungs. Lungs are pink organs located in the chest. The right lung contains three lobes and the left lung has two lobes. Each lobe contains a curtain called the pleural membrane, which prevents friction between the lobes and makes them slide on top of each other. Lungs include lung tissue, air passages or bronchi and air sacs or alveoli. The inner wall of the trachea is covered with a slimy membrane with fine hairs or cilia that trap and remove dust particles. The trachea is divided into two trachea or tracheal tree and sacs and trachea. The respiratory system consists of two main parts, the upper respiratory system and the lower respiratory system. In addition to these organs, certain muscles of the chest play a role in breathing, the most important of which is the diaphragm, which is located under the lungs and separates the chest from the abdomen. Smaller muscles between the ribs are also involved in breathing (1-4). Respiratory disease, which is generally related to lung disease, includes a group of diseases that cause lung dysfunction by involving part or parts of the respiratory system. Lungs are the most important part of the respiratory system, which plays a role in the exchange of respiratory gases to supply oxygen to different tissues of the body and remove carbon dioxide. Every year, lung diseases affect many people in society, which reduces the level of performance of the person in daily activities. Respiratory diseases are the most common cause of referral to general practitioners in England. The degree of respiratory dysfunction in a lung disease depends on the type of disease and the extent of the damage. Lung diseases can be upper respiratory tract infections, pneumonia or chronic obstructive pulmonary disease. Chronic respiratory diseases are among the 10 life-threatening diseases in the world. According to the statistics of the World Health Organization, hundreds of millions of people in the world suffer from chronic respiratory diseases. It is expected to become the third cause of death in the world after cardiovascular diseases and cancers by 2030 (5-9).

MATERIALS AND METHODS

Collection of samples

In this study, 31 asthmatic patients and 31 controls were examined. Pulmonary tests such as FEV1, FENO, ACT were examined in the patients, and sputum samples were taken and sputum cytology was taken to determine the percentage of lymphocytes (Lym), the percentage of macrophages (MO), the percentage of neutrophils (Neu) and the percentage Eosinophil (EO) was investigated. Also, the severity of shortness of breath, severity of cough, stage of asthma and duration of asthma and clinical information including age, gender, height, weight and body mass index of patients were obtained from their files.

STATISTICAL ANALYSIS OF DATA

In the description of the data, appropriate statistical tables and indices such as the mean, etc. have been used, and in the data analysis, the normality of the data has been investigated using the Shapirovix test, which confirmed the normality of the method. Appropriate parametric tests such as Student's test and analysis of variance (Tukey's test in pairwise comparisons) were used, and the Mann-Whitney test was used for non-normal data. The chi-square test was used in the analysis of data with a nominal scale and in cases where more than 20% of the frequencies. Expected tables were less than 5 (Cochran), and Fisher's Exact Test was used. A linear model has been used for general analysis. The software used in this research is SPSS v.26 and the significance level of the tests is less than 5% (values less than 5% are marked with "*" in the results).

Haemophilus influenza virus detection method

A real-time one-step RT-PCR method manufactured by AllplexTM (Cat. No. RP9801X) was used to detect and measure the Haemophilus influenza virus. The reagents used are shown in Table 1.

The basis of the PCR method is shown in Table 2. RT-PCR Mastermix prepared according to Table 2. The contents of the solution prepared in Table 2 were vortexed for 5 minutes. One microliter of RT-PCR Mastermix was poured into the PCR tube and then 8 microliters of nucleic acid samples were added to it. By centrifuging the tube, the rest of the steps were performed according to the relevant instructions to detect Haemophilus influenzae.

RESULTS

Analysis of the parameters

Distribution of main variables

In this study, in the control group, 14 people (45.2 percent) were men and 17 people (54.8 percent) were women, and in the asthma group, there were 19 people (61.3 percent) men and 12 people (38.7 percent). There were women, and (chi-square test) no statistically significant difference was observed between the two groups (Likelihood Ratio=1.63, P=0.309).

