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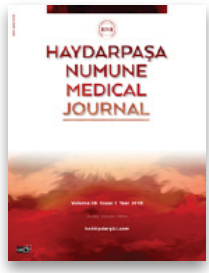

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ORIGINAL ARTICLE

Nasopharyngeal Aspiration and Nasopharyngeal Swab in the Diagnosis of RSV Children below Age 2 Presenting with Respiratory Infection Symptoms

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Abstract

Introduction: Bronchiolitis is a common, acute, contagious disease in infants and young children, which is associated with lower airway obstruction. Respiratory syncytial virus (RSV) is the main cause of viral lower respiratory tract infections in infants and children worldwide. RSV infections constitute 45-83% of all viral infections. One of the easiest and quickest methods to detect RSV is immunochromatography. Determination of the RSV antigen is frequently used in the samples taken from epithelial cells of the nose, nasopharynx or oropharynx where the virus is placed. In this study, although the aspiration of nasopharynx for the removal of the epithelium sample in RSV antigen determination is the gold standard, it was thought that nasopharyngeal swab could be used instead of nasopharyngeal aspiration to take an epithelial sample. By comparing the test results of the samples taken from both methods, we aimed to evaluate the usability of the nasopharyngeal swab as an alternative to aspiration.

Methods: In this study, 298 infants aged between 1-24 months who were admitted to the hospital presented with acute bronchiolitis, bronchopneumonia and upper respiratory tract infection were included. Both nasopharyngeal aspiration and swab samples were taken from each patient. Immunochromatographic methods were used in the first 15-30 minutes in the emergency laboratory. Statistical analysis of the results was performed using SPSS for Windows 10.0 statistical package program. Fisher's exact test, chi-square test and Mc Nemar tests were used in the comparisons. If the obtained p-value was less than 0.05, the result is significant; if the p-value was greater than 0.05, the result is considered meaningless.

Results: The frequency of RSV was 54% (161 cases). The maximum number of RSV detected was in the first six months of age. The test results were evaluated according to the time of onset of symptoms and 2-8 days were more RSV positive cases. Nasopharyngeal aspiration, which is the gold standard in the diagnosis of RSV, had a higher sensitivity and also lower false negativity than the nasopharyngeal swab. While 45.6% of RSV cases were determined by nasopharyngeal aspiration method, 39.6% of them were detected by nasopharyngeal swabs, and the difference between them was significant ($p<0.05$). When the cases were evaluated according to the age group and physical examination findings, within the first six months, the sensitivity of the swab samples taken from those who had pharynx hyperemia were higher, and the false negativity was lower.

Discussion and Conclusion: In our study, the findings suggest that the samples taken with nasopharyngeal aspiration yielded better results than the samples taken with the swab. However, in some special conditions and clinical symptoms, nasopharyngeal swabs have been shown to be preferable to aspiration which is a more traumatic method. It has been concluded that nasopharyngeal swabs can be applied superiorly to nasopharyngeal aspiration in children in the first six months of age, especially with pharynx hyperemia. Similar studies need to be performed with a higher number of cases because there is not enough study in the literature. The results obtained in our study will contribute to future studies.

Keywords: Bronchiolitis; immunochromatography; nasopharyngeal aspiration; nasopharyngeal swab; RSV.

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Respiratory syncytial virus (RSV) is the major cause of viral lower respiratory tract infections (LRTI) in infants and children worldwide. As a result of serological studies conducted in many regions of the world, it has been revealed that RSV infection can occur in all kinds of geographies and climates. In developing countries, approximately 4 million children die each year under the age of five due to LRTI, according to World Health Organization data[1].

RSV infection is frequently seen in children under 2 years of age, and nearly half of the infection progresses from the upper respiratory tract to the lower respiratory tract; and approximately 2% of the patients need to be hospitalized for this reason. On average, the mortality rate is 0.5-1.5% in those who are hospitalized[2,3]. The majority of RSV-related hospital admissions are infants younger than six months, especially born at the beginning of the RSV season[4].

Laboratory diagnosis of RSV infections is particularly important for public health and economically. With the rapid diagnosis of the infection, the discontinuation of the overuse of antimicrobial agents, early antiviral treatment in children with severe disease, prevention of nosocomial spread, and early discharge of children with appropriate treatment are provided[5].

