Identification of human metapneumovirus and *Chlamydophila pneumoniae* in children with asthma and wheeze in Singapore

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ABSTRACT

Introduction: The aim of our study was to determine if human metapneumovirus (hMPV) and *Chlamydophila pneumoniae* (CP) could be detected in Singaporean asthmatic children and wheezing infants during an acute asthma attack.

Methods: The study was performed on 30 older children (mean age 9.8 years) and 30 young children (mean age 1.3 years), who were admitted with an acute exacerbation of wheezing. Nasopharyngeal aspirates were collected and tested by polymerase chain reaction for CP, and for a panel of viruses (hMPV, respiratory syncytial virus, adenovirus, influenza virus types A and B, parainfluenza virus types 1 and 3, and rhinovirus).

Results: hMPV was isolated in eight out of 60 children (13.3 percent), while CP was isolated in two cases. Overall, 48/60 (80 percent) samples were positive for the presence of viruses.

Conclusion: In most of the children admitted because of acute wheezing, a virus could be detected. hMPV was isolated for the first time in Singapore in children who were admitted with an acute asthma attack.

Keywords: childhood asthma, *Chlamydophila pneumoniae*, human metapneumovirus, respiratory viruses, wheezing

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INTRODUCTION

Human metapneumovirus (hMPV) has been identified in many countries as a common respiratory tract virus, now one of the leading causes of lower respiratory tract infections in younger children.[1-4] *Chlamydophila pneumoniae* (CP) has also been isolated in children and its role in asthmatic exacerbations is being increasingly recognised.[5,10] The aim of our study was to determine if hMPV and CP could be detected in Singaporean asthmatic children and wheezing infants during an acute attack.

METHODS

30 older children, aged six to 16 years (median 9.8 years) and 30 young children, with median age of 1.3 years (range 0–3.4 years), were admitted to the National University Hospital, Singapore with an acute exacerbation of wheezing. They were randomly selected for the study between January 2004 and July 2005. Nasopharyngeal aspirates (NPA) were collected, and tested by polymerase chain reaction (PCR) for CP, and for the following panel of viruses: hMPV, respiratory syncytial virus (RSV), adenovirus, influenza virus types A (IA) and B (IB), parainfluenza virus (PIV) types 1 (PIV-1) and 3 (PIV-3), and human rhinovirus (HRV). Ethics approval was obtained from the hospital ethics board prior to the commencement of the study. Consent was obtained from parents of all the participating children.

Viral RNA in the NPA specimen was amplified by a two-step reverse transcriptase PCR (RT-PCR) protocol using reagents from Invitrogen, including reverse transcriptase and the relevant virus-specific primers. The RT-PCR products were analysed by electrophoresis in a 2% (w/v) agarose gel, with positive results identified as intense bands of diagnostic sizes after ethidium bromide staining.[3,6,7] For PCR detection of CP, DNA from each NPA specimen was isolated using a QIAamp DNA mini kit (Qiagen, Hilden, Germany). The AR39 reference strain of CP served as positive control. Nested PCR, initially using outer primers CRP-OU (5′-TTGTTCATGGGAACGTGCTT-3′) CRP-OD (5′-CTTGTAGGAGTTGTTTCTGG-3′) flanking a 1038-bp target fragment, and then using inner primers CRP-IU (5′-TGCTGCAATGTTTTGTGGAG-3′) and CRP-ID (5′-TCATAGGAACAGGTGCTGG-3′), was performed using the following cycling profile for

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both reactions, i.e. 95°C for one minute, followed by 35 cycles each of 95°C for 30 s, 55°C for 30 s, and 72°C for one minute or 30 s, with a final extension of 72°C for five minutes. Positive bands of 573 bp (including the positive control amplicon) were excised from the agarose gel, DNA extracted using a QIAquick gel extraction kit (Qiagen, Hilden, Germany), and the DNA sequence confirmed by direct sequencing using an ABI PRISM BigDye Terminator kit and an ABI PRISM 377 DNA sequencer (Perkin-Elmer, Foster City, CA, USA).

RESULTS

The results are summarised in Table I. hMPV was detected in eight out of 60 patients (13.3%). In addition, the hMPV-positive patients exhibited co-existence of other viruses in seven out of eight cases. These viruses were adenovirus (1), HRV (3), RSV (1), HRV, IA (1), HRV, PIV-3 and IA (1). CP was isolated in two cases, a 14-year-old girl and a three-month-old infant. Overall, 48/60 (80%) samples were positive for the presence of viruses. We also looked for any correlation between the presence of hMPV and the severity of asthma symptoms and other symptoms, e.g. fever and cough, compared to the non-hMPV cases, but no significant results were found due to the small sample size.