As can be seen in Table 3, there was no statistically significant difference (Student's test) between the two groups for the parameters of age, weight, height, body mass index and body surface index (P>0.05). In this

Table 1. Reagents used in the RT-PCR method

Symbol	Contents	Volume	Description
PRIMER	5X PR1 MOM	500 µL	MuDT Oligo Mix (MOM): . Amplification and detection reagent
ENZYME	Real time One step Enzyme	200 µL	Enzyme mix for one step RT-PCR
BUFFER	5X Real time One step Buffer	500 µL	Buffer for one step RT-PCR -Buffer containing dNTPs
CONTROL +	RP1 PC	80 µL	Positive Control (PC): -Mixture of pathogen and IC clones
CONTROL IC	RP-V IC	1000 µL	Exogenous Internal Control (IC) for All plex Respiratory Panel 1,2 and 3
WATER	RNase-free Water	1000 µL	Ultrapure quality, PCR-grade Negative Control (NC): . Sterilized water as Negative Control

Table 2. Preparation of RT-PCR Mastermix

5X RP1 MOM	5 µL
RNase-free Water	5 µL
5X Real time One step Buffer	5 µL
Real time One step Enzyme	2 µL
Total volume of One step RT-PCR Mastermix	17 µL

Table 3. Demographic parameters

Variable	Test		Asthma		Control	
	Average	standard deviation	Average	standard deviation	Average	standard deviation
Age	P=0.079	t=-1.78	14.13	58.58	52.03	14.72
Weight	P=0.893	t=-0.135	10.67	73.19	72.84	10.03
Height	P=0.188	t=1.33	8.08	163.45	166.48	9.78
BMI	P=0.302	t=-1.04	4.00	27.49	26.44	3.99
Body surface area index	t=0.339	P=0.736	0.15	1.82	1.83	0.15

study, based on the body mass index in the control group, 10 people (32.2%) were normal, 14 people (45.2%) were overweight 7 people (22.6%) were obese, and 9 people were in asthma group. (29.0%) were normal, 12 (38.7%) were overweight and 10 (32.3%) were obese.

Distribution of asthma duration, asthma severity and symptoms

The mean and standard deviation (minimum and maximum) of the duration of asthma in the examined patients are 11.13 and 6.36 (3 and 25) years, respectively. Other information is shown in Figure 4.

Distribution of asthma indicators

As can be seen in Table 5, for all variables such as ACT.Score.pre, FEV1.pre, FENO.pre, Lym percentage, Mq percentage, Neu percentage and Eo percentage, there is a statistically significant difference (Student's test) between the two groups ($P<0.05$**). Other results in this study show that:

- According to the ACT.Score. pre-index, in the control group, 31 people (100.0 per cent) were normal, 0 people (0.0 per cent) were abnormal, and in the asthma group, 0 people (0.0 per cent) were normal, 31 (100.0%) were abnormal.
- According to the FEV1.pre-index, in the control group, 24 people (77.4 per cent) were normal, 7 people (62.2 per cent) were abnormal, and in the asthma group, 0 people (0.0 per cent) were normal, 31 people (100.0 per cent) were abnormal.
- According to the FENO.pre-index, in the control group, 31 people (100.0 per cent) were normal, 0 people (0.0 per cent) were abnormal, and in the asthma group, 2 people (6.4 per cent) were normal, 29 people (63.6 per cent) were abnormal.

Investigating the effect of different variables on Hemophilus influenza infection

A linear model has been used to investigate the effect of quantitative variables on Hemophilus influenza. At first, the variables of sex, age, body mass index, body surface index, asthma severity, asthma duration, and ACT.Score.pre, FEV1.pre, FENO.pre, mQ, neu and Eo were entered into the model (Table 6). Based on the obtained results, it was found that the severity of asthma has a significant relationship with Hemophilus influenza ($P<0.05$).

Relationship of different variables with Hemophilus influenza infection

This section will investigate the relationship of different parameters with Hemophilus influenza infection. Figures 1 to 4 examine the relationship between the severity of Hemophilus influenza infection and the parameters of asthma severity, ACT.Score, FEV.pre, FENO.pre, %neu and %EO.

DISCUSSION

Among the vast number of chronic diseases, asthma is one of the most common chronic disorders of the respiratory system, which has a significant prevalence and incidence. Asthma is an intermittent, reversible, and obstructive disease of the airways, which is characterized by the excessive response of the bronchi to various stimuli. These changes cause narrowing of the airways and shortness of breath in the patient, and it is one of the most common chronic diseases worldwide, with approximately 300 million people worldwide suffering from this disease. Of this amount, 10 to 12

Table 4. Distribution of severity and symptoms of asthma

Variable		Number	Per cent
Asthma severity	1	5	16.1%
	2	11	35.5%
	3	6	19.4%
	4	9	29.0%
Cough	1	4	12.9%
	2	7	22.6%
	3	17	54.8%
	4	3	9.7%
Shortness of breath	2	10	32.3%
	3	21	67.7%