Various methods are available for RSV determination, such as nasal-wash fluid, nasopharyngeal swab and nasopharyngeal aspirates. Achieving nasopharyngeal secretions by aspiration method is stated as the gold standard in RSV determination[6].

Although nasopharyngeal aspiration sampling is the gold standart in RSV determination, it is a difficult method to apply, especially in small babies. During the procedure, a decrease in arterial oxygen saturation and vasovagal symptoms (bradycardia, hypotension, shock, etc.) may occur due to temporary hypoxemia. Pulmonary aspiration may develop during saline administration.

It is also a painful and invasive procedure that requires more materials, and with higher costs. It was thought that obtaining the material by swabbing instead of nasopharyngeal aspiration would be easier, economical, less invasive and painless. In this study, RSV antigen was analyzed with immunochromatographic rapid antigen test in materials collected by swab method and aspiration methods and the results of both material obtaining methods were compared.

Materials and Methods

Patient Group

A total of 298 infants aged 1 to 24 months who were admitted to the pediatric emergency unit of our hospital with acute bronchiolitis, bronchopneumonia and upper respiratory tract infection, and who were discharged from the emergency room without the need for long-term inpatient treatment or who were hospitalized in the infants' service, were included in the study.

Babies with a history of recurrent wheezing, those with serious comorbidities (sepsis, meningitis, etc.), infants with severe neurological and metabolic disorders, children with previously known immune deficiency, those younger than 1 month and older than 24 months of age, and babies whose family consent could not be obtained were excluded from the study.

Collection of Data

The families of the babies who met the inclusion criteria were informed about how to collect nasopharyngeal swab and aspiration samples, and their consent was obtained. The anamnesis of the patients in the study group were taken from their families in detail. As anamnesis information, age, gender, complaints at the time of application, duration of complaints, number of family members, number of siblings, who the primary care provider was and care conditions, nutritional history, whether the child has additional disease and the presence of concurrent upper or LRTI in family members was questioned and recorded. Physical examination findings of the cases were added.

Collection of Samples

Nasopharyngeal aspiration and swab samples were collected from the patients included in the study. Bloody samples were not included in the study as it may affect the test result while sampling.

Collection of Nasopharyngeal Aspirate

The baby was placed in the supine position and his hands and head are fixed by an assistant. A polyethylene nasogastric feeding catheter with a diameter of 6 or 8 FR suitable for the age of the child was shortened according to the length of the nasopharynx and advanced from any nostril to the nasopharynx. The nasopharynx was washed with 2 ml sterile injectors containing 0.9% NaCl (saline) and the fluid was aspirated and collected back into the injector.

Taking a Nasopharyngeal Swab

The baby is placed in the supine position and his hands and head are fixed by an assistant. With the help of a sterile throat swab stick with cotton tip and a tongue press, sam-

ples were taken from both medial tonsillar and posterior uvula regions of the nasopharynx by making 2-3 rounds with the stick.

Detection of RSV Antigen by Immunochromatographic Method in the Obtained Materials

Immediately after the samples were taken, they were studied in the emergency laboratory of our clinic in accordance with the prospectus information, with the Smartest Diagnostics RSV Strip rapid immunochromatographic test. Test results were recorded in the study form. Control line in samples studied with rapid immunochromatographic test which didn't turn positive were considered as inadequate samples and these cases were not included in the study.

Principle of the Immunochromatographic Test

Smartest Diagnostics RSV Strip rapid immunochromatographic test is used for qualitative detection of RSV fusion protein antigen in nasopharyngeal aspiration and swab samples. The RSV test strip contains a monoclonal antibody specific for RSV, which is conjugated to colloidal gold particles. There are also immobilized polyclonal antibodies in the test region of the strip. If RSV antigen is present in nasopharyngeal samples, this antigen forms an antigen-antibody complex with monoclonal antibodies conjugated with colloidal gold particles. This antigen-antibody complex moves towards the immobilized antibodies on the test strip. Pink-purple streaking in this area indicates that the test is positive. The remaining conjugate also moves towards a second antibody site on the test strip, called the control region, likewise creating a pink-purple streak. The presence of this second streak indicates that the test is working properly.

Statistical Analysis

Statistical evaluations of our study were made using SPSS for Windows 10.0 statistical package program. Fisher's Exact test, chi-square test and Mc Nemar tests were used for comparisons. The p value less than 0.05 is considered significant, and when it is greater, it is considered insignificant.

