

INFECTION RATES OF RESPIRATORY SYNCYTIAL VIRUS IN PEDIATRIC PATIENTS ATTENDING PHRA MONGKUTKLAO HOSPITAL, BANGKOK

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INTRODUCTION

Respiratory syncytial virus (RSV) is the single most common cause of acute respiratory tract infection in infants and young children, and often cause life threatening acute bronchiolitis or bronchopneumonia especially in very young children (Kapikan *et al.*, 1968; Kim *et al.*, 1971). RSV infection was reported to be a major share of the resultant morbidity and mortality in infants at the time when the level of maternally derived antibody was moderately high (Kim *et al.*, 1971).

In the temperate countries the epidemic forms of this infection occurs yearly for a period of three to four months (Kim *et al.*, 1971). In the tropics especially in Thailand, it has been commonly observed that children become ill of respiratory tract infections during the rainy season, but the nature of responsible causative agents are still unknown. The study by Chaivasu and co-workers (1981) showed that most people in Bangkok had neutralising antibodies against RSV indicating that RSV infection must be common among Bangkok population. The objective of this study was to determine the magnitude of this infection in pediatric patients in Bangkok during the rainy season.

MATERIALS AND METHODS

Nasopharyngeal secretions and sera were taken from 200 pediatric patients with upper respiratory tract infections attending the

Out Patients Department of Phra Mongkutklao Hospital during May and October 1982. The patients ages varied from 2 months to 13 years.

Nasopharyngeal secretions were placed into transport media (an equal mixture of medium 199 and Eagle's medium, 2 percent fetal calf serum, 0.002M glutamine and 20 mg/ml of gentamicin), and the specimens were transported in an ice bucket to the laboratory. The specimens were processed for virus isolation as described by Parrot *et al.*, (1979). Briefly, 0.1 ml of specimen containing transport medium was inoculated into a confluent monolayer of Hep-2 cells in a screw-capped tube containing an equal volume of medium 199 and Eagle's medium plus 10% fetal calf serum and 20 µg/ml gentamicin. Characteristic cytopathogenic effect (CPE) was observed 5 to 7 days after inoculation of the specimen.

Herpes simplex virus (HSV) and adenovirus were also isolated from the nasal secretions using a technique modified from that of Rawls (1979) and Kasel (1979). Briefly, the specimens from the transport media were inoculated into a confluent monolayer of Vero cells grown in medium 199 with 10% fetal calf serum and 20 µg/ml gentamicin. CPE was observed 2 to 3 days after inoculation of the specimen.

Antibodies against RSV, HSV and adenovirus were determined by ELISA as previously described (Tantivanich *et al.*, 1980). The RSV antigen (Behringwerke AG, Marburg, Germany) was used to sensitize the

plate at a dilution of 1 : 640, and the RSV titer of > 1 : 320 was considered significant. This titer was chosen after the preliminary finding that all 20 sera with complement fixing antibody titer of less than 1 : 10 had ELISA titer lower than 1 : 320.

RESULTS

Recovery of RSV, HSV, adenovirus and coagulase positive *Staphylococcus aureus*

from the nasal secretion is shown in Table 1. RSV was isolated at a higher rate (36%) than other pathogens. After age stratification, the positive isolation rate in the age group of 2 months to 1.4 year was higher than other 2 age groups, but this difference was not statistically different (Chi Square test, $p > 0.05$). Mixed infections of RSV and *S. aureus* was found only in a small percentage of cases (1.5%).

Table 1

Causative agents isolated from nasopharyngeal secretion of patients with upper respiratory tract infections.

Age group	No. tested	Number positive with						Staph. only	Total
		RSV	HSV	Adeno virus	RSV+ staph.	HSV+ staph.	Adeno virus + staph.		
2 mo - 1.4 yr	90	37 (41%)	1	6	1	1	1	4	51
1.5 - 3.9 yr	60	22 (36%)	1	2	1	1	-	4	31
4 - 13 yr	50	13 (26%)	1	3	1	1	1	-	20
All ages	200	72 (36%)	3 (1.5%)	11 (5.5%)	3 (1.5%)	3 (1.5%)	2 (1%)	8 (4%)	102 (51%)

Table 2

Geometric mean titer (GMT) of class specific anti RSV antibodies in patients with positive RSV culture.

Age group	IgM		IgG		IgM and IgG		IgM, IgG, IgA				
	No. pos.	GMT	No. pos.	GMT	No. pos.	GMT		No. pos.	GMT		
						IgM	IgG		IgM	IgG	IgA
2 mo - 1.4 yr (24%)	22	452.6	1	320	12	806.4	746.6	-	-	-	-
1.5 - 3.9 yr (22%)	13	545.4	1	1280	3	508.0	640	3	1015.9	1015.9	403.2
4 - 13 yr (16%)	8	538.2	1	640	8	697.9	586.9	-	-	-	-
All ages	43	470.9	3	640	23	621.0	621.0	3	1015.9	1015.9	403.2

Table 3

Geometric mean titer (GMT) of class specific anti RSV antibodies in patients with negative RSV culture.

