Evaluation of RSV Frequency in Acute Bronchiolitis By Different Methods

Akut Bronşiyolitte RSV Sıklığının Farklı Yöntemlerle Değerlendirilmesi

M.Hacımustafaoğlu*, S.Çelebi*, E. Aynacı*, N.Köksal*, M.Sınırtaş**, G.Göral**
*Uludağ Üniversitesi Tıp Fakültesi Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Bursa
** Uludağ Üniversitesi Tıp Fakültesi, Enfeksiyon Hastalıkları ve Mikrobiyoloji Anabilim Dalı, *Bursa, Turkey*

Summary

Aim: Etiological and epidemiological studies of respiratory syncytial virus (RSV) infections in our country are not abundant. The aim of this study was to determine the frequency and general epidemiological properties of RSV in acute bronchiolitis (AB) and to investigate the accuracy and cost-effectivity of various diagnostic methods.

A total of 76 children younger than 2 years of age who had the diagnosis of AB in the outpatient clinic or the emergency room were included. The criteria for the clinical diagnosis of viral AB were wheezing and sibilant rhonchi following a nonspecific viral upper respiratory infection of a few days along with hyperaeration on the chest X-ray. Patients with evidence of bacterial infection were excluded.

The nasopharyngeal secretions (NS) and sera of the patients were obtained and stored at -20°C to be subsequently tested for RSV antigen (Test Pack?, Abbott) in the NS and anti-RSV-IgG (two samples 14 days apart) as well as anti-RSV IgM antibodies in sera (ELISA, Euroimmune?). The assays were run simultaneously on the same batch.

Of the 76 patients with AB, 73.6% were male and 26.4% were female. Their mean age was 9.4±5.3 months (X±SD; range: 2-24). The age distribution was 2-6 months in 26.3% patients, 7-12 months in 52.6% patients, and 13-24 months in 21.1% patients.

RSV antigen in NS was positive in 36 (47.3%) patients, 4 of whom (5.2%) had anti-RSV-IgM in sera as well. Anti-RSV IgM was detected in an additional 3 patients whose NS were antigen-negative adding up to 39 (51.2%) patients with AB. Four patients tested positive for anti-RSV IgM only one of whom had a positive antigen in the NS. Out of 16 patients who had anti-RSV IgG concentration and were assayed twice after 14 days, 2 (12.5%) patients had increased concentrations of anti-RSV IgG in the second sample.

Male/female ratio was 31/8 in RSV positive patients, and 25/12 in RSV negative patients (p>0.05). The age

Geliş Tarihi: 14.08.2008
Kabul Tarihi: 08.09.2008

Correspondence Address
Yazışma Adresi
Dr. Mustafa Hacımustafaoğlu
Uludağ Üniversitesi Tıp Fakültesi, Çocuk Enfeksiyon Hastalıkları Bilim Dalı, *Bursa, Turkey*
Tel.: +90 224 442 88 75
Fax: +90 224 442 87 49
E-posta
mkemal@uludag.edu.tr

DİP NOT: Bu çalışma sadece bir sayı yayınlanıp yayından kaldırılan, artık yayın hayatında bulunmayan ve indekslenmemiş olan bir lokal dergide yayınlanmıştır.

Özet

Amaç: (Akut Bronşiyolitte RSV Sıklığının Farklı Yöntemlerle Değerlendirilmesi) Ülkemizde RSV için çocuklarda etyolojik ve epidemiyolojik yayınlar az sayıdadır. Bu çalışma bölgemizde A.bronşiyolitlerde RSV sıklığı, genel epidemiyolojik özellikleri, farklı tanı yöntemlerinin değerlendirilmesi ve maliyet-etkinlik analizlerinin yapılması için planlandı.