Ethics Committee Approval

In order to conduct our study, approval was obtained from the ethics committee of the Ministry of Health Istanbul Bakırköy Obstetrics and Pediatrics Training and Research Hospital.

Results

58.1% of the patients (173 cases) were male and 41.9% (125 cases) were female. Boy/girl ratio was 1.38, mean age was 7.3+5.0 (range: 30 days-23 months) months. The patients were divided into 2 groups according to their age: The number of those who were within the first 6 months of age was 158 (53%), and the number of those who were 7 months and over was 140 (47%). In general, the majority of the patients who applied to our clinic were in their first 6 months of age (Table 1). As a result, no significant difference was found between age groups and genders in terms of RSV positivity (p>0.05).

The period when the patients were admitted intensively for bronchiolitis and bronchopneumonia were in late autumn and in November, December and January in winter. Among 161 RSV-positive cases, 17 (85%) of 20 patients who presented in April were RSV positive, and 28 (70%) of 40 patients who presented in December were RSV positive, and they were found statistically more significant than in the other months (p<0.05).

In addition, when the 161 RSV positive cases were analyzed according to the distribution of the seasons, the number of RSV cases in the winter and spring was found to be significantly higher (p<0.05).

RSV positivity was found to be statistically significantly higher in patients who presented in the first 5 days af-

Table 1. Distribution of RSV positive cases by age group and gender

RSV	Negative		Positive		Chi-square	p
	Number of cases	%	Number of cases	%		
Age					0.30	0.582
First 6 months	75	47.5	83	52.5		
7 months and older	62	44.3	78	55.7		
Gender					0.119	0.730
M	81	46.8	92	53.2		
F	56	44.8	69	55.2		

ter the onset of their complaints compared to those who presented later, while there was no statistically significant difference in complaints such as fever, cough, runny nose, wheezing, shortness of breath and voice change.

Throat redness, postnasal drip and acute otitis media, which are among the upper respiratory tract physical examination findings, were found more frequently in RSV positive cases, while there was no difference between RSV (+) and RSV (-) cases in terms of tonsil redness and conjunctival redness.

When the RSV antigen test results of the cases were examined, the number of cases with RSV positivity in nasopharyngeal swab was 118 (39.6%) and the number of cases with RSV positivity in the nasopharyngeal aspiration was 136 (45.6%). While the number of positive results in the swab sample and negative results in the aspirate was 25 (15% of the RSV positive cases), the number of cases with positive results in the aspirate and the negative result in the swab sample was 43 (27% of the RSV positive cases), and the number of those detected positive by both methods was 93 (58% of RSV positive cases).

54% of the patients (161 cases) who were admitted to our clinic were the number of all RSV positive cases. Nasopharyngeal aspiration method was found to be slightly superior to the swab method in the detection of RSV positive cases (Table 2). The difference was statistically significant (p=0.038).

When both sampling methods were compared according to age groups (<6 months, >6 months); there were 83 cases with RSV positivity in the patient group younger than 6 months, and they constituted 51.5% of the total RSV positive cases. As can be seen here, RSV positivity in the age group of 6 months and under showed a slight increase in percentage. The number of RSV positive cases with nasopharyngeal aspiration in patients younger than 6 months was 67, and it constituted 80.7% of RSV positive cases under 6 months of age (Table 3).

The number of RSV positive cases with nasopharyngeal swab in patients younger than 6 months was 60, and it constituted 72.3% of RSV positive cases under 6 months of age (Table 3). In children younger than 6 months, no statistically significant difference was found between aspiration and swab methods in RSV detection (p>0.05) (Table 3).

Table 3. Comparison of aspiration and swab methods in patients younger than 6 months

Smaller than 6 Months	Nasopharyngeal aspirate		
	Negative	Positive	Mc Nemar p
Nasopharyngeal aspirate			0.337
Negative	75 (25.1%)	23 (7.7%)	
Positive	16 (5.3%)	44 (14.7%)	

There was also no statistically significant difference between aspiration and swab methods in detecting RSV in children older than 6 months (p>0.05) (Table 4).

