ORIGINAL ARTICLE

Epidemiological and clinical features of enteroviral acute lower respiratory tract infections in hospitalized paediatric patients in a Malaysian tertiary center

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ABSTRACT

Background: Human enteroviruses (HEVs) have been recognized to cause a significant number of respiratory tract infections in many regions. Previous studies conducted to analyse enteroviral respiratory tract infections focused on outbreaks. Data in the Southeast Asian region is still rather limited to date.

Objectives: We conducted a prospective analysis to understand the epidemiological characteristics of enteroviral lower respiratory tract infections (LRTIs) among paediatric patients admitted to Hospital Ampang, a tertiary hospital in Malaysia.

Methodology: Nasopharyngeal aspirates for common respiratory viruses and throat swabs for enteroviruses were obtained for rtPCR analyses. All positive enteroviral results were then cultured for species identification.

Results and conclusion: Of the total 211 recruited patients, enteroviral LRTIs made up 8%. The clinical features of enteroviral infections are mostly clinically indistinguishable from that of respiratory syncytial virus (RSV) infections. However, RSV appears to be more lymphocytosis causing than enteroviruses (EV). We found a higher asthma incidence within the enteroviral group compared to RSV group. Enteroviral infections continue to play an important role in LRTIs in children beyond infancy and up to school age. Among the enteroviral strains, EV71 contributes a major role in enteroviral LRTIs in our center. Routine testing for enterovirus would certainly help identify a significant proportion of unexplained viral LRTIs among paediatric patients. However, further cost analyses studies would be helpful to determine if incorporating testing for enteroviruses into routine respiratory viruses panel tests is economically feasible.

KEY WORDS:
Enterovirus, lower respiratory tract infections, epidemiology, paediatrics

INTRODUCTION

The human enteroviruses (HEVs) are RNA viruses belonging to the *Enterovirus* genus of the Picornaviridae family. Human enteroviruses are known to cause a wide range of disease syndromes involving many organs. These include hand-foot-mouth disease, myopericarditis, aseptic meningitis and neonatal sepsis.[1] However, HEVs are increasingly being found to be associated with acute respiratory tract infections as well. In fact, it contributes to a substantial number of paediatric wheezing-related respiratory infections besides respiratory syncytial virus (RSV).[2] Even though enteroviruses are commonly spread by the fecal oral route, many are also shed in respiratory and salivary secretions leading to transmissions by droplets.[1,3]

In recent years, enteroviral respiratory tract infections have been given significant attention with outbreaks of EVD68 infection in many areas of the world including Asia,[4,5,6] Latin America,[7,8] Europe[9,10] and USA.[11,12] This prompted the use of the terms 'emerging' and 're-emerging' to describe these enteroviral infections. [13] Many other different strains of enteroviruses including those belonging to Coxsackie A and B have been implicated in severe or fatal viral bonchopneumonias.[15,16] Use of broad-range molecular assays eg PCR has improved detection of enteroviruses and this has allowed for more accurate epidemiological data and clinical correlation.[4]

Data on enteroviral respiratory tract infections in South East Asia is still relatively scarce and focused on outbreaks.[4,5] In our study, we seek to analyse the epidemiologic and clinical characteristics of HEV infections amongst pediatric patients admitted to a tertiary center in Malaysia for viral lower respiratory tract infections between the months of June till August 2015. We would also make clinical comparisons between enteroviral and respiratory syncytial virus infections, the latter being one of the commonest causes for pediatric hospital admissions related to lower respiratory tract infections.

MATERIALS AND METHODS

We conducted a prospective recruitment of paediatric patients aged >1 month till <12 years who were admitted to the paediatric ward of Hospital Ampang, for lower respiratory tract infections from the months of June till August 2015. This is a tertiary government hospital which serves the district of Hulu Langat in the southeast region of the state of Selangor.

