Recurrence of Wheezing Episodes in Children with Respiratory Syncytial Virus and Non-Respiratory Syncytial Virus Bronchiolitis

Respiratuvar Sinsityal Virüs ve Respiratuvar Sinsityal Virüs Dışı Viral Bronşiyolitli Çocuklarda Tekrarlayan Hışıltı Atakları

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ABSTRACT Objective: It has been shown that many children who experience respiratory syncytial virus (RSV) infection in infancy develop recurrent wheezing episodes and asthma later. Viral role in pathogenesis of asthma is still a debate. We aimed to investigate the recurrence of wheezing episodes, atopy and serum cytokine levels in children encountered with RSV infection. Additionally, we aimed to compare these parameters in children with non-RSV bronchiolitis. **Material and Methods:** The study was conducted between January 2006 and November 2008 in Dokuz Eylul University hospital. Seventy children aged between 0-36 months who were diagnosed with acute bronchiolitis for the first time were recruited for the study. Nasopharyngeal lavage fluid was analyzed with polymerase chain reaction (PCR) for RSV antigen in all patients. Serum immunoglobulin E, total eosinophil count, interleukin (IL)-4, IL-13 and γ-interferon (IFN-γ) levels were obtained at the last examination of the patients after a follow up period of 1-3 years. **Results:** Recurrence of a wheezing episode was observed in 35% and 53.3% of the patients in RSV group and non-RSV group, respectively (p= 0.064). Mean serum IFN-γ and IL-4 levels were detected to be significantly higher in non-RSV group. No significant relation was detected between recurrence of wheezing episodes and total eosinophil count, serum IgE, IL-4, IL-13, and IFN-γ levels. **Conclusion:** No difference could be detected between RSV and non-RSV bronchiolitis regarding subsequent wheezing episodes. Although serum IL-4 and IFN-γ levels were higher in non-RSV bronchiolitis group, no significant correlation was observed between these parameters and recurrence of wheezing episodes.

Key Words: Asthma; bronchiolitis

ÖZET Amaç: Süt çocukluğu döneminde respiratuvar sinsityal virus (RSV) infeksiyonu geçiren çocukların daha sonra hışıltı atakları ve astım geçirdikleri gösterilmiştir. Astımın patogenezinde virüslerin rolü halen tartışmalıdır. RSV infeksiyonu ile karşılaşan çocuklarda hışıltı ataklarının tekrarlamasını, atopi ve serum sitokin düzeylerini araştırmayı ,ayrıca bu parametreleri RSV-dışı bronşioliti olan çocukların sonuçları ile karşılaştırmayı amaçladık. **Gereç ve Yöntemler:** Çalışma Ocak 2006 ile Kasım 2008 arasında Dokuz Eylül Üniversitesi Hastanesinde gerçekleştirildi. İlk kez akut bronşiolit tanısı koyulan 0-36 ay yaş aralığında 70 çocuk çalışmaya alındı. Tüm hastalarda nazofarenjiyal lavaj sıvısı RSV antijeni bakımından polimeraz zincir reaksiyonu (PCR) ile incelendi. Bir ila üç yıllık takip süresinden sonra hastaların son muayenelerinde serum immunoglobulin E, total eozinofil sayısı, interlökin (IL)-4, IL-13 ve interferon-γ (IFN-γ) düzeylerine bakıldı. **Bulgular:** RSV grubunda ve RSV-dışı grupta hışıltı atağı tekrarı sırasıyla hastaların %35'inde ve %53.3'ünde gözlendi (p= 0.064). Ortalama serum IFN-γ ve IL-4 düzeyleri RSV-dışı grupta belirgin olarak yüksek bulundu. Hışıltı ataklarının tekrarı ile total eozinofil sayısı, serum IgE, IL-4, IL-13 ve IFN-γ düzeyleri arasında önemli bir ilişki saptanmadı. **Sonuç:** RSV ve RSV-dışı bronşiolit arasında müteakip hışıltı atakları bakımından fark saptanamadı. RSV-dışı bronşiolit grubunda serum IL-4 ve IFN-γ düzeyleri daha yüksek olmakla birlikte bu parametrelerle hışıltı ataklarının tekrarı arasında önemli bir korelasyon gözlenmedi.