When we compared both methods according to upper respiratory tract symptomatology; while there was no statistically significant difference between both sampling methods and RSV positivity in cases under 6 months of age, nasopharyngeal swab method and total RSV positivity were found to be statistically highly significant in cases where we detected pharyngeal hyperemia in patients under 6 months of age (p<0.001). In the group with or without pharyngeal hyperemia with over 6 months of age, there was no statistically significant relationship between RSV positivity with nasopharyngeal aspiration or swab method (p>0.05) (Table 5).

Total RSV positivity was found to be statistically highly significant with both sampling methods in cases with postnasal drip under 6 months of age (p<0.001). In this group, no superiority of each technic to each other could be determined. RSV positivity was found with a higher rate in children with postnasal drip in the 6-24 months age group in samples taken by nasopharyngeal aspiration method

Table 2. Comparison of both sampling methods in RSV positive patients

RSV antigen	Those who had nasopharyngeal aspiration sampling		Significance
	Negative	Positive	Mc Nemar p
Thosw who had nasopharyngeal swab sampling			
Negative	137 (46%)	43 (14.4%)	0.038*
Positive	25 (8.4%)	93 (31.2%)	

Table 4. Comparison of aspiration and swab methods in children older than 6 months

Bigger than 6 Months	Nasopharyngeal aspirate		
	Negative	Positive	Mc Nemar p
Nasopharyngeal aspirate			0.061
Negative	62	20	
Positive	9	49	

(p<0.05). Nasopharyngeal aspiration method was found to be superior to nasopharyngeal swab in this group (p<0.05).

Discussion

Bronchiolitis is a common, acute, contagious disease in infants and young children that involves the lower respiratory tract and results in obstruction of the small airways. Viral agents are often held responsible in the etiology of LRTI. RSV is also a major cause of viral LRTI in infants and children worldwide. RSV infections constitute 45-83% of all viral infections. RSV is followed by parainfluenza virus, influenza A and B and adenoviruses[7].

The estimated number of infections would reach the peak in December and January. Considering the seasons, RSV infections are common in late autumn, winter and early spring, reaching a peak in the winter months[8,9]. In a study conducted in Istanbul province in our country, 88% of the cases with acute bronchiolitis were reported to be admitted to the clinic between November and April[10]. In our study, RSV bronchiolitis cases were seen more frequently in winter and spring, especially in December and April, compared to other months.

In the RSV direct antigen screening study using the rapid immunochromatographic test, cases that were detected by nasopharyngeal swab and aspiration methods alone or jointly were accepted as RSV positive. RSV positive cases constituted 54% (161 cases) of all cases.

In a study conducted by Dereli et al.[11] in Izmir in the winter season of 1993-1994, they found RSV positivity to be 29.2% in 19 of 65 patients hospitalized for acute bronchiolitis between 2-24 months of age, with cell culture and direct fluorescent antibody method. In another study conducted by Yarkın et al.[12] in the Çukurova region, they detected RSV by RSV IgM ELISA in 20 (24.7%) of 81 children under 2 years

Table 5. Comparison of nasopharyngeal aspiration and swab methods in patients with pharyngeal hyperemia who are younger than 6 months and older than 6 months

Less than 6 months	Pharyngeal hyperemia Absent		Pharyngeal hyperemia Present			
	Number of cases	%	Number of cases	%	Chi-square	p
Nasopharyngeal swab					19.24	0.001
Negative	63	78.8	35	44.9		
Positive	17	21.3	43	55.1		
Nasopharyngeal aspirate					2.51	0.113
Negative	51	63.8	40	51.3		
Positive	29	36.3	38	48.7		
RSV					12.34	0.001
Negative	49	61.3	26	33.3		
Positive	31	38.8	52	66.7		
Bigger than 6 months					**Chi-square**	**p**
Nasopharyngeal swab					1.46	0.226
Negative	41	64.1	41	53.9		
Positive	23	35.9	35	46.1		
Nasopharyngeal aspirate					0.74	0.388
Negative	35	54.7	36	47.4		
Positive	29	45.3	40	52.6		
RSV					0.82	0.364
Negative	31	48.4	31	40.8		
Positive	33	51.6	45	59.2		

of age with lower respiratory tract infection. When studies conducted in other countries were evaluated in terms of RSV, in the study conducted by Zelaya et al.[13] from El Salvador, 32 (76%) of 42 children under 12 months of age, who were hospitalized due to lower respiratory tract infection were found to have RSV infection with DFA. In a study conducted by Kuroiwa et al.[14] in Japan, RSV antigen was found positive by immunochromatographic method in 66 (64.7%) of 102 children between the ages of 8 days and 9 years with symptoms of lower and upper respiratory tract infections. In another study conducted by Aldous et al.[15] in 2004 in the USA, RSV antigen was investigated by immunochromatographic method in 310 children aged between 15 days and 6 years who presented with symptoms of upper and lower respiratory tract infection, and 102 of them (32.9%) were found to be positive.