Table 5. Asthma indices

Variable	Test		Asthma		Control	
	probability value	Test statistics	standard deviation	Average	standard deviation	Average
ACT.Score. pre	P=0.0001**	=24.69	2.12	11.03	1.40	22.29
FEV1.pre	P=0.0001**	=11.59	13.98	51.35	9.15	86.03
FENO.pre	P=0.0001**	=07.95	23.48	43.10	4.12	7.71
%Lym	P=0.0001**	=-4.69	8.54	21.26	3.92	13.32
%Mq	P=0.0001**	=45.94	5.55	22.45	5.00	81.77
%neu	P=0.0001**	=-25.82	9.92	36.45	1.69	3.74
%Eo	P=0.0001**	=-17.04	5.95	18.71	.18	1.03

percent are adults and it is expected that 100 million people will be added to the population of asthma patients in the world by 2025. In a report published in 2003, the prevalence of asthma in Iran in the entire population was estimated at 5%. This disease is classified into different types based on the severity of clinical symptoms, and all its forms are considered in this study. Asthma is a major problem in most parts of the world and its diagnosis and treatment is still a health problem. A large number of people suffering from this disease die every year. The death rate due to asthma is increasing in most countries. In Iran, according to the statistics reported by the Asthma and Allergy Clinic, 10% of Iranians have asthma. In the United States, one out of every 20 people has asthma, and 14 to 15 million people with asthma live in the United States. Asthma changes the family life and social activities of the sufferers and limits the physical activity of the patients, which leads to mental problems such as anxiety, depression and sadness in the patients. In this way, asthma affects various aspects of the patient's life (10-13). Bizintino and his colleagues investigated the relationship between rhinovirus C and the severity of asthma symptoms. In this study, 128 children with acute asthma in the age range of 2-16 years were investigated. The results of this study showed that children with chronic asthma and infected with rhinovirus C had more severe asthma attacks, so it can be concluded that rhinovirus C causes an increase in the severity of asthma symptoms(14).

Carroll and colleagues investigated the relationship between bronchitis and the risk of developing asthma in infants. In this study, 90,341 children were examined, 18% of whom had bronchitis. The results showed that children who were hospitalized with bronchiolitis during infancy, compared to children who did not have any symptoms of bronchiolitis, showed an increase in asthma in early childhood,

and this study confirms that diseases Pulmonary infections play an important role in the occurrence of asthma (15).Gerke and his colleagues investigated the relationship between seasonal influenza and the severity of asthma symptoms. The results of this study showed that based on time series regression models, there is a strong and significant relationship between the simultaneous activity of influenza and the incidence of asthma hospitalization. The results of this study confirmed that influenza activity is associated with an increase in the severity of asthma symptoms and these results show that improvement in the monitoring, analysis and prevention of influenza can reduce hospitalization in asthma patients (16).

CONCLUSION

Asthma is one of the common non-communicable diseases characterized by reversible airflow obstruction. It causes many problems for all age groups, from infancy to old age, and the people of Iran are not exempt from this. This disease can be controlled through simple education, and by using simple solutions, the suffering of these patients can be alleviated, finally, the country can be freed from spending huge costs in the field of acute asthma attacks and the absence of sufferers from work and social activities. Various studies have shown that the occurrence of viral infections is related to the severity of asthma symptoms, so its progress can be prevented by controlling viral factors. Therefore, in this study, the severity of persistent severe asthma symptoms with Haemophilus influenza infection was investigated. The results of this study showed that in patients with asthma, the percentage of people with

Table 6. Variables included in the model to investigate the severity of asthma with Haemophilus influenzae

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	2853.989[a]	12	237.832	1.831	.119
Intercept	36.873	1	36.873	.284	.601
Gender	9.757	1	9.757	.075	.787
Age	7.082	1	7.082	.055	.818
BMI	27.189	1	27.189	.209	.653
Body surface area index	.266	1	.266	.002	.964
ACT.Score.pre	12.361	1	12.361	.095	.761
FEV1.pre	12.492	1	12.492	.096	.760
FENO.pre	79.307	1	79.307	.611	.445
Lym	110.375	1	110.375	.850	.369
mQ	285.373	1	285.373	2.198	.156
Neu	219.391	1	219.391	1.689	.210
Eo	80.363	1	80.363	.619	.442
Asthma severity	1209.413	1	1209.413	9.313	.007
Error	2337.512	18	129.862		
Total	29670.535	31			
Corrected Total	5191.501	30			