Anahtar Kelimeler: Astım; bronşiyolit

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Acute bronchiolitis (AB) is the most common lower respiratory tract infection seen among infants and is caused by inflammation of small airways. Bronchiolitis manifests in a typical seasonal pattern with peaks during winter and fall. It is one of the principal causes of hospital admissions in infants under the age of 1 year.[1] Acute bronchiolitis is mainly caused by viruses, and respiratory syncytial virus (RSV) accounts for 50-90% of the cases.[2-4] Other viruses such as rhinoviruses, enteroviruses, human metapneumovirus and human bocavirus are also found in infants with this condition.[5-7]

It has been shown that many children who experience RSV infection in infancy develop subsequent wheezing episodes later and the risk for asthma is increased in those infants.[8-13] Wheezing usually re-occurs after acute bronchiolitis in 50% of the cases. Increased frequency of wheezing episodes and asthma among the children with bronchiolitis history could not be explained with only atopy and family history. It is still unknown whether bronchiolitis triggers the immune response to involve an asthmatic process later or whether RSV infection induces the asthmatic susceptibility of the infants which was presented before.[1] Mast cells, activated eosinophils and activated T-helper lymphocytes (Th) are increased in the airways of asthmatic individuals. T-helper lymphocytes which produce proinflammatory cytokines [Interleukin (IL)-4, IL-5, IL-13] and chemokines are involved in this inflammatory process.[1] Respiratory syncytial virus infection triggers a complex immune response. It is postulated that the balance between Th1 and Th2 is directed towards Th2, and with the aid of cytokines involving in asthma pathogenesis, allergic inflammation begins after RSV infection.[14]

The aims of this study are (i) to detect the relationship between recurrence of wheezing episodes in children encountered with RSV infection and atopy as well as serum cytokine levels, (ii) to compare these children with those did not encounter RSV bronchiolitis, (iii) and to discuss the role of cytokines in the etiopathogenesis of recurrent wheezing.

MATERIAL AND METHODS

This study was conducted between January 2006 and November 2008 in the Department of Pediatrics, Medical Faculty, Dokuz Eylul University. The study was approved by the ethics committee of the hospital. The parents of all infants were asked to participate in the study and gave their informed consent.

Children aged between 0-36 months who were diagnosed with bronchiolitis for the first time according to the history obtained from parents were recruited for the study. Following evaluation in the Department of Pediatric Pulmonology and Allergy, Department of Pediatric Emergency Medicine or pediatric inpatient unit, nasopharyngeal lavage fluids were analyzed with reverse transcriptase-polymerase chain reaction (RT-PCR) for RSV antigen in all patients. Nasopharyngeal aspirates were collected with a disposable catheter which was inserted in the nostril to the depth of 5-7 cm and drawn back after a gentle suction with an injector. The specimens were transported to the laboratory at room temperature. RSV was detected by using an RT-PCR. The patients were evaluated biannually by the same physician. The patients with a history of prematurity and an acute bronchiolitis episode before and patients with chronic diseases (chronic lung disease, asthma, cystic fibrosis, congenital heart defects) were excluded.

Patients were followed for 1-3 years. Data regarding gender, gestational age, birth weight, exposure to cigarette smoke, duration of breastfeeding, maternal education level, mean monthly family income, number of family members, properties of the house dwelled in and the presence of a feathery pet and toy at home were noted. Family history for asthma, allergic rhinitis and atopy, age at the time of bronchiolitis were also recorded at the first evaluation. Frequency of wheezing episodes was recorded during the follow-up period following acute bronchiolitis.