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Nasopharyngeal aspirates were sampled from all recruited patients for common respiratory viruses and throat swabs were obtained for enterovirus detection. The samples were collected within 72 hours of admission to avoid picking up nosocomial viral infections. Nasopharyngeal aspirates were sampled with a disposable mucus aspirator while throat swabs were taken with a flocked swab. The nasopharyngeal aspirates were sent for pan-respiratory virus assay. Throat swabs were placed in viral transport medium then sent to the lab immediately and were kept at 4°C during transportation. Once received in the lab, the integrity of the samples were checked again by the presence of ice. PCR was conducted on the same day of receiving the sample if possible and if not, the samples were kept at 4°C for processing the next day. The turnaround time for PCR is 72 hours.

Detection via immunofluorescence antigen testing was performed for a panel of 7 common respiratory viruses which include RSV, adenovirus, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, influenza virus A and influenza virus B.

The throat swabs were sent for a multiplex pan-enteroviral rtPCR which detects strains of HEVs. The primers were targeted at detection of the 5'-untranslated region of the enteroviral genome. Reverse transcription was performed at 45°C for 15min followed by pre-denaturation at 95°C for 2 min, denaturation at 95°C for 15 sec and annealing at 55°C for 30 sec. This would then be run for a total of 45 cycles. Enteroviral isolation was also performed using cell culture assays.

The sequences of the primers used were:
1. RT Pan EV forward: 5'-TCA GGC CTG AAT GCG GCT AAT-3'
2. RT Pan EV Reverse : 5'-ACA ATA TTG TCA CCA TAA GCA GCC A-3'
3. RT Pan EV Probe: 5'-(FAM)AAA CAC GGA(ZEN) CAC CCA AGT AGT CGG(IBFQ)-3'

Lower respiratory tract infections was defined as "An acute illness with at least 1 lower respiratory tract symptom reported by a caregiver or study personnel (fast or difficulty breathing, chest wall indrawing) and/or abnormal auscultatory findings (crackles/crepitations or bronchial breath sounds)".[17,18] This may include bronchiolitis, bronchitis and pneumonia.[19]

A data collection sheet for each recruited patient was prepared to include demographic data of age, gender and registration number. Other information included were chief presenting features and duration of hospital stay, oxygen requirement, nebulization and diagnosis upon discharge. As for laboratory data, columns were prepared for inclusion of absolute neutrophil, lymphocyte, total white cell count, blood culture and also respiratory viruses and enteroviral results.

The study protocol was approved by the Malaysian Research Ethics Committee and informed consent was obtained from all parents or guardians prior to sampling.

Statistical analysis

Viral LRTIs were distributed according to different age categories for comparison. Quantitative variables were subsequently described with median and mean. For further analyses, qualitative data was cited as number and percentage. Clinical characteristics of enteroviral lower respiratory tract infections were compared with that of RSV (as they were the two largest group of patients in this study). In terms of blood tests, only blood results of patients who were positive for respiratory viruses were analysed. Fischer exact test was performed for qualitative variable comparison and t test was performed for comparison of means of quantitative variables. All statistical analyses were performed using Minitab version 17 (Minitab Inc 2015). A p value of <0.05 was regarded as statistically significant.

RESULTS

Epidemiological findings

Out of a total of 462 eligible patients who were admitted to the Paediatrics General Ward in Hospital Ampang from the months of June till August 2015, a total of 211 patients consented and were recruited into this study (recruitment rate 46%). Patients who were not recruited included those who did not consent and those who were discharged before sampling was able to be performed (for example over the weekend or public holiday). A total of 68 patients were tested positive for one or more type of respiratory viruses. Of these, enterovirus was the second largest group 26% after RSV 51%. Other smaller groups include influenza (3%), adenovirus (3%) and parainfluenza 1% (Fig 1). Co-infections made up about 16% of the cases. Enterovirus was found to be positive in 8% of the total recruited patients.

RSV infections rapidly declined beyond three years. Enteroviral respiratory cases was highest in the 1-3 years category and cause infections up till >5 years of age (Fig 2). All enteroviral co-infections were with RSV and 89% of non-enteroviral co-infections involved RSV.

Clinical findings

Respiratory infections caused by EV and RSV were the largest groups in our population and had many similarities. In both groups of EV and RSV infections, there were more males than females. Consistent with the pattern of age distribution in Fig 2, the mean age for RSV, 12.6 months is lower than that of EV LRTIs, 26.6 months ($p=0.005$) and the oldest patient in the former category was 3.5 years as compared to >8 years in the latter.