Acil ve normal çocuk polikliniğine başvuran ve klinik olarak akut viral bronşiolit tanısı konan 2 yaşından küçük 76 çocuk çalışmaya alındı. Birkaç gündür devam eden nonspesifik viral ÜSYE sonrası, wheezing olan ve/veya muayenede yaygın sibilan ronküs saptanan ve PA akciğer grafisinde havalanma artışı olan bebekler klinik olarak akut bronşiolit tanısıyla çalışmaya dahil edildi. Muhtemel başka bakteriyel enfeksiyonu kanıtlanmış olan olgular çalışmaya dahil edilmedi.

Olguların nazofarengeal sekresyonları; RSV antijeni (Test Pack^{R}, Abbott) bakılmak, serumları; anti-RSV-IgG (14 gün arayla 2 kez) ve anti-RSV-IgM antikorları (ELISA, Euroimmun) bakılmak için derin dondurucuda (-2^{o}C) saklandı. Sonuçlar daha sonra ve toplu olarak çalışıldı.

Çalışmaya alınan 76 akut bronşiolitli olgunun %73.6'sı erkek, %26.4'ü kız olup tanı anındaki ortalama yaşları 9.4±5.3ay (X±SD)(dağılım; 2-24) arasındaydı. Yaş dağılımı %26.3 olguda 2-6 ay, %52.6olguda 7-12 ay, %21.1olguda 13-24 ay idi.

Nazofarengeal sekresyonda RSV antijeni 36 olguda (%47.3), serumda anti-RSV-IgM 4 olguda (%5.2) pozitif saptandı. Anti-RSV-IgM pozitif saptanan üç ek olguyla beraber RSV akut bronşiolit olgu sayısı 39' a (%51.2) yükseldi. Anti-RSV-IgG düzeyi çalışılabilen 16 olguda çift serum örneğinde anti-RSV-IgG titre artışı 2 olguda (%12.5) saptandı.

RSV pozitif saptanan olgularda erkek/kız oranı 31/8, RSV negatif saptanan olgularda erkek/kız oranı 25/12 bulundu (p>0.05). RSV ve non-RSV olgular arasında başvuru yaşları arasında anlamlı fark yoktu (p>0.05). Akut bronşiolit tanısı konan 76 olgunun 70'i (%92)

of patients with and without RSV was not significantly different (p>0.05). Seventy out of 76 (92%) patients presented between October and April. The month of presentation between the RSV positive and negative patients was not different (p>0.05). Forty-two (55%) patients were hospitalized and 34 (45%) were treated as outpatients.

The cost of antibiotics and diagnostic tests were calculated for each patient. The cost of antibiotics was 45+30 USD in RSV positive patients, and 90.5+90.5USD in RSV negative patients. One test for RSV antigen cost ~13.3 USD, anti RSV IgM 4.58 USD, and anti-RSV IgG (two assays) 6.6 USD. The total cost even when all tests were done on one patient was less expensive than an unnecessary antibiotic treatment.

RSV was detected in approximately half of the patients with AB. When compared to fast RSV antigen detection by EIA, serologic tests (ELISA IgM and IgG) had a high frequency of false negativity. Antigen detection in NS should be the preferred method for the diagnosis of RSV in AB which is highly cost-effective especially during the epidemic months.

(*J Pediatr Inf 2008; 2: 156-61)*

Key words: *Respiratory syncytial virus, diagnosis, epidemiology*

Ekim-Nisan ayları arasında başvurdu. RSV saptanan ve saptanmayan olgular arasında başvuru ayları arasında anlamlı bir farklılık yoktu (p>0.05). Olguların 42'si (%55) kliniğe yatırılırken 34'ü (%45) ayaktan takibe alındı.

Olguların antibiyotik ve tanı yöntemleri ölçüm maliyetleri hesaplandı. Sadece antibiyotik maliyeti RSV saptanan olgularda 45+30 USD, RSV saptanmayan olgularda 90.5+90.5 USD bulundu. RSV antijen (bir kez) çıplak maliyeti 13.3 USD, anti RSV-IgM (bir kez) 4.58 USD, anti-RSV-IgG (iki kez) 6.6 USD bulundu. Bu değerlerle; bütün tanı kitleri aynı hastada kullanılsa bile, lüzumsuz verilecek olan antibiyotik maliyetinin daha altında bulundu.