DISCUSSION

We were able to detect viruses in 80% of the children who were admitted because of acute wheezing (i.e. acute asthma), suggesting that viral respiratory infections are closely linked to acute wheezing. Moreover, in the present study, hMPV has been isolated for the first time in children with asthma and wheeze in Singapore. Our incidence reports are fairly similar to that of other studies.[8] Interestingly, in our study, hMPV exists merely as a co-infection with other respiratory viruses, a finding that was only reported occasionally, but coincides with reports of dual infection by hMPV and other viruses.[9] The identification of CP also suggests that colonisation or infection with this antibiotic-treatable bacterial pathogen exists in childhood as early as infancy, a finding that is commonly ignored. In summary, it seems that both hMPV and CP play roles in asthma and wheeze in children in Singapore. Whether or not hMPV and CP contribute to the overall increase in asthma symptoms in children worldwide, including Singapore, needs to be explored.

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Table I. Individual results in the 60 patients.

No.	IA	IB	PIV-1	PIV-3	HRV	RSV	hMPV	Ade	CP
p1	-	-	-	-	-	-	+	+	-
p2	-	-	-	-	-	-	-	-	-
p3	-	-	-	-	+	-	-	-	-
p4	-	-	-	-	+	-	-	-	-
p5	-	-	-	-	+	-	-	-	-
p6	-	-	-	-	+	-	-	+	-
p7	-	-	-	+	+	-	-	-	-
p8	-	-	-	-	-	-	-	-	-
p9	-	-	-	-	-	-	-	-	-
p10	-	-	-	-	-	-	-	-	-
p11	-	-	-	-	-	-	-	-	-
p12	-	-	-	-	+	-	-	+	+
p13	-	-	-	-	-	-	-	-	-
p14	-	-	-	-	-	-	-	-	-
p15	-	-	-	-	+	-	-	-	-
p16	+	-	-	-	+	-	-	-	-
p17	+	-	-	-	+	-	-	-	-
p18	+	-	-	+	+	-	+	-	-
p19	+	-	-	-	-	-	-	-	-
p20	+	-	-	-	+	-	+	-	-
p21	-	-	-	-	-	-	-	-	-
p22	+	+	-	+	-	-	-	-	-
p23	+	+	-	-	-	-	-	-	-
p24	+	-	-	-	+	-	-	-	-
p25	+	-	-	-	-	-	-	-	-
p26	-	-	-	-	-	-	-	-	-
p27	-	-	-	-	+	-	-	-	-
p28	-	-	-	-	-	-	-	-	-
p29	+	+	-	-	-	-	-	-	-
p30	-	-	-	-	+	-	-	-	+
p31	-	-	-	-	-	-	-	-	-
p32	-	-	-	-	-	-	-	-	-
p33	-	-	-	+	+	-	-	-	-
p34	-	-	-	-	+	-	-	-	-
p35	-	-	-	-	+	-	-	-	-
p36	-	-	-	-	+	-	-	-	-
p37	-	-	-	-	+	-	-	-	-
p38	-	-	-	-	-	-	+	-	-
p39	-	-	-	-	+	-	-	-	-
p40	-	-	-	+	+	-	-	-	-
p41	+	-	-	-	+	-	-	-	-
p42	-	-	+	+	-	-	-	-	-
p43	-	-	-	+	-	-	-	-	-
p44	-	-	-	+	-	-	-	+	-
p45	-	-	-	-	+	-	+	-	-
p46	-	-	-	+	-	-	-	-	-
p47	-	-	-	-	+	-	+	-	-
p48	-	-	-	-	+	-	-	-	-
p49	-	-	-	-	+	-	-	-	-
p50	-	-	-	-	-	+	+	-	-
p51	-	-	-	+	+	-	-	-	-
p52	-	-	-	-	+	-	-	-	-
p53	-	-	-	-	+	+	-	-	-
p54	-	-	-	-	+	-	+	-	-
p55	-	-	-	-	+	-	-	-	-
p56	-	-	-	-	-	+	-	-	-
p57	-	-	-	-	+	-	-	-	
p58	-	-	-	-	+	-	-	-	
p59	-	-	-	-	+	-	-	-	
p60	-	-	-	-	+	-	-	-	
	11	3	1	10	33	3	8	4	

\+ positive result
\- negative result

IA: Influenza A; IB: Influenza B; PIV-1: parainfluenza type 1 ; PIV-3: parainfluenza type 3; HRV: Human rhinovirus; RSV: Respiratory syncytial virus; hMPV: Human metapneumovirus; Ade: Adenovirus; CP: *Chlamydophila pneumoniae*

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