In terms of clinical course, there was no statistically significant difference between the two in terms of duration of fever, length of hospital stay and also number of days of oxygen requirement. (Table I). However, it is interesting to note that despite no difference found in terms of the mean total white cell count, enteroviral infections demonstrated a higher neutrophil to lymphocyte ratio with the reverse being true for the RSV infections.

There is a higher proportion of ex premature patients being infected by RSV compared to EV ($p = 0.0003$). Asthma was found to be higher in the EV group as compared to RSV and

Table I: Comparison of characteristics between paediatric patients who tested positive for enterovirus and RSV lower respiratory tract infections

	Enterovirus	RSV	P value
Total number, n	17	34	0.011
Age (mths) mean [range]	26.6 (3-101)	12.6 (2-38)	0.005
Gender ratio (M/F)	2.4	1.4	0.21
Fever (days) mean [sd]	3.7 [2.3]	3.9 [3.1]	0.88
LOS (days) mean [sd]	4.4 [1.7]*	3.9 [3.1]	0.49
O2 support (days) mean [sd]	1.6 [2.1]	1.8 [2.0]	0.70
TWC (x10^3/μL) mean [sd]	12.3 [4.3]	12.3 [4.0]	0.98
ALC (x10^3/μL) mean [sd]	3.9 [2.1]	6.1 [2.8]	0.006
ANC (x10^3/μL) mean [sd]	12.3 [4.0]	4.8 [3.0]	0.044
Past medical history :			
Prematurity[a] n= (%)	1 (5.9)	20(17.6)	0.0003
Recurrent[b] viral LRTI n= (%)	3 (17.6)	8 (23.5)	0.73
Asthma[c] n= (%)	3 (17.6)	0 (0)	0.03
Diagnoses :			
Pneumonia[d] n= (%)	5 (29.4)	15 (44.1)	0.37
Bronchiolitis[e] n= (%)	6 (35.3)	16 (47.1)	0.55
AEBA n = (%)	3 (17.6)	0 (0)	0.03
Episodic wheeze[f] n= (%)	3 (17.6)	3((8.8)	0.38

LOS : length of stay, O2 : oxygen, TWC : total white cell count, ALC : absolute lymphocyte count, ANC : absolute neutrophil count, LRTI : lower respiratory tract infection, AEBA : acute exacerbation of bronchial asthma.
Statistical significance is taken as p<0.05
Total number of patients recruited (swabs taken) 211. Total number with positive PCR result for one or more respiratory viruses 68.
[a] Prematurity defined as birth at less than 37 completed gestation weeks
[b] Recurrent viral LRTI defined as 3 or more lower respiratory tract infections in a year
[c] Asthma defined as a history of recurrent wheeze, cough, chest tightness or breathlessness in a child more than 2 years and demonstrating reversibility with bronchodilator and variability
[d] Pneumonia is defined as symptoms of lower respiratory tract illness of acute onset caused by infection with accompanying radiological infiltrates
[e] Bronchiolitis is defined as acute infection of the small airways in children less than 2 years old which may be accompanied by rhonchi and crepitations.
[f] Episodic wheeze is defined as discrete episodes of wheezy attacks during viral illness and with the child well between episodes and occurring in the preschool age between 1 and 5 years.
* One patient with enteroviral LRTI required ventilation in the ICU.

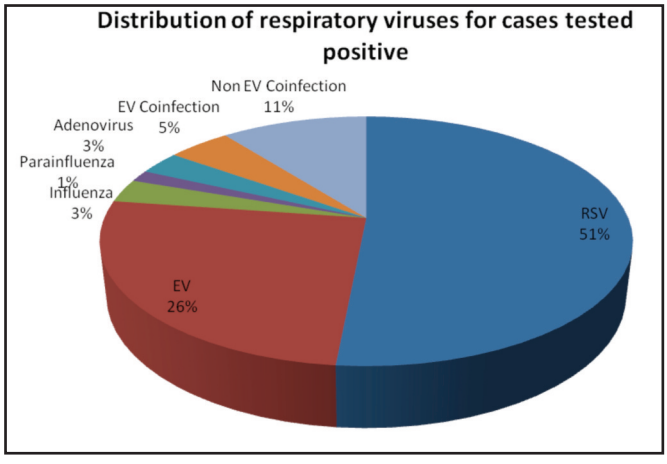

Fig. 1: Distribution of respiratory viruses for cases tested positive.