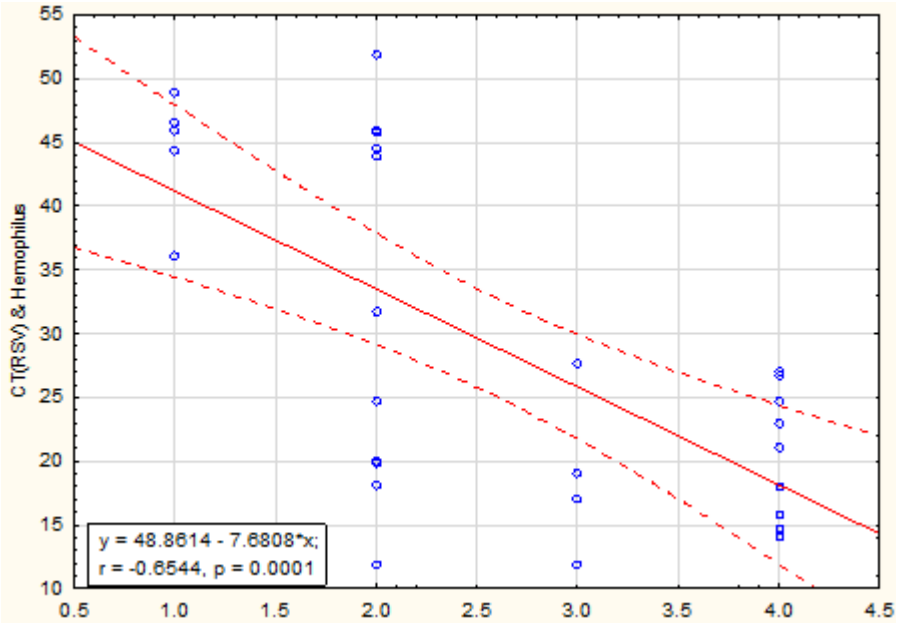

Fig 1. Correlation of asthma severity with Haemophilus influenza

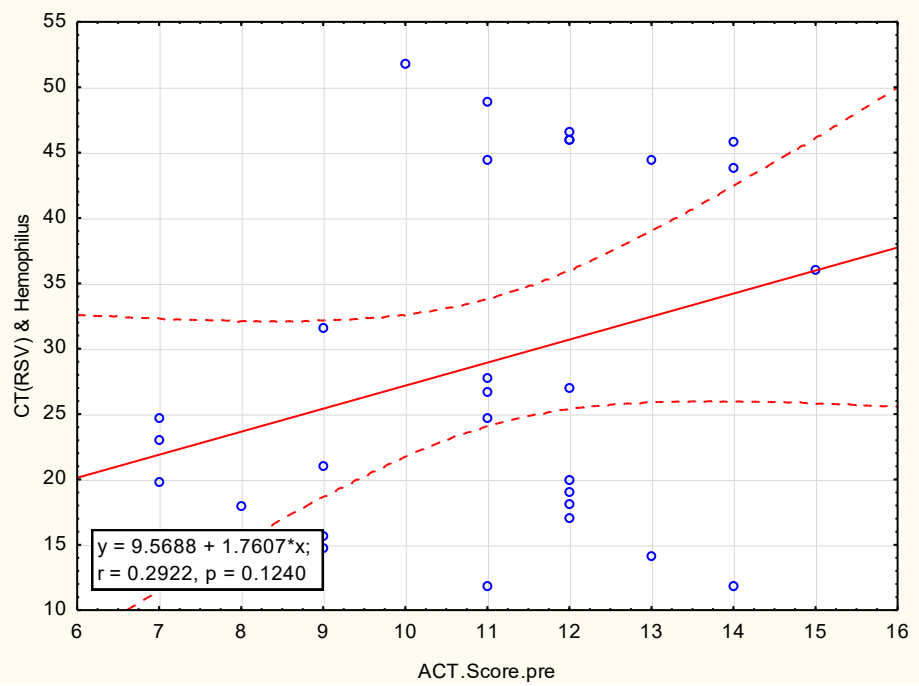

Fig 2. Correlation of ACT. Score pre with Haemophilus influenza

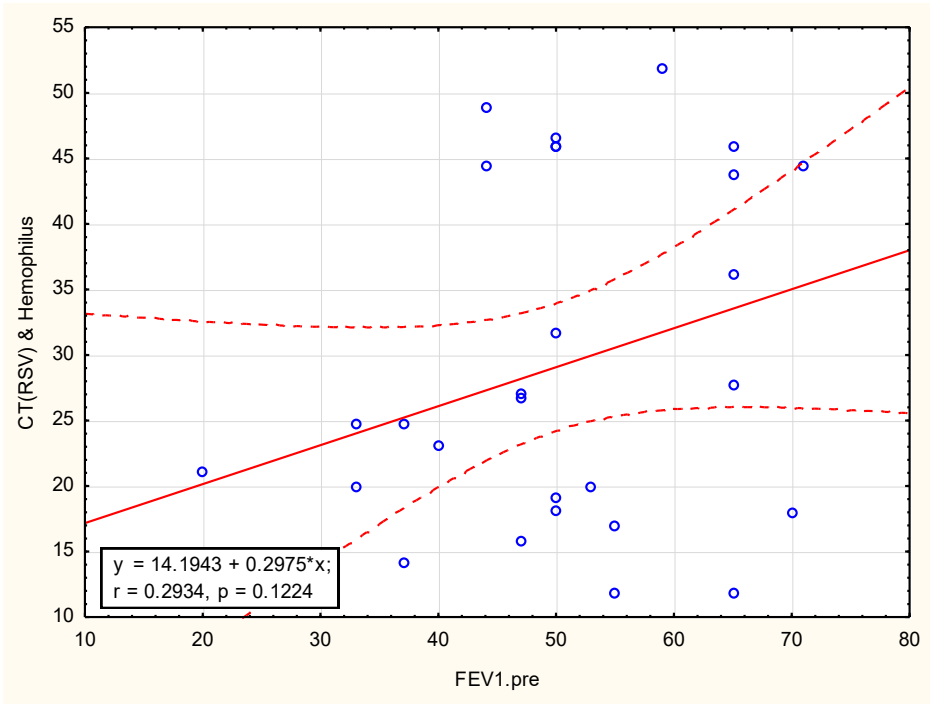

Fig3. FEV1.pre association with Haemophilus influenza

Haemophilus influenza is 71%, and Haemophilus influenzae virus was not observed in 29% of the remaining asthma cases. The relationship between the severity of asthma, cough and shortness of breath with Haemophilus influenza infection showed that with the increase in asthma symptoms, the severity of the infection increases, and no significant relationship between cough and shortness of breath with Haemophilus influenza was observed. Therefore, the results of this study clearly show that Haemophilus influenza virus causes worsening asthma symptoms in patients. Also, the relationship between asthma severity variables, ACT. Score, FEV.pre, FENO. pre, and %Eo with Haemophilus influenza infection were studied and the results showed that: There is a significant relationship between the severity of asthma symptoms and Haemophilus influenza infection (P =0.0001).

