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Exploring the Correlation between the Cycle Threshold value of Syncytial Virus and Paraclinical Characteristics of Patients with Severe Asthma

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Abstract

Background and objectives

Asthma is an important chronic disease of the respiratory tract that can present with different severities. Several factors play a role in its exacerbation; yet, the role of human respiratory syncytial virus (HRSV) on persistent severe asthma is not fully understood. Therefore, this study aimed to investigate the association of HRSV threshold cycles (Ct) with persistent severe asthma.

Materials and methods

In this study, 100 severe asthma were enrolled, and 45 adults infected with HRSV participated; their demographics (age, sex, body mass index), asthma control status (measured by asthma control questionnaire and asthma exacerbation severity classification), lung tests (spirometric breathing test and exhaled nitric oxide), were recorded. A sputum sample was collected from all participants; after extracting the RNA, real-time PCR was performed and the association of Ct values with the study variables was studied.

Results

The analysis revealed significant correlations between HRSV and clinical parameters such as BMI, FEV1, and exacerbation severity. Specifically, there was a reverse correlation between HRSV and BMI (r=0.492, p=0.03) as well as exacerbation severity (r=-0.481, p=0.02). Additionally, a positive correlation was found between HRSV and PEF (r=0.633, p=0.04), and a notable correlation with FEV1 (r=-0.502, p=0.02).

Conclusion

The study highlights that Human Respiratory Syncytial Virus (HRSV) significantly impacts respiratory health and may worsen asthma in affected patients. Therefore, effective preventive measures, such as vaccination or early treatment, are essential for better asthma management.

Keywords: Respiratory Syncytial Virus, Human, Asthma, Signs and Symptoms, Respiratory

Introduction

Asthma is one of the most common diseases in the world with an incidence of 43.12 million cases in 2017 and a prevalence of 3.57% worldwide with an increasing trend [1]. Novel medicine has reduced the mortality rate; yet, it is responsible for 0.5 million deaths annually. The mortality rate increases with aging, peaking after 80 years, and is higher in higher in women [1]. Asthma results in a substantial morbidity and disease burden, as it requires frequent visits to the emergency department and decreased social productivity (missing school or work days) [2].

Several risk factors have been identified for the prevention of asthma, including environmental factors, like tobacco smoke exposure, air pollution, and occupational exposure, dietary factors, like obesity, and fruit and vegetable intake, and maternal factors, such as feto-maternal health, breastfeeding, and childhood vaccination [3]. Furthermore, when the disease occurs, some factors make the patients' condition worse, identified as risk factors for exacerbation. The most important of these factors are pulmonary comorbidities, which include upper respiratory tract diseases, like obstructive sleep apnea, allergic/nonallergic rhinitis, chronic rhinosinusitis, and nasal polyposis; and middle or lower respiratory tract diseases, such as chronic obstructive pulmonary disease, allergic and bronchopulmonary aspergillosis. Extrapulmonary comorbidities, including anxiety, depression, gastro-esophageal reflux disease, obesity, cardiovascular, and metabolic diseases are also important factors of severe asthma [4].

Different types of lower tract respiratory infections are associated with higher incidence or severity of asthma [5]; the significance of this issue has resulted in the suggestion of the term "infection-related asthma" [6]. Human respiratory syncytial virus (HRSV) is the leading cause of infectious bronchiolitis [7]; infection with this virus in early life (infancy) is suggested to be a predictor of asthma in later non-atopic asthma, presenting in childhood (school-age) [8] and/or adulthood [9, 10]. Because effective treatment of HRSV may be able to prevent the incidence of asthma or reduce its severity, it is important to study this association. However, review studies have referred to insufficient evidence in this regard, due to the methodological issues of the currently available literature [11]. Therefore, it is important to provide more evidence in this regard. Accordingly, the present study aimed to investigate the effect of HRSV and the viral load on the asthma status of patients with persistent severe asthma.

Materials and Methods

The present study involved 100 severe asthmatic patients with exacerbations accompanied by viral cold symptoms, including nasal obstruction, nasal discharge, scratchy throat, body aches, and, in some cases, fever. These patients were referred to the subspecialty lung clinic in Mashhad, Iran, between October 1 and November 15, 2023, for evaluation of the Human Respiratory Syncytial Virus (HRSV). All participants were over 18 years old and had confirmed severe asthma, having undergone lung HRCT either at the time of the survey or within the month prior. Participants were excluded from the study if they had abnormal lung HRCT due to (infection, emphysema, bronchiectasis, cancer, or other lung diseases), any underlying diseases (diabetes, heart failure, kidney or liver diseases), elevated total IgE, positive IgG aspergillosis, bacterial pharyngitis, were current or former smokers, had not taken an influenza vaccine shot in September, or not taken 3 doses of Corona vaccine shots. Only patients with positive HRSV tests were included in the study.

