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Klinik Araştırma

Viral serology of patients admitted with asthma exacerbation

Astım atağı ile başvuran hastalarda viral seroloji

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ABSTRACT

Objective: One of the factors which triggers asthma exacerbation is viral infections. Rhinovirus and Respiratory Syncytial Virus are frequent isolates. In this study active bacterial agents in acute asthma exacerbations and detection of the dominant viruses in our region were investigated.
Methods: This study was performed with 35 randomly selected pediatric patients. Twenty-one of them were admitted with acute exacerbation of asthma, and followed up in our allergy outpatient clinic between October 2011-February 2012 with diagnosis of asthma. As the control group 14 non-asthmatic patients who suffered from respiratory tract infections were included. Patients' nasopharyngeal swab specimens were studied for the viral serologic panels with the method of multiplex PCR.
Results: General characteristic features of the groups were similar. In asthmatic group 12 patients (57%), in the control group 3 patients (21%) had virus positive results. The most commonly encountered virus in the asthmatic group were Respiratory Syncytial Virus (n:5; 41.6%) and Human Rhinovirus (n:4; 33.3%), in the control group Adenovirus (n:1; 33,3%), Influenza A Virus (n:1; 33.3%), Influenza B Virus (n:1; 33.3%) were detected.
Conclusion: Viral infections are the leading factors of asthma exacerbations. To determine the agent may be important for the prophylaxis and new therapies for the attacks. Viruses were isolated from a small number of patients in our study but studies with the large number of populations with different asthma phenotypes are needed to develop effective treatment strategies against asthma attacks which are triggered with specific viral pathogens seen regionally.

Key words: Children, asthma, asthma exacerbation, viral infections, multiplex PCR analysis

ÖZET

Amaç: Astım atağını tetikleyen faktörlerden birisi de viral enfeksiyonlardır. En sık etken olarak Respiratuar Sinsityal Virus ve Rhinovirus izole edilmektedir. Bu çalışmada akut astım atağındaki aktif ajanlar ve bölgemizdeki dominant virüsler araştırılmıştır.
Yöntemler: Çalışmaya randomize olarak 35 pediatrik hasta alınmıştır. Çocuk Alerji Polikliniği'ne de astım tanısıyla takipli hastalardan Ekim 2011-Şubat 2012 tarihleri arası akut astım atağı kliniği ile başvuran 21 hasta, kontrol grubu olarak astımı olmayan solunum yolu enfeksiyonu şikayetleri ile genel çocuk polikliniklerine başvuran 14 hasta alınmıştır. Tüm hastaların nasofarengeal sürüntü örneklerinde multipleks PCR yöntemi ile viral seroloji paneli çalışılmıştır.
Bulgular: Grupların genel karakteristik özellikleri benzerdi. Astım atağı ile gelen hastaların 12'sinde (%57), kontrol grubunda 3 olguda (%21) virüs izole edildi. En sık çok izole edilen virüs astmatik grupta Respiratuar Sinsisyal Virus (n:5; %41.6), Human Rhinovirus (n:4; %33.3) iken kontrol grupta, Adenovirus (n:1; %33), Influenza A Virus (n:1; %33), Influenza B Virus (n:1; %33) olarak dağılım göstermekteydi.
Sonuç: Viral enfeksiyonlar astım ataklarının önde gelen nedenleridir. Etkeni saptamak ataklardan korunma ve yeni tedavilerin geliştirilmesi için önemli olabilir. Çalışmamızda küçük sayıda hastada virüs izole edilmiştir fakat bölgesel olarak görülen farklı viral patojenlerin tetiklediği astım ataklarına karşı etkin tedavi stratejileri geliştirmek için, farklı astım fenotipleri ile daha geniş populasyonlu çalışmalarına ihtiyaç vardır.

Anahtar kelimeler: Çocuk, astım, astım atağı, viral enfeksiyonlar, multipleks PCR analizi

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INTRODUCTION

Bronchial asthma is a chronic disease of airways which progresses with eosinophilic and epithelial desquamation. It is the most common chronic respiratory disorder in childhood.

Environmental factors are thought to be more effective than genetic factors. Atopy and allergy are thought to be risk factors for the development of asthma. Immunological sensitization plays a role in the development of asthma due to exposure to allergens at an early age. As a result of genetic predisposition, atopy, exposure to allergens, environmental factors, chronic inflammation form and bronchial hyperresponsiveness develop. As a result of inflammatory process wheezing, shortness of breath, episodes of cough in the night and morning develop. Asthma exacerbations are episodes of acute bronchospasm attacks during the course of the disease [(1)]. The most common reasons of the exacerbations are the environmental factors of viral which viral infections are most commonly seen [(2)].

Considering the asthma attacks can increase in frequency, and severity with viral infections, which can affect the patients, it is important to determine the involvement of these infections for the prevention and management of asthma attacks.