Sonuç olarak; A.bronşiyolitlerin yaklaşık yarısında RSV etyolojisi saptandı. EIA ile hızlı RSV antijen saptanmasına kıyasla, serolojik tetkikler (ELISA IgM ve IgG) yüksek oranda yanlış negatif bulundu. Tanıda nazofarengeal sekresyonda antijen arama yönteminin özellikle RSV nin epidemi yaptığı aylarda ilk tercih edilecek yöntem olması gerektiği kanısına varıldı.

(Çocuk Enf Derg 2008; 2: 156-61)

Anahtar kelimeler: *Respiratory sinsityal virus, tanı, epidemi-yoloji*

Introduction

Acute bronchiolitis (AB) is a frequent infection of the lower respiratory tract which involves in-flammation of the bronchioles. It is a clinical syndrome that affects infants younger than two years of age characterized by rapid and labored respiration with wheezing. Typically, the initial symptoms are those of a viral infection including nasal discharge and cough sometimes associated with low grade fe-ver. The specific symptoms of AB ensue in a few days with tachypnea, dyspnea, and wheezing.

The most frequent cause of AB is respiratory syncitial virus (RSV). Other viruses responsible in a small number of patients include adenovirus, enterovirus, influenza, and parainfluenza. Approximately 90,000 children are admitted to the hospital annually with AB and 4500 die in the U.S.A.[1]. Unfortu-nately, there is no effective control method against this community problem. The causative agent of AB and viral pneumonia is RSV in 12-52% of the patients (2-5). This frequency may be higher in hospitalized patients, and RSV in inpatients has been reported to be responsible in 50-90% of bronchitis, 5-40% of pneumonia, and 10-30% of tracheabronchitis (6,7). The cumulative incidence of RSV in respiratory infections in Turkey is between 11-43%8,9.

The golden standard for the diagnosis of RSV is culture as in all viral respiratory infections. How-ever, it is not widely used because the results are obtained in 3-7 days, the method is affected by the environmental conditions, and it is not available in many hospitals. The fast antigen detection in NS may be done by enzyme linked immunosorbent assay (EIA) or immunofluorescence (IFA; direct or indirect). The sensitivity and specificity of antigen tests in comparison to culture ranges between 60-95% (frequently 80-90%) (6,10). The demonstration of increasing RSV-specific IgG titers (2-4 fold) or the detection of RSV-specific IgM in sera is also important. However, the infection may not always be as-sociated with seroconversion especially in young infants. Routine RSV culture or ELISA RSV antigen detection is not widely available in Turkey.

On the other hand, immunotherapeutic modalities for AB have recently evolved tremendously. Ga-maglobulin preparations with high concentrations of preventive antibodies (Respigam) or humanized monoclonal RSV specific antibody (Palivizumab; Synagis) may be used prophylactically (11). Research on the parenteral and nasal RSV vaccines is currently at the stage of phase I and II. There is no effective and routine vaccine at present, but there is much hope for the near future (6,12). Understanding the epidemiology of RSV in our population will guide us in using costly therapeutic agents as well as timing the vaccinations which may be available in the future.

In this prospective study, we aimed to investigate the role of RSV in patients with clinical AB in Bursa as well as its epidemiology and to compare the diagnostic valves of value of different methods.

Material and Methods

A total of 76 infants younger than two years of age who were seen with the clinical diagnosis of vi-ral AB at the Pediatric Emergency RoomSadece acilde değil of Uludag University Hospital between December 1999-March 2001 were included. Those patients with nonspecific viral upper respiratory infections followed by wheezing and diffuse sibilant rhonchi on physical examination and hyperaeration on the chest X-ray were diagnosed as AB. A verbal approval was obtained from the parents of these infants and a study form was filled out. The clinical and laboratory evaluation of the patients were done according to this form. The patients with positive bacterial throat or blood culture, high CRP (>1 mg/dl) or probable other bacterial infection were excluded.