The diagnosis of asthma was confirmed based on clinical symptoms, the results of physical examination (wheezing on auscultation), and the results of the spirometry test (forced expiratory volume in one second [FEV1] < 60% or the ratio of FEV1 to forced vital capacity [FVC] < 75%). They were treated with Seroflo (manufactured by Cipla Company, Mumbai, India), an inhaled corticosteroid (ICS) and long-acting beta-agonist (LABA) combination containing fluticasone (250µg) and salmeterol (50µg). If the initial treatment was ineffective, a high dose of ICS in Seroflo (500µg) was administered. If the symptoms persisted, a second controller medication such as montelukast (10mg, manufactured by Pursina Pharmaceutical Company, Tehran, Iran) or tiotropium bromide (Spiriva trademark, manufactured by Boehringer Ingelheim Co, Germany) might have been added [12]. During the pretrial stage, individuals suspected of having viral cold symptoms had two swab samples collected from their nasopharynx and oropharynx for real-time polymerase chain reaction (rt-PCR).

As we found no similar study, we selected 45 patients as the pilot sample size. The eligible patients were enrolled using a convenient and sequential sampling method, after they were informed about the study objectives and signed the written informed consent.

The patients' demographics (age, sex, body mass index [BMI]), asthma exacerbation severity based on (dyspnea/body position, speech, level of consciousness, respiratory rate, accessory muscles in use, heart rate, PEF % predicted, spO2 in air) [13], lung tests (spirometric breathing test and exhaled nitric oxide) were recorded in the study's checklist. Asthma Control Questionnaire (5-item version; ACQ-5) was used for the evaluation of clinical symptoms, a self-report questionnaire, which evaluated the one-week recall of five characteristics (daily activities, shortness of breath, frequency of symptoms [including cough, chest heaviness, wheezing and pain], frequency of need for adjuvant drugs, and asthma control); the patient is asked to mark the response of each question based on a five-point Likert scale (1 indicating uncontrolled and severe cases to 5 indicating complete control). The total scores of each dimension are calculated, which falls within a range of 5-25; higher scores indicate better control, and scores ≤19 indicate good asthma control. This tool was designed to modify the original 7-item questionnaire as a valid tool for evaluating asthma control [14].

For measurement of the fractional exhaled nitric oxide (FeNO) that evaluates the level of airway inflammation, the NObreath device from Badfon Company (UK) was used (accuracy of ±5 ppb) FeNO levels of < 25 of parts per billion (ppb) is considered normal, 25 ppb - 50 ppb as intermediate, and > 50 ppb high [15]. Lung function was evaluated using a super-spirometry micromedical type spirometer (MicroMedical W3550, UK) to measure FEV_1 and PEF values.

To evaluate HRSV (Human Respiratory Syncytial Virus) infection, sputum samples were collected from all participants using a sampler. The volume of each sample was measured with a calibrated cup. The samples were then homogenized by shaking for 15 minutes. To remove cellular and mucus contaminants, the samples were filtered through a 48-µm nylon gauze filter and subsequently centrifuged at 1400 rpm at 4°C for 10 minutes. The resulting supernatant was frozen at -24°C.

RNA was extracted using the QIAamp Viral RNA Mini Kit (Cat. no. 52904, Qiagen GmbH, Hilden, Germany). For real-time PCR, the GA SARS Flu & RSV OneStep RT-PCR Kit was employed. This kit is designed for the simultaneous detection of SARS-CoV-2, influenza A, influenza B, and RSV (Respiratory Syncytial Virus) by specifically identifying target gene sequences. It contains a mixture of primers and probes consisting of six pairs, which target the conserved regions of the N2, N1, and ORF10 genes in SARS-CoV-2; the M2 gene regions in influenza A; the M1 gene regions in influenza B; and the L gene regions in RSV. These targets are amplified using the CFX RT-PCR detection system (Corbett, USA). Notably, samples were collected before the administration of any antiviral treatment.

Threshold cycle (Ct) values were reported, with values less than 35 considered positive. Values below 20 indicate a high viral load, reflecting high gene expression.

Statistical analysis

After gathering all the necessary information, we input the data into the statistical software SPSS version 24 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp. Released 2013). We used appropriate statistical tables and indicators such as mean and standard deviation (SD) to describe the data. To ensure accuracy, we first assessed the normality of the data using the Shapiro-Wilk test. We also

utilized a linear, logistic, and polynomial regression model to review the association of variables and calculated the beta coefficient with a 95% confidence interval (CI). The significance level of the tests was set at less than 0.05 and all statistical tests were two-sided.