A blood sample was obtained at the last follow-up visit of each patient. Total eosinophil count, serum immunoglobulin E (IgE), IL-4, IL-13, and interferon-γ (IFN-γ) levels were studied. Interleukin-4, IL-13 and IFN-γ levels were analyzed

by enzyme-linked immunosorbent assay (ELISA) technique using commercial kits (Bioo Scientific, Austin, TX, USA). The lower detection limits for these assays were: 4 pg/mL for IL-4, 30 pg/mL for IL-13 and 10 pg/ mL for IFN-γ.

Skin prick tests (SPT) were performed following the recommendations by the European Academy of Allergy and Clinical Immunology in each patient in order to evaluate atopy.[15] A commercial extract (Allergopharma, Germany) was used with the skin prick/puncture method. A standard panel of respiratory allergens consisting of mites, grass mix, mold mix, cat and dog dander, cockroach and certain food allergens (cow's milk, egg white, cocoa, banana and tuna fish) were included in skin prick test. The SPT was considered positive when the mean diameter of the wheal was at least 3 mm when read after 15 minutes.

STATISTICAL ANALYSIS

Data were analyzed with SPSS for Windows, version 15.0. Descriptive statistics were used to demonstrate demographic characteristics. Differences in clinical characteristics between patients with RSV and non-RSV bronchiolitis were analyzed using Chi-square test for categorical variables and Mann Whitney-U test for numerical variables. Kruskal Wallis test was used for comparison of the groups with recurrent wheezing episodes. A p value of <0.05 was accepted as statistically significant.

RESULTS

Of the 212 patients who met the inclusion criteria, 70 patients (40 children in RSV group and 30 in non-RSV group) accepted to participate in the study. Forty-one (58.6%) of these children were boys. There was not a statistical difference between the RSV and non-RSV group by means of gender and other demographic characteristics except birth weights and admission age (Table 1). The mean age of the children at admission was 6.46 ± 6.78 months (range: 1-33 months) in the RSV group while it was 9.53 ± 7.55 months (range: 1-30 months) in the non-RSV group. The mean admission age of the RSV group was smaller than the non-RSV group (p= 0.031). Other demographic characteristics of study group can be seen in Table 1.

The follow-up period was 18.97 ± 7.95 months (range: 12-42) for the whole study group. RSV group was followed up for a mean period of 19.7 ± 8.91 months (range: 12-42 months) while non-RSV group was followed up for a mean period of 18 ± 6.47 months (range: 12-33 months). In RSV group, 26

TABLE 1: Demographic characteristics of the study group.

	RSV group	Non-RSV group	p
Age at admission (month) mean ± SD	6.46 ± 6.78	9.53 ± 7.55	0.031
Sex n (%)			
Female	17 (42.5)	12 (40)	1
Male	23 (57.5)	18 (60)	
Way of delivery n (%)			
Vaginal delivery	11 (27.5)	12 (40)	0.311
CS	29 (72.5)	18 (60)	
Birth weight (g) mean ± SD	3366 ± 531.28	3075.73 ± 512.96	0.015
Prenatal exposure to cigarette smoke n (%)			
Yes	9 (22.5)	7 (23.3)	1
No	31 (77.5)	23 (76.7)	
Family history of atopy n (%)			
Yes	27 (67.5)	21 (70)	1
No	13 (32.5)	9 (30)	
Breastfeeding period (month) mean ± SD	11.02 ± 5.59	13. ± 7.38	0.22

children (65%) were followed up for one year (12-23 months) while 12 (30%) were followed up for two years (24-35 months) and 2 (5%) were followed up for three years (36 months and further). In non-RSV group, 24 children (80%) were followed up for one year while six (20%) were followed up for two years.

Serum IL-13 level was not different in non-RSV and RSV groups (p= 0.565) (Table 2). The serum IL-4 level was higher in non-RSV group compared to RSV group (p= 0.002). The IFN-γ level was also significantly higher in non-RSV group compared to RSV group (p= 0.003) (Table 2). Serum IgE level and total eosinophil count did not differ between the groups (p= 0.39, p= 0.121, respectively) (Table 2). Skin prick test positivity was similar in two groups, one patient in each group was sensitized to grass mix. Symptoms of allergic rhinitis were noted in one of these patients in the RSV group.