Blood was drawn and nasopharyngeal secretions (NS) was obtained at the time of presentation. Normal saline 2 ml was dispersed from one nostril and at least 1 ml of NS was obtained from the other nostril through an aspiration catheter to be saved in plastic tubes. Blood samples were centrifuged at 3000 rpm for 5 minutes and the supernatant was separated. All samples were stored at -200C until they were assayed.

Those patients with an O_2 saturation of lower than 90% and no clinical improvement after three doses of inhaled salbutamol or racemic epinephrin were admitted to the hospital.

All patients were tested for the presence of RSV antigen in NS, and anti-RSV IgM antibody in sera. Two blood samples obtained 14 days apart were tested for anti-RSV IgG in 16 patients. All tests were performed in the Serology Laboratory of Microbiology Department at Uludag University. RSV antigen was detected by using Test Pack® (Abbott). Euroimmune® kits were used to detect anti-RSV IgG and anti-RSV-IgM antibodies in sera. Anti-RSV-IgG levels of above 20 RU (relative units)/ml were con-sidered positive.

The results were studied by standard statistical methods. (SPSS statistics program, version 6.0, Chicago, IL, USA) This study was approved by the Committee of Ethics of the Uludag University Faculty of Medicine.

Results

Out of the 76 patients with AB, 56 (73.6%) were male and 20 (26.4%) female. The mean age at di-agnosis was 9.4±5.3 (X±SD)(range: 2-24 months). The age distribution was 2-6 months in 20 (26.3%) patients, 7-12 months in 40 (52.6%) patients, and 13-24 months in 16 (21.1%) patients.

RSV antigen in NS were found positive in 36 (47.3%) patients. Anti-RSV IgM was detected in an additional 3 patients whose NS were antigen-negative adding up to 39 (51.2%) patients with AB. Four patients tested positive for anti-RSV IgM only one of whom had a positive antigen in the NS. Out of the16 patients whose anti-RSV IgG concentration could be assayed two times 14 days apart, 2 (12.5%) patients had increased concentrations of anti-RSV IgG on the second sample. The initial anti-RSV IgG was negative (<20RU/ml) in both patients which increased to 32 and 34 RU/ml. One of these patients tested positive and the other negative for RSV antigen in NS. However, the RSV antigen negative pa-tient had a positive IgM. Of the 16 patients who had two IgG tests, 5 (31%) had positive and 11 (69%) had negative RSV antigen test in NS.

Male/female ratio was 31/8 in RSV positive patients, and 25/12 in RSV negative patients (p>0.05). Seventy out of 76 (92%) patients presented between October and April and 6 (8%) between May and September. Among the 70 patients seen between October and April, 36 (51%) had RSV and 34 (49%) did not. Similarly, among the 6 patients who presented between May and September, 3 (50%) was posi-tive and 3 (50%) negative for RSV. The month of presentation between the RSV positive and negative patients was not different (p>0.05). However, 36 out of 39 (92%) of the patients with RSV presented between October and April.

The mean age of the patients positive for RSV was 9.6±6 (2-24 months), and the mean age of RSV negative patients was 9.2±4.7 (2-24 months). The age of patients with and without RSV was not signifi-cantly different (p>0.05). Forty two (55%) patients were admitted to hospital and 34 (45%) were treated as outpatient.

The cost of antibiotics and diagnostic tests were calculated for every patient. The cost of antibiotics (excluding the cost of injections and personnel) was 45±30 USD in RSV positive patients, and 90.5±90.5USD in RSV negative patients. One test for RSV antigen cost ~13.3 USD, anti RSV-IgM 4.58 USD, and anti-RSV-IgG (two assays) 6.6 USD. The total cost even when all tests were done on one patient was less expensive than an unnecessary antibiotic treatment.