The Black Sea region of Turkey is at a higher risk for allergies because of its geographic situation, A study has not been made about this subject in this region of our country yet. So in this study we aimed to find out which viral factor is dominant in asthma exacerbations in our location.

MATERIAL AND METHODS

Study population

Thirty five patients were randomly selected for the study, 21 of whom were admitted with acute asthma attack to the Children's Allergy Outpatient Clinic of Samsun Maternity and Children Hospital between October 2011-February 2012. Fourteen patients were selected as the control group who had clinical symptoms of respiratory infections admitted to the General Pediatric Outpatient Clinics of Samsun Maternity and Children Hospital during the same period.

Subjects

Age, gender, biochemical and C-reactive protein (CRP) test results were recorded, and nasopharyngeal swab samples were obtained from all of the patients at the time of the admission to the hospital.

Asthma group was treated at the allergy clinic, and the control group was treated at the general oupatient clinics. They were sent home after stabilization of the symptoms. Asthma group was followed up for 4 months for recurrent attacks.

Detection of respiratory viruses

Nasopharyngeal swab samples were taken from the patients and studied at Institute of Refik Saydam Hygiene Center Laboratory. Viral DNA/RNAs were isolated automatically using EZ1 XL (Qiegen) with the virus RNA/DNA isolation kits (Qiagen, EZ1 Virus Mini Kit v2.0).

Procedure of mixture was made with the isolates by automatic Qiagility pipetting device (Qiagen) with Seeplex RV15 ACE Detection PCR kits (Seegene). Then all of the PCR mixtures were amplificated at 2720 ABI Thermal cycler device (Applied Biosystem) by the method of poymerase chain reaction (PCR) analysis. Samples were examined at Invitrogen E-Gel Agarose System after electrophoresis procedure was made at 2% agarose gels.

As the viral panel, Respiratory Syncytial Virus A (RSV A), Respiratory Syncytial Virus B (RSV B), Influenza A, Influenza B, Adenovirus A/B/C/D, Parainfluenza Virus Type 1.2.3.4, Coronavirus 229E/NL63, Coronavirus OC43, Rhinovirus A/B/C, Bocavirus Type 1.2.3.4, Metapneumovirus, Ebstein-Barr Virus, Enterovirus A/B/C were studied from the nasopharengeal swabs.

Statistical Analysis

All statistical analyses were performed using SPSS Windows version 16.0 (SPSS Inc., Chicago, IL, USA). The chi square test was used to compare percentages and p values of less than 0.05 were considered significant.

RESULTS

Age range of the patients admitted to the pediatric allergy outpatient clinic with acute asthma exacerbations was 10-160 months, and mean age was 64.7 months. In the control group age range was 12-144 months and the mean age was 85.7 months (p=0.167). The male/female ratio was 11/10 (53%, 47%) in the asthmatic, and 10 /4 (%71, %21) in the control groups (p=0,26).

Mean leukocyte count (WBC) was 10072/mcl in asthmatic, and 9475/mcl in the control group (p=0.682). Mean CRP level was 0.86 mg/dl in the asthmatic, 1 mg/dl in the control (p=0.062) groups. Mean eosinophil count was 286/mcl, in the asthmatic and 264/mcl in the control (p<0.01) group (Table 1).

Viruses were isolated from 12 patients (%57) in the asthmatic, and 3 (%21) in the control group (p<0.01). Virus panel results in the asthmatic group were Respiratory Syncytial Virus (RSV) (n:5; 41.6%), Human Rhinovirus (HRV) (n:4; 33.3%), Influenza A Virus (n:2; 16.6%), Adenovirus (n:1; 8.3%). In the control group Adenovirus (n:1; 33.3%), Influenza A Virus (n:1; 33.3%), Influenza B Virus (n:1; 33.3%) were detected (Table 2).

Table 1. Characteristic features of the patients in the groups.

	Asthmatic (n:21) Mean±Standart deviation	Control (n:14) Mean±Standart deviation	P
Age (month)	64.7±37.959	85.7±44.617	0.167
Gender (M/F)	11/10	10/4	0.26
WBC/mcl	10072±4400.108	9475±3828.787	0.682
CRP mg/dl	0.86±2.026	1±2.202	0.062
Eosinophil/mcl	286±264.619	264±184.132	<0.01

Table 2. Distribution of viruses isolated in the groups.

	Asthmatic (n=12)	Control (n=3)
Virus isolated	12(57%)	3(21%)
RSV	5(41.6%)	0(0%)
HRV	4(33.3%)	0(0%)
Influenza virusA	2(16.6%)	1(33%)
Influenza virusB	0(0%)	1(33%)
Adenovirus	1(8.3%)	1(33%)

None of the virus isolated or non-isolated patients with asthma attack were hospitalized, all patients were sent home after stabilization with outpatient treatments.