Age group	IgM		IgG		IgM and IgG		
	No. pos.	IgM	No. pos.	GMT	No.pos.	GMT	
						IgM	IgG
2 mo - 1.4 yr	2	452.5	3	403.2	4	640	640
1.5 - 3.9 yr	3	806.4	2	452.6	4	538.2	452.6
4 - 13 yr	3	508.0	2	320	3	806.4	320
All ages	8	586.9	7	390.1	11	640	467.0

The immunoglobulin class specific antibodies against RSV were determined in all 72 patients with positive RSV isolation (Table 2). Sixty nine patients (95.8%) had IgM antibodies, 43 of them had IgM antibody alone and the remaining 26 patients had a mixture of IgM antibody and other immunoglobulin classes. There was no significant difference among various age groups with respect to the proportion with IgM antibody. ($p > 0.05$, chi square analysis). Likewise the geometric mean titer among the various age group was not significantly different as determined by student's T test ($p > 0.05$). In the same age group, it was found that those with mixed classes of immunoglobulins had IgM geometric mean titer higher than those with IgM alone, but T test analysis showed that these differences were not statistically significant.

Immunoglobulin class specific antibodies were also determined in 26 RSV negative patients. Table 3 shows that 73% of them had IgM antibodies of which 30.7% had IgM only and 42.3% had a mixture of IgM and IgG. There was no significant differences in the stratified age groups with respect to the

seropositive rate or the geometric mean titer ($p > 0.05$ by Fisher's exact test or by Student T test). Among these 26 patients, 19 had no other infection whereas 3 were positive for coagulase positive *S. aureus*, 1 for *Streptococcus pneumoniae*, 1 for *Acinetobacter anitratum* and 2 for *Escherichia coli*.

Four of 11 patients with positive isolation of adenovirus had RSV antibodies, three of them (age 4 months, 10 months and 13 months) had IgG antibodies each with a titer of 1 : 320, and the remaining patient (age 6 years) had IgM antibody with a titer of 1 : 1280. One of two patients who had mixed infections with adenovirus and *S. aureus* had IgM antibody against RSV with a titer of 1 : 1280. Two cases with mixed HSV and *S. aureus* infections also had RSV antibodies, one had both IgM and IgG with a titer of 1 : 1280 and 1 : 640 respectively, and another case had only IgM antibody with a titer of 1 : 320.

DISCUSSION

In the present study, it was shown that during the rainy season, RSV was isolated

from nasopharyngeal secretions of 75 of 200 children (37.5%) with upper respiratory tract infections, of whom 95.8% had IgM anti-RSV antibody. This finding indicated that RSV is likely to be the most common pathogen responsible for the upper respiratory tract infections in children in Bangkok during the rainy season. Though the infants appeared to be more commonly affected than those in other age groups, these differences were not statistically significant ($p > 0.05$). RSV has been recognized as the most important cause of severe respiratory illness in infants and young children (Kim *et al.*, 1971). In our study, the severity of this infection in infants and young children has not been determined, because the patients had not been admitted to the hospital and follow-up study had not been carried out. It should be worth investigating to see whether in Thailand RSV infections in the infant is as severe as those in other countries.

In this study, it was shown that causative agents could be demonstrated only in 102 patients (51%). It was possible that infections caused by pathogenic agents not included in the study were responsible. Nevertheless the finding that IgM anti-RSV antibody was present in 19 of 26 culture negative patients (73%) suggested that these patients might have recently acquired the RSV infection and that at the time of taking specimens, the patients might have been able to control the infection, as a result of which RSV culture became negative.

The demonstration of mixed infections of RSV and other pathogens indicated posed a basic question which agent was the primary cause and which one played a more important role in causing the clinical symptoms.

SUMMARY

Respiratory syncytial virus (RSV) and other pathogens were isolated from naso-

pharyngeal secretions from 200 pediatric patients attending the Out Patient Department of Phra Mongkutklao Hospital with symptoms of upper respiratory tract infections. Their sera were also taken for determination of class specific immunoglobulin antibody titers. The positive isolation rates were 36% for RSV, 5.5% for adenovirus 1.5% for herpes simplex virus (HSV), and 4% for *Staphylococcus aureus*. One to 5.5% of these patients had mixed infection. Ninety five percent of patients with positive RSV isolations had IgM antibody which was found only in 30.7% in patients with negative RSV isolations. This result indicated that RSV was likely to be the most common pathogen responsible for the upper respiratory tract infections in children in Bangkok during the rainy season.

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