Discussion

AB is one of the most common causes of hospitalization before one year of age and the most af-fected age range is between 2 and 6 months (11). RSV is also encountered in adults and it has been found to be positive in 7% aged 18-60 years with 84% symptomatic (74% upper respiratory infection, 26% lower respiratory infection, and 40% febrile) (13).

In many countries, RSV has been reported to be the most common cause of AB and the role of other viruses is very limited. The possibility of RSV causing lower respiratory infections (AB, pneumo-nia) is higher in younger infants. RSV infections manifest as lower respiratory infections in 32% of in-fants younger than 1 year and 23% of the infants 3-4 years old (14). In 10 developing countries, RSV has been responsible for 70% of the lower respiratory infections in patients younger than 5 years of age (15). RSV positivity in respiratory infections in Turkey is between 11-50% (8,9).

Seasons are important for RSV infections. RSV epidemics in northern hemisphere occur between October and June. The time interval between the epidemics may as short as 7-12 months or as long as 13-16 months (15). Similarly, 92% of our patients with AB presented between October and April. There-fore, it may be assumed that the epidemic season in our region is from October to April. Therefore, in terms of cost-effectivity, it may be appropriate to use the RSV diagnostic methods for patients with AB and viral pneumonia especially during this time period. In addition, high risk patients may require pro-phylactic measures such as palivizumab and RSV-specific immunoglobulin between these months.

RSV positivity has been detected at least once in 25% of small premature babies and 52% of infants with bronchopulmonary dysplasia in Germany (2). In the same country, RSV has been detected by PCR in 12% of hospitalized children younger than two years of age over a period of three years. An underlying risk factor has been defined in 25% of these patients (3). Among the patients with respiratory infection less than two years of age in Brazil, RSV has been detected in 21% over a period of three years (4). The inci-dence of RSV in NS in a similar group of infants in Jordan has been reported to be 21% (5).

In our study, RSV incidence was 51.2% of which 47.3% was defined as antigen positivity in NS (by EIA), and 3.9% defined as anti-RSV IgM positivity in serum. This is the highest incidence defined to date in Turkey. This study is also the first prospective study in our country which includes the second large patient population.

AB is a relatively benign disorder and less than 5% of the patients without a risk factor require hos-pitalization. The high rate of admission (50%) in our study could be due to the fact that our hospital is a tertiary care center and more severe cases were referred.

It is important to detect RSV in patients with AB. Fast and accurate methods to diagnose RSV en-ables the physician to take precautions to prevent dissemination of the disease in the hospital and select appropriate antiviral treatment in high risk patients such as premature, immune compromised, post-transplant patients as well as those with congenital heart disease. Viral culture is the most accurate method for the diagnosis of viral respiratory infections, and RSV can be demonstrated by culture. How-ever, there are some disadvantages of culture for RSV such as unstability and sensitivity of the microorganism to environmental insults, delays in obtaining the results (3-7 days), and the necessity for a good laboratory and experienced staff (16). The possibility of cultivating the microorganism in viral cultures is highest in the earlier stage of infection because of viral shedding and decreases after 1-3 days from the beginning of the symptoms. This factor is important in high risk patients as well as epidemiological studies (17). In addition, viral culture is not available in most hospitals on a routine basis.

In general, the sensitivity of antigen detection methods (IFA or EIA) is considered to be higher than viral culture (16). When urgent or early diagnosis is important especially during RSV seasons, fast antigen tests are preferred. The sensitivity and specificity of antigen tests in comparison to culture for the diag-nosis of RSV ranges between 60-95% (frequently 80-90%) (6,10). Although IFA is reported to be slightly more sensitive than EIA in some studies, the specificity, predictive value, and the clinical interpretation of the two methods are comparable. The fact that EIA is more effective in suboptimal samples, its sim-plicity, the presence of an objective end-point, compatibility for automation, and availability of the re-sult in 15-20 minutes are the advantages of this method. Therefore, it may be performed at bedside to provide rapid diagnosis (16,18,19). The sensitivity and specificity of EIA for RSV (Abbott Test Pack®) com-pared to culture are 86.8-97%, and 88-98% respectively (20-22). The detection of RSV by reverse tran-scriptase PCR (RT-PCR) method provides high sensitivity and specificity, but it is expensive, has con-tamination risk, and requires high technology laboratories. Therefore, it is not a routine diagnostic method (17).