If the analysis was made according to the years of follow-up, serum IL-4 and IFN-γ levels were higher in non-RSV group compared to RSV group in the subsets that were followed up for one year (p= 0.04 and p= 0.02, respectively). Serum IL-4 level was also significantly higher in non-RSV group compared to RSV group in the subsets that were followed up for two years (p= 0.04). Serum IL-13 level, serum IgE level and total eosinophil count were not different in RSV and non-RSV group subsets followed up for one and two years. No comparison could be made in the groups of children followed up for three years because insufficient number of patients.

Allergic rhinitis, eczema and recurrence of wheezing episodes were noted for each patient. No patient was diagnosed with eczema. Of the patients in RSV group, 65% did not develop any further wheezing. However, 30% had wheezing episodes fewer than three and 5% developed three or more wheezing episodes. Of the patients in non-RSV group, 46.7% did not develop further wheezing. On the other hand, 30% had wheezing episodes fewer than three and 23.3% developed three or more wheezing episodes. No difference was noted between the groups for recurrence of wheezing episodes (p= 0.064) (Table 3).

No significant relation was noted between birth weight, way of delivery, age at admission, maternal education level, exposure to prenatal or postnatal cigarette smoke, duration of breastfeeding, mean monthly family income, properties of the house dwelled in, number of family members and recurrence of wheezing episodes (p> 0.05). Recurrence of wheezing episodes was also found out to be unrelated to total eosinophil count, serum IL-13, serum IL-4, IFN-γ or serum IgE levels (p> 0.05). In addition, no relation was found between skin

TABLE 2: Laboratory findings of the study group.

	RSV group Mean ± SD	Non-RSV group Mean ± SD	p
IL-13 (pg/mL)	22.61 ± 34.17	63.68 ± 267.82	0.565
IL-4 (pg/mL)	20.43 ± 45.57	40.77 ± 50.37	0.002
IFN-gama (pg/mL)	90.12 ± 82.3	162.57 ± 98.0	0.003
IgE (IU/mL)	98.8 ± 207.59	267.62 ± 1006.29	0.39
Total eosinophil count (/mm³)	213.22 ± 192.19	240.40 ± 137.40	0.121

TABLE 3: Recurrence of wheezing episodes in RSV positive and non-RSV group.

	RSV group n (%)	Non-RSV group n (%)	p
No recurrence	26 (65)	14 (46.7)	
Recurrence of wheezing episode < 3	12 (30)	9 (30)	
Recurrence of wheezing episode > 3	2 (5)	7 (23.3)	
Total	40 (100)	30 (100)	0.064

test positivity and recurrence of wheezing episodes ($p > 0.05$).

DISCUSSION

Lower respiratory infections in infancy are thought to be related to recurrent wheezing and asthma thereafter. There are a number of epidemiological studies regarding the role of respiratory viruses and especially RSV in recurrent wheezing.[8,10,16-19] In one of the studies investigating the role of RSV in the development of asthma, 12 studies were reviewed and increased risk of asthma and recurrent wheezing after RSV infection was found in the first 36 months, but the relation showed a tendency to decrease with age.[18] Henderson et al.[17] similarly found a relation between RSV bronchiolitis before 12 months of age and later asthma, but no relation with atopy. Respiratory syncytial virus bronchiolitis is thought to be characterized by an excess of type 2 cytokines.[14] However, whether RSV bronchiolitis causes asthma by deviating subsequent immune responses towards a type 2 cytokines or whether asthma and bronchiolitis simply have same risk factors, with bronchiolitis being the first presenting illness in a child already at risk of developing asthma as a result of impaired type 1 immunity is a matter of debate.