Although the RSV rate in our study (54%) was in line with the results of the general literature, it had higher rates than in Dereli et al.[11], Yarkın et al.[12] and Aldous et al.[15]'s studies, and showed consistency with the rates in Zelaya et al.[13] from El Salvador and Kuroiwa et al.[14] from Japan's studies.

Routinely used methods in the laboratory diagnosis of RSV infections are: isolation of the virus in cell culture, direct or indirect fluorescent antibody method, rapid antigen detection tests with enzyme immunoassay, antigen determination by immunochromatographic test, serological methods and PCR method. Shell-vial cell culture has been developed as a rapid culture technique as an alternative to classical cell culture, and research on the use of molecular techniques continues in recent years.[16]

One of the most commonly used methods in the laboratory diagnosis of viral infections is viral antigen determination. Various methods are used for viral antigen determinatio. Immunofluorescence, enzyme immunoassay and immunochromatographic methods are currently the most commonly used methods due to their advantages[17]. Immunochromatographic test has advantages over other RSV antigen diagnosis methods.

In the direct immunofluorescence method, the need for an experienced technician, the need for fluorescence microscopy, and the experience to collect sample containing sufficient amount of nasopharyngeal epithelial cells constitute the disadvantages. The ELISA method may be preferred in that its interpretation is objective, it is faster, simpler, and allows a larger number of samples to be analyzed together than direct immunofluorescence. However, there are studies reporting that the sensitivity is less with this method, especially in samples taken with swab[18].

In the immunochromatographic method, the person performing the test does not need to be an expert, there is no need for experience, special laboratory conditions and equipment. The fact that this method is easily accessible, applicable and economical can be counted among its advantages. The test can be applied in a practical way in as little as 15 minutes. No cross reaction with antigens of other viruses has been observed. The sensitivity of this test ranges from 85-95% and its specificity varies between 95-99%[19]. The method of detecting RSV antigen by immunochromatography should be preferred in emergency departments where patient admissions are intense, due to its rapid results, high sensitivity and specificity, being economical and practical.

Throat, nose, nasopharynx swabs, respiratory tract epithelium samples taken by aspiration and washing methods can be used for rapid diagnosis of the virus. The number of epithelial cells taken with these techniques is important[17]. In the literature, obtaining epithelial samples by nasopharyngeal aspiration method is stated as the gold standard in RSV determination[6]. Our study revealed that nasopharynx aspiration is more sensitive than nasopharyngeal swab method. There are studies showing that more epithelial cells are obtained with a nasopharyngeal swab compared to a nasal swab[20]. Among these methods, nasopharyngeal swab is more advantageous because it is easy and painless, does not require special training, and is economical.

Test results in cases of bronchiolitis may be affected by age, symptoms and sampling location. In our study, it was observed that false negativity was lower in nasopharyngeal swab samples taken from patients with pharyngeal hyperemia with 6 months of age or lower, than in nasopharyngeal aspiration. No significant difference was found in children older than 6 months with pharyngeal hyperemia.

Especially in young children (under 6 months of age), aspiration method is a difficult method to apply, sufficient sample collection requires experience, requiring more equipment, and is a more invasive, more painful method, with higher cost, which accelerates the emergence of vasovagal symptoms (bradycardia, hypotension, shock, etc.), and which can cause local trauma, cough attacks and temporary hypoxemia. The nasopharyngeal swab method can be used as an alternative to aspiration method in cases with pharyngeal hyperemia under 6 months of age. Since there is no previous study on this subject in the literature, it is thought that the results obtained will contribute to the

data in future studies. Controlled studies are needed with higher number of cases to make clearer recommendations.

Ethical Committee Approval: In order to conduct our study, approval was obtained from the ethics committee of the Ministry of Health Istanbul Bakırköy Obstetrics and Pediatrics Training and Research Hospital.

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Conflict of Interest: None declared.

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