Myanmar Health Sciences Research Journal, Vol. 30, No. 1, 2018

Molecular Detection of Human Rhinoviruses in Children with Influenza-like Illness Attending Yangon Children's Hospital, 2016

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Influenza-like illness (ILI) is caused not only by influenza virus but other viruses including human rhinovirus (HRV). HRV usually causes common cold and exaggerates asthmatic attack and otitis media in children. The aim of this study was to determine the prevalence and clinical severity of HRV among children with influenza-like illness. It was a cross-sectional study conducted at Out-Patient and Emergency Department of Yangon Children's Hospital (YCH). Nasopharyngeal swab samples were obtained from a total of 153 children with ILI from January to December, 2016. Viral RNA was extracted by QIAamp® RNA Mini kits. Non-coding region of HRV gene was detected by conventional RT-PCR using Qiagen One Step RT-PCR kit. Of 153 cases, HRV was detected in 42 cases (27.5%). Males were slightly more affected than females with the ratio of male to female, 1.2:1. The maximum number of HRV cases was found in the children aged less than 5 years that accounted for 71.4%. During the study period, HRV positive cases were detected in rainy season and winter season peaking in June, November and December. Fever, cough and rhinorrhoea were observed as the main symptoms of HRV infections that were responsible for 100%, 100% and 81% of HRV cases, respectively. Gastrointestinal symptoms such as diarrhoea and vomiting were observed in 7.1% and 2.4%, respectively, of HRV-positive cases and fast breathing was observed in 2 HRV cases (4.8%). There was no HRV-positive case that presented with tightness of chest. Most of the HRV-affected children presented with low grade fever (mean=100.7ºC, SD±0.85). Clinical diagnosis of HRV cases included acute viral infection (AVI), acute respiratory infection (ARI), pneumonia and dengue haemorrhagic fever grade 1 (DHF I) accounting for 83.3%, 7.1%, 4.8% and 4.8%, respectively. This study provided baseline information about ILI cases due to human rhinovirus that would be useful for the assessment of HRV outbreak and management of children with influenza-like illness.

Key words: Human rhinovirus, Influenza-like illness, Children, Outbreak

INTRODUCTION

Influenza-like illness (ILI) is the definition used for the surveillance of influenza worldwide.[1] The viral aetiology of ILI includes not only the influenza viruses but other respiratory viruses such as human rhinoviruses (HRV), adenoviruses, respiratory syncytial virus (RSV), human enterovirus, human metapneumovirus, parainfluenza viruses and human coronavirus 229E or OC43.[2, 3] The frequency distribution of such causal viruses differs geographically. In Brazil, influenza and HRV were found to be most prevalent among ILI 39 cases. A study in Gabon stated that adenoviruses were found to be predominant among children with ILI.[4] During 2010-2013, influenza virus and

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RSV Leyte Island of the Philippines.[5] In Myanmar, 22% of ILI cases were known be caused by influenza viruses.[6] According to the studies conducted at Yangon Children's Hospital from 2013 to 2015, 6-9% of children attending YCH were due to influenza viruses. However, the aetiology of the remaining large proportion of ILI cases has not been studied.[7-9]

Human rhinoviruses (HRVs) are the common viruses causing ILI and responsible for 25% ILI cases.[10] They usually cause common cold in children and adults and trigger the asthma attacks.[11] Since common cold and influenza have some similar clinical features, there may be many cases of HRV in ILI cases with no laboratory-confirmed influenza virus.[12]

Besides, HRVs can also be regarded as the predominant viruses among children with otitis media (OM). Among the children with asymptomatic OM with effusion, HRVs were found up to 40% of the cases.[13] Besides, children with HRV infection cause more severe clinical course than other respiratory viruses and cause significant co-morbidities.[14] These findings highlight the important role of HRVs in respiratory diseases. In Myanmar, there were a few studies on HRVs among children with acute respiratory infection (ARI). A study conducted at Yangon Children's Hospital in 2014-2015 found HRVs as the predominant virus in ARI cases. They accounted for 18% of all ARI cases and 71% of the ARI cases with multiple viral infections.[15] However, there are very limited local data of ILI cases due to HRV in Myanmar. Therefore, the HRV should be studied among ILI cases to generate the baseline data for the assessment of the disease outbreak and management of ILI cases in clinical settings.

MATERIALS AND METHODS

Study design

It was a cross-sectional study conducted at Out-Patient and Emergency Department of Yangon Children's Hospital (YCH). Nasopharyngeal swab samples were obtained from a total of 153 children who attended YCH with ILI from January to December, 2016.

Inclusion criteria

Children of any age, of any sex, attending Yangon Children's Hospital with influenza like-illness were included in the study.

Working definition of influenza-like illness

Influenza-like illness was defined as fever more than or equal to 38ºC with cough or sore throat or both.[16]

Exclusion criteria

Children with cleft palate or oral thrush were excluded from this study.