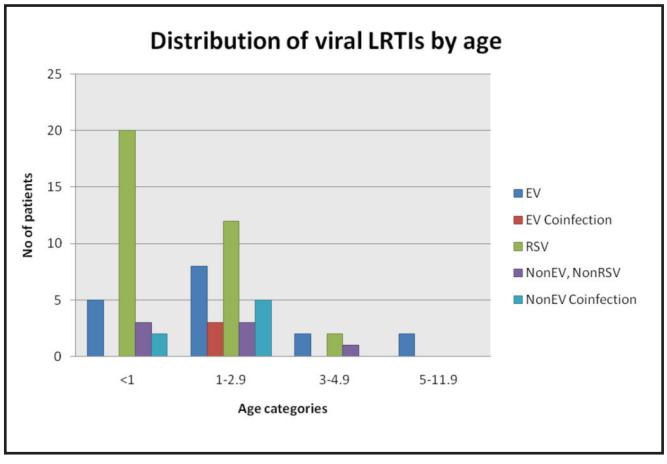

Fig. 2: Number of patients in each category of viral lower respiratoy tract infections.

it follows that a statistically higher proportion of EV patients had exacerbation of asthma in this study (P=0.03). A majority of patients in both groups were diagnosed to have bronchiolitis and pneumonia with almost equal proportions between the two. In these categories rates of both types of infections did not differ significantly. (p=0.37, p=0.55 respectively). None of the patients had positive blood cultures for bacteria in this study.

All cases of positive enteroviral PCR were cultured for viral identification. Of the 17 cases of enteroviral infections, 7 were culture positive on viral culture assays (4 EV71, 2 CoxB4 and 1 Cox B5). EV71 was the most frequent causative agent, followed by Coxsackie B4 and Coxsackie B5. However, further identification of the culture-negative subtypes by CODEHOP PCR could not be performed at our center at present due to lack of funding for certain primers.

DISCUSSION

In many parts of the world, recent attention has been given to picornaviruses like human rhinoviruses and enteroviruses as significant contributors to influenza-like illnesses (ILIs) as they represent a significant proportion of infected patients who present with both upper and lower respiratory tract symptoms.[7,8,15] However, there are not many studies examining this in the Southeast Asian region. Recently, there are reports of sporadic outbreaks of EVD68 infections and a renewed interest to explore the circulation of respiratory-related enteroviruses in the region.[4,5]

In this prospective study, we aimed to investigate the prevalence and clinical characteristics of enteroviral lower respiratory tract infections among paediatric patients in a tertiary hospital setup in Malaysia. Out of the 211 patients recruited with swabs taken, 8% were positive for enteroviral infections. When compared with other positive viral results, enterovirus made up 26% of the cases. This makes up about half of RSV related LRTIs (51%) and EV and in our cohort, appears to be commoner than the other respiratory viruses commonly tested in the standard PCR panel. In the literature, the prevalence ranged from 3% among ILI cases in one study[8] up to 12% among bronchiolitis cases[20] and as high as 25% among cases of acute expiratory wheezing in children.[21] Enterovirus co-infection made up 5% among patients tested positive in our cohort. In all cases, co-infection was with RSV. Co-infections with other viruses have been reported in other studies and these encompass a wide range including, but not limited to, human metapneumovirus, RSV, adenovirus, parainfluenza and influenza.[7,8,22]

As is widely known, there is a clear pattern of decline of RSV cases in our cohort as the patients age increase and this is most rapid beyond the age of three years. However, in comparison, enteroviral cases predominate in the age group of one to three years and, though declining in subsequent years, continue to cause infections up to school age. The cause of this is unclear but could be related to factors like the mode of transmission of the individual viruses or the virulence of the strains.