Results

Data from 100 asthmatic patients were analyzed; 45 of the patients were infected with HRSV, 8 with influenza A/B/, 17 with SARS-Cov2 and 30 with other viruses may include (adenovirus, *Chlamydia pneumonia*, endemic coronavirus, rhinovirus, human metapneumovirus, *Mycoplasma pneumonia*, and human parainfluenza virus) (Table.1). the mean age of the participants was 58.58±14.13 years and about half were women (54.8%). Table 2 provides a complete description of the participants' demographics and baseline characteristics (Table 2).

Table 1: Classified viruses using the RT-PCR kit

Viruses	N=100
HRSV	45
Influenza A/B	8
SARS-Cov2	17
other	30

HRSV: Human Respiratory Syncytial Virus, SARS-Cov2: Severe acute respiratory syndrome coronavirus 2

Table 2: Demographic characteristics, PCR, and respiratory results of the participants

	Variable	Categories		Value
Baseline characteristics	Gender, N(%)	Male		20(44.4)
		Female		25(66.6)
	Age (years), mean±SD			58.58±14.13
	BMI (kg/m^2) mean±SD			32.49±4.18
PCR results	HRSV CT values	X<20	N (%)	29(64.4%)
			mean±SD	17.58±4.66
		20<X<35,	N (%)	16(36.6%)
			mean±SD	28.48±4.39
Respiratory results	ACT score, mean±SD			11.03±2.12
	FEV$_1$, mean±SD			51.35±13.98
	PEF, mean±SD			48.22±14.27
	FeNO	X<25	N (%)	43.10±23.48
			mean±SD	
		25<X<50	N (%)	7 (15.5%)
			mean±SD	
		X>50	N (%)	29 (84.5%)
			mean±SD	
	Asthma exacerbation severity	Mild/Moderate		17(37.7%)
		Severe		25(55.5%)
		Life-threatening		3(0.06%)

BMI: body mass index, HRSV CT: Cycle Threshold value of Human Respiratory Syncytial Virus (HRSV), ACT: Asthma Control Questionnaire FEV1: Forced expiratory volume in 1 second, PEF: Peak expiratory flow, FeNO: Fractional exhaled nitric oxide

The mean ± standard deviation (SD) of body mass index (BMI) was 32.49 ± 4.18. The HRSV (human respiratory syncytial virus) cycle threshold (CT) results showed that 64.4% of patients had high loads of RSV, while 36.6% had CT values between 20 and 35, indicating positive results.

Spirometry results demonstrated that all patients exhibited abnormal forced expiratory volume in one second (FEV1), with a mean of 51.35 ± 13.98. Peak expiratory flow (PEF) was also abnormal in all patients, with a mean of 48.22 ± 14.27. Additionally, the Asthma Control Test (ACT) score was abnormal in all patients, with a mean of 11.03 ± 2.12. The frequency of abnormal fractional exhaled nitric oxide (FeNO) levels above 50 and the mean values are presented in Table 2.

The analysis of the relationship between HRSV and various clinical parameters, including BMI, FEV1, and the severity of exacerbations, revealed noteworthy correlations. Specifically, a reverse correlation was observed between HRSV and BMI, indicated by a correlation coefficient of r=0.492 with a significance level of p=0.03. Similarly, the exacerbation severity also displayed a reverse correlation with HRSV, yielding a coefficient of r=-0.481 and a significance level of p=0.02.

In addition to the observed inverse relationships, a positive correlation was identified between HRSV, PEF, and FEV1. The correlation coefficients were quantified as r = 0.633 with a p-value of 0.04 for HRSV and PEF, and r = -0.502 with a p-value of 0.02 for HRSV and FEV1. These results suggest a significant association. For more detailed results, please refer to Table 3.

Table 3: The relationship between the cycle threshold of HRSV and asthma characteristics

variable	p-value	coefficient
Gender (Male/Female)	0.37	0.267
Age(years)	0.71	0.185
BMI (kg/m^2)	0.03	-0.492
ACT (score)	0.06	-0.763
FEV1%	0.02	0.502
PEF%	0.04	0.633
FeNO (ppb)	0.84	-0.284
Exacerbation severity	0.02	-0.481

Discussion

Measurement of Ct values (by rtPCR) in the present study showed that adult patients with higher HRSV viral load have higher exacerbation severity and worse ACT scores. This finding shows the important association between HRSV infection and asthma. Evidence has suggested that infecting the epithelial layer of the airways with HRSV induces an innate and adaptive immune response that results in the exacerbation of asthma [16, 17]. An animal study (on mice) showed that RSV infection increases the mRNA expression of interleukins (IL), including IL-17A and the transcription factor of box P2, and thus increase the T-helper17 cell levels; and decrease T cells in the lymph nodes that can result in compromising asthma tolerance [18]. These may be the appropriate cellular mechanisms for justification of the association of Ct with asthma severity, as suggested by the results of the present study. Clinical evidence has suggested RSV-induced resistance to steroids that can, then again, result in exacerbation of asthma with a different mechanism [19].