DISCUSSION

Asthma attacks are generally trigerred by viral infections in childhood. Our study also supported this idea. We aimed to find which virologic factors trigerred asthma exacerbations of our pediatric patients living in our region.

Asthma is a chronic inflammatory disease of the airways characterized by recurrent attacks. Three mechanisms are responsible. These are, reversible airway obstruction, airway inflammation and increased sensitivity of the airways. It is the most common chronic disease in childhood [3].

The starting age of the disease is about 1 year in 30% of the patients. First symptoms occur in 80-90% of the patients before 4-5 years of age. The prevelance of the disease is observed 2 times more in the boys than girls at the early ages. This ratio gradually becomes equal towards the adolesant ages than reverses in adulthood.

Among the etiologic factors gender, allergy, atopy, diet, race, socioeconomic status, smoking, air pollution, exercise, perinatal factors, bronchial hyperresponsiveness, stress, drugs and viral infections can be enumerated.

Clinically, coughing attacks, wheezing, shortness of breath are seen. These symptoms usually occur at

night and early morning hours. The most important and the most common environmental trigger of acute exacerbation of asthma, except allergens, even in the patients who are under treatment, are the viral infections [1,2]. So our study performed a regional research of virologic factors which trigerred our children's asthma exacerbations

However respiratory infections clinically differ from each other. Most of them cause acute upper or lower respiratory illnesses, wheezing, bronchiolitis, pneumonia, cold and flu-like symptoms, bronchopneumonia, dyspnea and asthma exacerbations. In winter these viruses also aggravate the symptoms of asthma. In asthmatic patients clinical findings may differ according to the agent, its viremia time and localities. With seasonal occurence, the frequency of attacks may increase. HRV makes a peak in autumn, RSV in winter and spring time. In younger ages, Respiratory Syncytial Virus can be serious enough to necessitate hospitalizations. Altough Adenovirus and Influenza viruses are seen less than others, they may be more severe in asthma. Some Adenovirus subtypes may be fatal (10%) in younger ages.

Risk factors that cause the development of these viral infections are nursery at home, smoking, dusty and crowded environment, having large number of siblings and low socioeconomic status [4]. Viral infections change the autonomic innervation of the respiratory tract in favor of the cholinergic system, disrupt epithelial integrity, increase airway inflammation, and accelerate development of exacerbations. General opinion is that viral respiratory infections do not cause asthma but they are important triggers in asthma [2,3].

Epidemiologic studies show that 80% of childhood asthma exacerbations are associated with viral upper respiratory truct infections [5]. In our study viral serology was positive in 57% of the patients in the asthmatic group. Among respiratory viruses HRV found to be the most common agent in 24.5% [6], and 50% [7] of the patients, respectively. Ozcan et al. [8] isolated HRV from 53.8% of the patients with acute exacerbations of asthma, and found Rhinovirus in 35.6% of the patients.

In a study performed in patients aged 3 months and 16 years, viral causative agent was detected in 78% of the patients. RSV was found about 40% of them as the most frequent viral agent [6]. We also isolated RSV mostly (41.6%), then HRV (33.3%), Influenza virus (16.6%), Adenovirus (8.3%) in descending order of frequency. As is known, RSV is seen in younger ages, when the study groups' age range included smaller patients [6], RSV was found to be the dominat agent as in our study. Generally, studies have demonstrated that in about 2.4 % of asthma attacks adenovirus were detected (8). In a study made by Sackesen et al. [9], the frequencies of Adenovirus in asymptomatic and symptomatic asthmatic patients, healthy controls and wheezy children were found to be 33.3, 71.4, 37 and 62.96%, respectively. Adenovirus was detected in higher number of patients with asthma exacerbation and in children with wheezing than in patients without asthma exacerbation and in the healthy controls ($p<0.05$). We isolated Adenovirus from 8.3% of the asthmatic patients which was relatively higher when compared with other studies. As Adenovirus is a serious respiratory airway pathogen, in our region this agent may be an important risk factor for the asthmatic patients.

In younger children the cause is the Respiratory Syncytial Virus in the foreground. Among other factors, Influenza virus, Parainfluenza virus, Metapneumovirus, Respiratory Syncytial Virus, Bocavirus, Adenovirus, Human Coronavirus, Enterovirus, bacterial agents as Mycoplasma Pneumoniae, Chlamidydophila Pneumoniae have been identified. But these agents have not been associated with the severity of attacks [10]. In our study we did not isolate Parainfluenza virus, Bocavirus, Metapneumovirus, Coronavirus and Enterovirus. We didn't study bacterial agents cultured from nasopharengeal swabs.

Rakes et al. [11] found most commonly HRV in cultures obtained from children with asthma attacks.