Enteroviruses have been known to be a significant causative agent for bronchiolitis and wheezing like illnesses.[2,15] This could make it difficult to distinguish from RSV which is generally regarded as the most important causative agent for bronchiolitis. Our data shows many similarities between the clinical course of both viral lower respiratory tract infections. However, it is interesting to note that RSV was more lymphocytosis-causing than EV. Further studies would be required to see if this pattern remains consistent in a larger cohort of patients.

One patient with enteroviral LRTI in our cohort was ventilated in the ICU. There were no patients in the RSV group who required intensive care. In a study by Asner *et al*, there was an overall more severe course of enteroviral/rhinoviral infections compared to other viruses.[23] They demonstrated a statistically higher oxygen requirement in enteroviral /rhinoviral infections compared to RSV but in terms of ICU admissions there was no difference between the two.

There were no statistically significant differences in terms of bronchiolitis and pneumonia rates between the two groups. However, in our cohort, there was a significantly higher proportion of asthma cases within the enteroviral group. This may be explained in part by the rapid decline of RSV rates above three years and a persistence of enteroviral cases even up to school age when diagnoses of asthma become clearer. Whether enterovirus plays a role in asthma exacerbations or pathogenesis remains unclear. However, one study has implicated EVD68 as a causative agent for cases of severe exacerbation of asthma.[24] Determination of a causal link between enteroviral LRTIs and asthma exacerbations would require a well-designed prospective study among bigger samples of paediatric patients with asthma in the future.

It is important to note that EV71 remains a major contributor to enteroviral respiratory tract infections in our center. EV71 is a known causative agent of hand-foot-mouth disease and aseptic meningitis but has recently also been implicated in respiratory tract infections in various cohorts internationally.[25,26] However, the proportion of EV71 in respiratory tract infections vary between these studies.[8,26] There remains about half of enteroviral-infected patients who did not give a positive yield by culture. This is consistent with the fact that detection of species by viral culture is lower than that compared to PCR as demonstrated in various studies.[8,15]

Although a majority of enteroviral infections are self-limiting, there are cases of potentially life-threatening enteroviral infections particularly among immunocompromised groups eg neonates. There are various proposed therapies for treatment of enteroviruses. These include interferon, immunoglobulins, capsid-inhibiting compounds and protease inhibitors.[27] Pleconaril, an oral viral capsid function inhibitor, has been shown to be well-tolerated and reduced duration and severity of illness in adults with colds caused by picornaviruses.[28] Recently, it has also been tested in neonates with enteroviral sepsis and demonstrated better survival.[29] However, whether pleconaril treatment beyond the neonatal group would improve outcome remains to be elucidated by future studies.

We recognize certain limitations to our study. The short duration does not allow us to discuss seasonality of enteroviral infections in comparison to other viruses. Our panel of respiratory viruses also did not cover other classes of respiratory viruses like rhinoviruses, human metapneumoviruses, bocavirus and coronaviruses. Lack of current funding has also limited identification of more subtypes of enteroviruses which were PCR positive but culture negative.

Enteroviruses contribute a significant proportion of hospital admissions in our cohort of children with LRTIS. Being commoner than other respiratory viruses (besides RSV), it would seem appropriate to propose that EV be incorporated into our standard panel of respiratory viruses. However, further cost analyses would be required before this is put into routine clinical practice. With increasing awareness towards enteroviral LRTIs, it is hoped that more viral isolation studies and viral subtype analyses would be carried out in the future to fully map the epidemiological burden of this pathogen.

CONCLUSION

This study provides a perspective of the burden of enteroviral respiratory tract infections amongst Malaysian paediatric patients in a tertiary hospital setting. These infections are relatively common in our center (about half the number of RSV-related LRTIs) and is clinically difficult to distinguish from infections from other common respiratory viruses particularly RSV. Comparing EV and RSV, the latter resulted in more lymphocytosis. As enteroviral respiratory tract infections contribute to a significant proportion of hospital admissions, it follows that incorporating EV into the routine respiratory viruses panel would help identify unexplained viral LRTIs. However, further cost-benefit studies would be required to see if this is economically feasible.

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