In the present study, we documented the association between severe asthma and high HRSV viral load; however, we did not evaluate the causative relationship between them to clarify which one has caused the other. Besides the mechanisms presented for the effect of HRSV on the exacerbation of asthma, some others have suggested that asthma also impairs the appropriate antiviral response. Evidence suggests that the function of eosinophils that capture viruses is impaired in asthma, considering the hyperinflammatory responses, which can result in a more severe HRSV infection in patients with asthma [20]. Furthermore, evidence has documented the impaired response of the goblet and multiclitaed cells in patients with asthma that deteriorates the antiviral response of epithelial cells to HRSV infection [16, 17]. Therefore, the association between high viral load and severe asthma in the present study may be the result of the predisposition of patients with severe asthma to severe HRSV infection, due to the impaired antiviral response in these patients.

Several studies have referred to the role of HRSV infection in infancy in the presentation of asthma later in life [8-10]. These studies confirm the negative effect of HRSV on the lungs which predisposes the individual to developing asthma. Nevertheless, we have investigated the concomitant effect of the virus and its load on asthma severity but has not been investigated profoundly. Another study in China evaluated 88 children with asthma exacerbation and 43 children with stable asthma; they found HRSV in 25.2% of patients (16.1% type A and 9.1% type B) [21]. Although this study emphasizes the concomitancy of asthma and human rhinovirus infections (including HRSV), which is consistent with the results of the present study, the positivity rate of HRSV type B in their study was much lower than that in the present study. This could be because we have selected patients with refractive asthma, while they have also included patients with stable asthma. Furthermore, their study population was children, while we evaluated adult patients. In another study, the sputum sample of patients admitted with respiratory diseases was evaluated, 23% of whom had asthma exacerbation. This study revealed RSV A and B in sputum and nasal samples of patients [22]; although this study referred to the significance of RSV as an important respiratory virus, it did not specifically define the value of RSV in patients with asthma to be comparable with the results of the present study. Investigating the sputum sample of 103 asthmatic patients with 30 control subjects showed rhinovirus in 9.4% of mild and 16% of moderate asthma and 6.7% of the controls; while none of the viruses separated were RSV [23]. This finding could be because they did not include patients with severe asthma.

The present study showed a high viral load in most of the patients. Another study on HRSV showed that patients with a higher viral RNA concentration had a higher risk of complications and respiratory insufficiency [24], which will in turn deteriorate the condition of patients with asthma. Therefore, it is necessary to pay greater attention to the concomitant infection of HRSV in these patients. Considering the significant effect of asthma severity on patients' morbidity and mortality, several studies have focused on factors that predispose patients to asthma exacerbation. Among several factors investigated, infections are one of high significance, as they are treatable and their treatment can reduce the severity of asthma and the following complications. In line with the strong association between HRSV and asthma, efforts have been also made to reduce the epithelial damage, caused by HRSV to reduce its negative impact on asthma [25]. This may be the key to reducing the rate of asthma exacerbation in the future. However, more studies are required in this regard to determine the exact pathophysiology of the interaction of the virus with an allergic host and the development or exacerbation of asthma by HRSV infection [26].

This study shows a high proportion of HRSV (Human Respiratory Syncytial Virus) infections in asthmatic patients compared to other viruses. Given the association of HRSV with more severe exacerbations, it would be advisable to vaccinate patients with severe asthma against this virus.

The main strength of the present study was the novelty of investigating Ct values of HRSV in patients with asthma and evaluating its effect on asthma control and severity. Evaluating both objective and subjective variables for asthma severity was another strength of the present study. However, this study was not exempt from limitations; the first limitation was related to the small sample size of the study which limited studying the associations in subgroups (due to the small number). However, this sample size was appropriate was appropriate for the pilot study on this subject. Another limitation of the present study was the lack of follow-up to determine the effect of HRSV treatment on the course of asthma disease. Furthermore, the cross-sectional nature of the study limited suggestion of causative relationships between the variables and the outcome.

Conclusion

The current study indicates that Human Respiratory Syncytial Virus (HRSV) is a significant respiratory infection that can occur alongside asthma and may contribute to the risk of severe uncontrolled asthma in patients. Therefore, it is crucial to pay closer attention to this infection, particularly in individuals with asthma. Implementing effective preventive measures, such as vaccination or early treatment, may be essential for reducing the severity of asthma and improving the management of the condition.

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Conflict of Interest
The authors declare no conflicts of interest.

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Data Availability
All necessary data will be readily available in an Excel file upon request. For any research reviews or contributions, please do not hesitate to contact Mahsa Manafi Varkiani at the following email address: mahsafall_2005@yahoo.com.

AI Assistance Disclosure
Not applicable.

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