Many viral infections may be diagnosed by the detection of specific IgM antibodies with rapid sero-logic tests on acute phase blood samples. IgG and IgM concentrations measured by different methods such as IFA and EIA as well as neutralization and complement fixation are consistent. However, EIA is the method that is most commonly used (16). RSV IgM antibodies generally appear 5-8 days after the be-ginning of the symptoms and stay in blood for weeks. Therefore, especially in young infants, blood tests performed early in the course of the disease may not reveal antibodies. In contrast, culture and antigen tests are more advantageous at this stage because of higher viral shedding. In addition, false positive RSV IgM tests have been reported in the first month of life because of cross-complex-binding due to the presence of maternal RSV IgG (16). This situation may occur with both IFA and EIA methods. Cranage and Gardner have detected RSV IgM antibodies in 73% of their patients by EIA whereas Hornlesh has detected in 77%23, (24). On the other hand, Welliver has reported the frequency as 13% in 1-3 month-old infants, 50% in 3-6 month-olds, and 71% in 6-12 month-olds (25). In Branderburg's study, 45 babies were followed from birth to 6 months and 32 developed RSV infections only one of whom (3%) tested posi-tive for RSV-IgM. A wide range of 0-22% IgM positivity has been determined by various methods (such as viral neutralization, RSV IgM, RSV IgG, and ELISA) (26). The diagnosis of RSV in patients with AB by RSV IgM positivity differs from 3 to 71% and increases with age. Meurman et al have reported RSV IgM antibodies in 73% of their patients which appeared in serum 10-20 days after the beginning of the symptoms. Some patients may not develop antibodies until the 20th day while others may continue having positive antibodies 68 days after (27). In this study and others, IgM response has been lower in in-fants younger than one year of age and the

antibody concentration has increased with age (25-27). The fre-quency of IgM positivity in our study was markedly lower than other studies. This could be due to the young age of our patients (mean 9 months, 79% less than 12 months) as well as testing early in the course of the disease. These results as well as our findings show that IgM response is not reliable in early diagnosis and its sensitivity is variable.

Specific RSV IgG antibodies may be helpful in diagnosis. A positive result or a 4-fold increase in 2-4 weeks may imply the diagnosis. However, time required for antibody formation, the probable inhi-bition of antibody formation in the infant by maternal antibodies, and drawing blood twice are the dis-advantages of this method. In addition, maternal antibodies in the first few months of life may cause false positive results. Meurman's study has shown increasing IgG concentrations in 92% of the patients with positive RSV antigen in NS. The maximum increase has occurred at 20-30th days of the disease, but antibody increase has not happened in infants younger than 4 months of age who had high maternal antibody titers (27). In general, diagnostic serologic response has been detected in 50% of infants between 1-3 months of age, and 85-90% in older infants (16). The fact that only two of our patients had increasing IgG concentrations could be due to the small number of patients, early testing at 14 days instead of 4 weeks, or inhibition by maternal antibodies.

Although the incidence of RSV in boys and girls are equal, severe bronchiolitis is more common in males. The higher incidence of AB (73%) in males in our tertiary care center supports this view.