Rhinovirus-induced wheezing was also demonstrated as an important risk factor for the development of asthma and the risk shows a tendency to persist with increasing age.[20-22] In a prospective study on children from birth to 6 years of age, Lemanske et al.[23] concluded that the occurrence of rhinovirus-associated wheezing during infancy was the most significant risk factor for asthma and preschool childhood wheezing. In studies comparing the role of bronchiolitis caused by RSV and viruses other than RSV in recurrent wheezing episodes, it is shown that infections other than RSV confer a substantially greater risk of recurrent wheezing compared to RSV infections.[24,25] In our study, no statistical difference could be detected between RSV and non-RSV bronchiolitis regarding recurrence of wheezing episodes. The viral etiology of non- RSV illness was not demonstrated in our study but it is likely that most of them were caused by rhinoviruses or enteroviruses. Although statistical difference was not shown in our study, it is noted that recurrence of wheezing episodes were observed in 53.3% of the non-RSV group while it was noted in 35% of the RSV positive group. We think that the number of our study group, major limitation of our study, might have caused this conclusion.

Interleukin-13 dependent mechanisms are shown to be related with airway eosinophilia and enhanced airway hyperresponsiveness during reinfection in a mouse model of neonatal RSV infection.[26] In several studies, it was shown that IL-4, IL-13 and IFN-γ levels increased after RSV infection.[27-29] However no relation was shown between IL-4, IFN-γ levels and recurrent wheezing episodes.[30] In our study, IL-13 levels were not different in RSV positive and negative cases but IL-4 and IFN-γ levels were significantly higher in the non-RSV group. Serum IL-4 levels were higher in both subsets of the non-RSV group followed up for one and two years. however this difference did not predict any parameter in the follow up. In our previous study, we investigated the relationship between serum IL-4, IL-13, IFN-γ levels and recurrence of wheezing episodes in infants with acute bronchiolitis, and found out a positive correlation between serum IL-13 levels and the number of wheezing episodes.[31] We could not study the viral etiology in that study but we supposed that RSV had been the causative agent in the majority of cases. In the study of Castro et al.[32] 206 infants were followed up after a severe RSV infection, and IL-2, IL-4, IL-13 and IFN- γ were studied immediately after the infection, at the age of 2, 4 and 6. The authors found that IL-13 expression was lower in the group of infants who developed asthma later and denied the role of Th2 type immune response in development of asthma after RSV infection. In current study, we evaluated the relationship between IL-13, IL-4, IFN-γ and recurrence of wheezing episodes, but no significant relation was established. Our results might have been different if we could compare the basal levels at the admission of the patient with the levels at the last follow-up visit. Unfortunately we could only study the levels at the

last follow-up visit. No relation was noted between RSV infection and atopy and skin test positivity, similar to previous studies.[33]

In our study, we investigated the relation between the demographic features and recurrence of wheezing episodes. However, no relation was observed between gender, gestational age, birth weight, exposure to cigarette smoke, duration of breastfeeding, maternal education level, family income, number of family members, properties of the house dwelled in, presence of a feathery pet at home, family history of asthma, allergic rhinitis and atopy, age at the time of bronchiolitis and recurrence of wheezing episodes. The limitations of our study might be an explanation for these conclusions. First of all, two-thirds of the patients fulfilling diagnostic criteria could not participate in the study. Secondly, the follow-up period of one year in some children may be regarded as short and the follow-up periods are not the same. This difference between the follow-up periods may limit the present results. Thirdly, we could not determine the viral etiology of bronchiolitis in non-RSV group because rhinoviruses and other respiratory viruses were not routinely studied in our hospital during the study period. Unfortunately, we do not have frozen samples for further research. On the other hand, our patient population consisted of patients with varying severity. As a consequence, we think that this issue prevented the study from bias of severe cases who were at the risk of recurrent wheezing episodes.

In conclusion, our results show that both RSV and non RSV bronchiolitis have a similar potential roles for recurrence of wheezing episodes. However, further larger studies are needed to determine the viral role in the pathogenesis of wheezing recurrence and asthma.

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