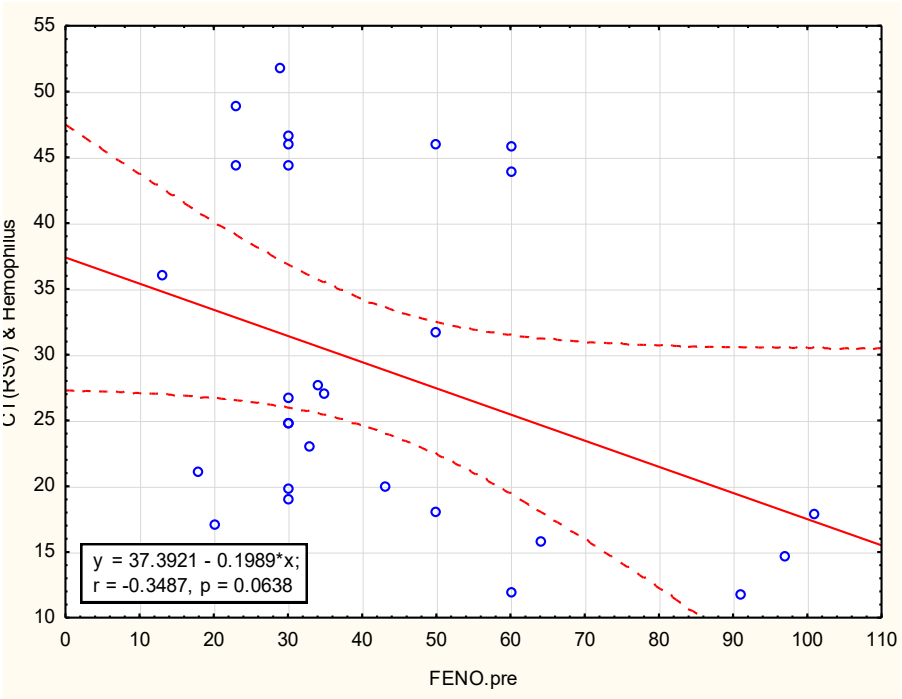

Fig4. FENO.pre association with Haemophilus influenza

Acknowledgements

The authors extend their deepest appreciation to the patients for their valuable contribution to this study.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

The authors of this article have not received any financial support from private or government sources.

REFERENCES

1.C. Drain, "Physiology of respiratory system related to anesthesia," in *CRNA: the clinical forum for nurse anesthetists*, 1996, pp. 163-180.

2.G. Shorten, N. Opie, P. Graziotti, I. Morris, and M. Khangure, "Assessment of upper airway anatomy in awake, sedated and anaesthetised patients using magnetic resonance imaging," *Anaesthesia and intensive care,* vol. 22, pp. 165-169, 1994.

3.S. Isono, "Obesity and obstructive sleep apnoea: mechanisms for increased collapsibility of the passive pharyngeal airway," *Respirology,* vol. 17, pp. 32-42, 2012.

4. D. J. Minnich and D. J. Mathisen, "Anatomy of the trachea, carina, and bronchi," *Thoracic surgery clinics,* vol. 17, pp. 571-585, 2007.

5.G. Raghu and K. K. Brown, "Interstitial lung disease: clinical evaluation and keys to an accurate diagnosis," *Clinics in chest medicine,* vol. 25, pp. 409-419, 2004.

6.V. Cottin, L. Wollin, A. Fischer, M. Quaresma, S. Stowasser, and S. Harari, "Fibrosing interstitial lung diseases: knowns and unknowns," *European Respiratory Review,* vol. 28, 2019.

7.A. Sciriha, S. Lungaro-Mifsud, P. Fsadni, J. Scerri, and S. Montefort, "Pulmonary Rehabilitation in patients with Interstitial Lung Disease: The effects of a 12-week programme," *Respiratory Medicine,* vol. 146, pp. 49-56, 2019.

8.D. B. Coultas, R. E. Zumwalt, W. C. Black, and R. E. Sobonya, "The epidemiology of interstitial lung diseases," *American journal of respiratory and critical care medicine,* vol. 150, pp. 967-972, 1994.

9.A. M. Fendrick, A. S. Monto, B. Nightengale, and M. Sarnes, "The economic burden of non–influenza-related viral respiratory tract infection in the United States," *Archives of internal medicine,* vol. 163, pp. 487-494, 2003.

10.C. S. Murray, G. Poletti, T. Kebadze, J. Morris, A. Woodcock, S. Johnston*, et al.*, "Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children," *Thorax,* vol. 61, pp. 376-382, 2006.

11.D. Belgrave, J. Henderson, A. Simpson, I. Buchan, C. Bishop, and A. Custovic, "Disaggregating asthma: big investigation versus big data," *Journal of Allergy and Clinical Immunology,* vol. 139, pp. 400-407, 2017.

12.E. M. Mäkikyrö, M. S. Jaakkola, and J. J. Jaakkola, "Subtypes of asthma based on asthma control and severity: a latent class analysis," *Respiratory research,* vol. 18, p. 24, 2017.

13.K. Larsson, B. Ställberg, K. Lisspers, G. Telg, G.

Johansson, M. Thuresson*, et al.*, "Prevalence and management of severe asthma in primary care: an observational cohort study in Sweden (PACEHR)," *Respiratory research,* vol. 19, p. 12, 2018.

14.J. Bizzintino, W. Lee, I. Laing, F. Vang, T. Pappas, G. Zhang*, et al.*, "Association between human rhinovirus C and severity of acute asthma in children," *European Respiratory Journal,* vol. 37, pp. 1037-1042, 2011.

15.K. N. Carroll, P. Wu, T. Gebretsadik, M. R. Griffin, W. D. Dupont, E. F. Mitchel*, et al.*==Foster, J. E. Cavanaugh, and P. M. Polgreen, "Association of hospitalizations for asthma with seasonal and pandemic influenza,» *Respirology,* vol. 19, pp. 116-121, 2014.