Cost-effectivity is the most important data of medical treatment all over the world. The rational use of resources without disregarding scientific approach is most important in countries with limited re-sources. Rapid diagnosis of viral lower respiratory tract infections will avoid unnecessary and costly antibiotic therapy and repetitive microbiological, serological, and other laboratory tests. In addition the duration of hospital stays and nosocomial infections along with their economic burden will decrease. No studies of cost-effectivity has been done regarding this issue in our country. One of the few studies on this subject has demonstrated that rapid diagnosis of RSV, influenza A and B, parainfluenza, and adenovirus has proven cost-effective even in uncomplicated patients fascilitating a mean reduction of 0.9 days in hospital stay and a reduction of 18% in total cost (28). In our study, the cost of antibiotics was 45±30 USD in RSV positive patients, and 90.5±90.5 USD in RSV negative patients. One test for RSV antigen cost ~13.3 USD, anti-RSV IgM 4.58 USD, and anti-RSV IgG (two assays) 6.6 USD. The total cost even when all tests were done on one patient was less expensive than the cost of an unnecessary antibiotic excluding the cost of injections and staff. The benefits certainly would be much higher if po-tential nosocomial infections and additional laboratory tests were taken into account. The cost-effectivity of the serologic tests in routine care was thought to be lower in younger infants because of high frequency of false negative results. However, antigen test in NS in all infants was very cost-effective in preventing unnecessary antibiotic therapy.

In conclusion, the incidence of AB particularly those caused by RSV was highest between October and April. RSV was the causative agent in approximately half of all AB patients. The frequency of false negativity was very high in serologic tests (ELISA IgM and IgG) compared to rapid antigen test by EIA in NS. In light of our findings and the relevant literature, we suggest 1) not utilizing viral culture on a routine basis because it requires time and equipment, and 2) preferring practical and rapid antigen test in NS as the first line diagnostic method, because RSV IgM and increase in RSV IgG antibodies are less sensitive. The rapid antigen test is extremely cost-effective for the diagnosis of RSV especially in epi-demic seasons.

References

1. Update: RSV activity-United States, 1996-97 season. MMWR 1996; 45: 1053-5.
2. Berner R, Schwoerer R, Schumacher RF, Meder M, Forster J. Community and nococomially ac-quired respiratory syncytial virus infection in a German paediatric hospital from 1988 to 1999. :Eur J Pediatr 2001; 160: 541-7.
3. Weighl JA, Puppe W, Schmitt HJ. Incidence of respiratory syncytial virus-positive hospitalizations in Germany. Eur J Clin Microbiol Infect Dis 2001; 20: 452-9.
4. Cintra OA, Owa MA, Machado AA, et al. Occurrence and severity of infections caused by subgroup A and B respitatory syncytial virus in children in southeast Brasil. J Med Virol 2001; 65: 408-12.
5. Bdour S. Respiratory syncytial virus subgroup A in hospitalized children in Zarga, Jordan. Ann Trop Paediatr 2001; 21: 253-61.
6. Hall CB. Respiratory Syncytial virus and parainfluenza virus. N Engl J Med 2001; 344: 1917-28.
7. Hall CB, McCarthy CA. Respiratory syncytial virus. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's Principles and practice of infectious disesases, 5th ed. Vol. 2. Phila-delphia: Churchill Livingstone, 2000: 1782-801.
8. Yılmaz G, Uzel N, Işık N, Baysal SU, Aslan S, Badur S. Viral lower respiratory tract infections in children in İstanbul, Turkey. Ped Infect Dis J. 1999; 18: 173.
9. Baskın H, Çakır N, Amato Z, Yuluğ N. The diagnosis of Respiratory syncytial virus in a developing country. (Gelişmekte olan bir ülkede Respiratuvar Sinsitiyal Virus tanısı). Türk Mikrobiyol Cem Derg 1996; 26: 114-6.
10. Kellogg JA. Culture vs direct antigen assays for detection of microbial pathogens from lower respira-tory tract specimens suspected of containing the respiratory syncytial virus. Arch Pathol Lab Med 1991; 115: 451-8.
11. American Academy of Pediatrics. Respiratory Syncytial Virus. In: Pickering LK, ed. 2000 Red Book: Report of the Committe on Infectious Diseases. 25 th ed. Elk Greve Village, IL: American Acad-emy of Pediatrics; 2000: 483-7.
12. Piedra PA. Respiratory syncytial virus vaccines: recent developments. Pediatr Infect Dis J 2000; 19: 805-8.
13. Hall CB, Long CE, Scnabel KC. Respiratory syncytial virus infections in previously healthy working adults. Clin Infect Dis 2001; 33: 792-6.