And they also found synergistic relationship between presence of allergen specific IgE, eosinophilia and acute virus-induced wheezing. Although general properties were similar in both of the groups we found significant differences between the eosinophil counts of the groups (p<0.01). So this result supports the the role of eosinophilia in asthma.

In a prospective cohort study, patients with the diagnosis of asthma at the ages of 6-14 years were followed up for a period of 1 year. They were assessed according to their gender, levels of control of asthma treatment, their therapies, asthma symptoms, presence of atopy, and lung function test results. Their nasal swab samples were studied by immunfluorescence and PCR methods. Virus detection rates were found to be equal in the non-asthma respiratory illnesses and asthma exacerbations (34.8-39.2%) [12]. We isolated viruses from 57% of the patients in the asthmatic, and 21% of the control groups. There was a significant difference in virus detection rates between asthmatic and control groups (p<0.01). These results were also compatible with the literature.

The pathogenetic mechanism of virus in acute asthma exacerbation was unclear. Type 3 IFNλ were found higher in HRV+ patients with wheezing than HRV- ones. And these patients were clinically worse. Interestingly HRV+ cases had fewer attacks of wheezing during follow up than HRV-ones [13]. HRV was believed to be asthmogenic [14]. Our patients were followed up for 4 months after the attack. In none of them attacks recurred.

In a large study performed during the 2003-2009 Influenza seasons and 2009 pandemics, the records and the diagnosis of the patients aged between 2-17 years with asthma, status asthmaticus or acute asthma attack were examined. During the influenza season 32% of the children hospitalized with Influenza had asthma, while during the 2009 pandemics 44% had asthma. Compared with asthmatic children with seasonal Influenza, a higher proportion with 2009 pandemic H1N1 Influenza required intensive care (16 vs 22%; p=0.01) and were diagnosed with pneumonia (40 vs 46%; p=0.04), whereas equal proportions had respiratory failure (5 vs 5%; p=0.8) and died (1 vs 1%; p=0.4). More asthmatic children with Influenza A (seasonal or pandemic) had diagnoses of asthma exacerbations compared with those with Influenza B (51 vs 29%; p<0.01) [15]. In our study Influenza virus was detected in 2 asthmatic patients (16%). None of the virus detected patients were hospitalized or had bad prognosis.

As it is known that HRV and Influenza Virus are the most frequent virus in the children with upper airway tract infections, Influenza Virus was shown to be the most frequent one [16,17]. We also isolated Influenza Virus as the dominant agent from the control group (Influenza A 33.3%, Influenza B 33.3%). The etiolo-gic differences between asthmatic and control groups might be due to the patients response to the viral agent, immunity and asthma pathology.

In an analysis of gene expression of Rhinovirus in asthmatic and normal individuals' bronchial epithelial cells, up and down-regulated genes were found corresponding to the immune response. Probably the changes in the Rhinovirus infected cells were also present in the cells before the impact of infection [20,21].

The role of respiratory viruses in exacerbations of asthma depends upon the host's immune preexisting immunity, asthma phenotype, environmental factors and infectious agents. Determination of the agent in the seasons with higher prevalence of asthma (such as HRV in autumn) is not necessary. If required, quick recognition of spesific viral syndromes (Influenza virus during the winter months) with rapid tests may be necessary to begin early antiviral treatment [22].

There is not a clear idea about the benefits or harms of Influenza vaccine in preventing acute asthma attacks. It is believed that Influenza vaccine do not cause an acute asthma attack [21]. There are studies showing that the vacination reduced the rate of acute asthma exacerbations about 22-41% [22]. Even vaccination do not decrease the frequency of asthma

attack, but it can prevent the complications of infections and increase the patient's quality of life [23].

It is unnecessary to use antibiotics during an attack because of the low incidence of bacterial infection. In our study none of the virus isolated or non-isolated patients with asthma attack hospitalized, all patients were sent home after stabilization with outpatient treatments. Exacerbations associated with viral infections are still not well responsive to asthma treatment and the new antiviral therapies, researche, vaccines are needed which are specific for unique viral pathogens [24].

CONCLUSION

Viral infections are the most important triggering factors of asthma and knowing their impact on disease may be helpful in protecting children from attacks. Better understanding of these impacts will effect the new approaches for the primary prevention of asthma exacerbations.

The results of our study are interesting because such a study has not been made in our region yet. Besides, the results were similar to the literature findings, except rates of RSV, Influenza and Adenovirus So this study is important for emphasizing the importance of such viruses which are not seen frequently (RSV, Adenovirus, Infuenza virus etc). We isolated viruses from a small number of patients, so studies with a large number patients with different asthma phenotypes are needed to develop effective treatment strategies against asthma attacks which are triggered with spesific viral pathogens seen regionally.

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