14. Wohl MEB. Bronchiolitis. In: Chernick V, Boat TF, eds. Kendig' s Disorders of the Respiratory Tract in Children Philaedelphia: Saunders Company, 1998, pp:473-85.
15. Eric A, F Simoes. Respiratory syncytial virus infection. Lancet 1999; 354: 847-52.
16. Tristram DA, Weliver RC. In: Manual of Clinical Microbiology. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds.). 6th ed. ASM Press, Washington DC, 1995; 932-9.
17. Abels S, Nadal D, Stroehle A, Bossart W. Reliable detection of Respiratory Syncytial virus infection in children for adequate hospital infection control management. J Clin Microbiol 2001; 39: 3135-9.
18. Ahluwalia G, Embree J, McNicol P, Law B, Hammond GW. Comparison of nasopharyngeal aspirate and nasopharyngeal swab specimens for respiratory syncytial virus diagnosis by cell culture, indirect immunoflurescence assay, and enzyme-linked immunosorbent assay. J Clin Microbiol 1987; 25: 763-7.
19. Takimoto S, Grandien M, Ishida MA, et al. Comparison of enzyme-linked immunosorbent assay, indirect immunoflouresscence assay and virus isolation for detection of respiratory viruses in nasophary-geal secretions. J Clin Microbiol 1991; 29: 470.
20. Dominguez EAL, Taber LH, Couch RB. Comparison of rapid diagnostic techniques for respiratory syncytial and influenza A virus respiratory infections in young children. J Clin Microbiol 1993; 31: 2286-90.
21. Krilov LR, Lipson SM, Barone SR, Kaplan MH, Clamician Z, Harkness SH. Evaluation of a rapid diagnostic test for respiratory syncytial virus RSV): potential for bed side diagnosis. Pediatrics 1994; 93: 903-6
22. Olsen MA, Shuck KM, Sambol AR. Evaluation tf Abbott TestPack RSV for the diagnosis of respira-tory synctyial virus infections. Diagn Microbiol Infect Dis 1993; 16: 105-9.
23. Cranage MP, Gardner PS. Systemic and cell-mediated antibody responses in infants with respiratory syncytial virus infections. J Med Virol 1980; 5: 161-70.
24. Hornsleth A, Friis B, Grabualle PChr, Krasilnikof PA. Detection by ELISA of IgA and IgM antibod-ies in secretion and IgM antibodies in serum in primary lower respiratory syncytial virus infection. J Med Virol 1984; 13: 149-61.
25. Welliver RC, Kaul TN, Putnam TI, Sun M, Riddlesberger K, Ogra P. The antibody response to primary and secondary infection with respiratory syncytial virus: Kinetics of class-spesific responses. J Pediatr 1980; 96: 808-13.
26. Branderburg AH, Groen J, Steensel-Moll HA, et al. Respiratory syncytial virus spesific serum anti-bodies in infants under six months of age: Limited serological response upon infection. J Med Virol 1997; 52: 92-104.
27. Meurman O, Ruuskanen O, Sarkkinen H, Hanninen P, Halonen P. Immunoglobulin Class-Spesific Antibody Response in Respiratory Syncytial Virus Infection Measured by Enzyme Immunoassay. J Med Virol 1984; 14: 67-72.
28. Woo PCY, Chiu SS, Seto WH, Peiris M. Cost-effectiveness of rapid diagnosis of viral respiratory tract infections in pediatric patients. J Clin Microbiol 1997; 35: 1579-81.