Specimen collection and transport

After getting the informed consent, the medical history of child was taken from the parent or guardian and medical record. Nasopharyngeal swab specimen was taken from child according to the guidelines laid down by CDC.[17] The specimen was transported with Viral Transport Media (VTM) to the laboratory of Virology Research Division in cold condition. The VTM was prepared according to the guidelines laid down by WHO.[18] The VTM fluid was stored at -70ºC before sample processing was done.

Detection of human rhinovirus

Viral RNA was extracted from nasopharyngeal swab specimens by using RNA extraction kit (QIAmp® Viral RNA Mini Assay) according to the manufacturer's instructions. Non-coding region of human rhinovirus RNA was detected by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using specific primers. The reaction mixture contained 7.5 µl of distill H2O, 5 µl of 5X buffer, 5 µl of Q solution, 1 µl of dNTP, 0.25 µl of sense primer (CCA ACA GTA GAC CTG GCA GATG), 0.25 µl of anti-sense primer (ACG GAC ACC CAA AGT AGT TGGT), 1 µl of enzyme mixture and 5 µl of RNA template. HRV positive and negative RNA templates

were used for quality control.[19] Reverse transcription temperature was 50ºC for 45 minutes and initial denaturing temperature was 95ºC for 10 minutes. Cycling temperature was 94ºC for 30 seconds, 58ºC for 30 seconds and 72ºC for 1 minute for a total of 40 cycles. Amplicons were mixed with loading dye and were subjected to 2% agarose gel electrophoresis at 100 volts for 45 minutes. Reaction bands were visualized by molecular imager (Gel DocTM XR+, Bio-Rad).

Data entry and analysis

Data entry and analysis was done by using Statistical Package for Social Science (SPSS) version 15.0. Fisher's Exact Test was applied to determine the significance of association of data.

Ethical consideration

Ethical approval to conduct the study was obtained from Ethics Review Committee of Department of Medical Research.

RESULTS

When 153 ILI cases were tested with RT-PCR, 42 cases (27.5%) revealed non-coding region of HRV gene. Therefore, HRV was found to be responsible for 27.5% of children with ILI.

Among 42 HRV-positive cases, 23 cases (54.8%) were males and 19 cases (45.2%) were females. Ratio of male to female was 1.2:1. However, sex preponderance was not observed among them because males were also more affected in HRV negative cases. Regarding age group, majority of HRV-positive cases were under 5 years of age that accounted for 71.4%. There were 11 HRV-positive cases (26.2%) in the age group 5 to 9 years and one HRV-positive case in age group 10 to 12 years that included only a few ILI cases (Table 1).

During the study period from January to December 2016, HRV positive cases were mainly detected in the rainy season from June to September. The maximum number of positive cases was found in June that accounted for 46.1% (Fig. 1).

Table1. Demographic characteristics of influenza-like illness (ILI) cases

	HRV cases (%)	
	Positive	Negative
Sex		
Male	23(54.8)	63(56.8)
Female	19(45.2)	48(43.2)
Age (years)		
0-4	30(71.4)	66(59.5)
5-9	11(26.2)	39(35.1)
10-12	1(2.4)	6(5.4)

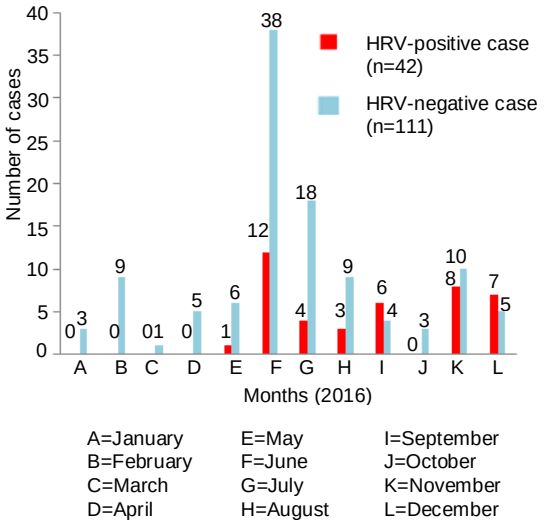


Fig. 1. Monthly distribution of influenza-like illness (ILI) cases

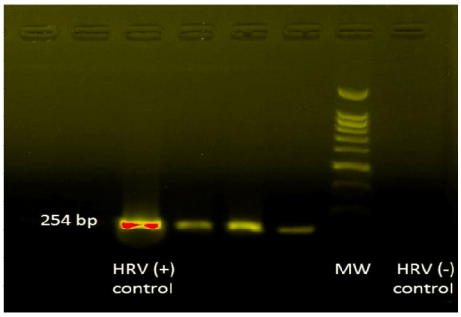


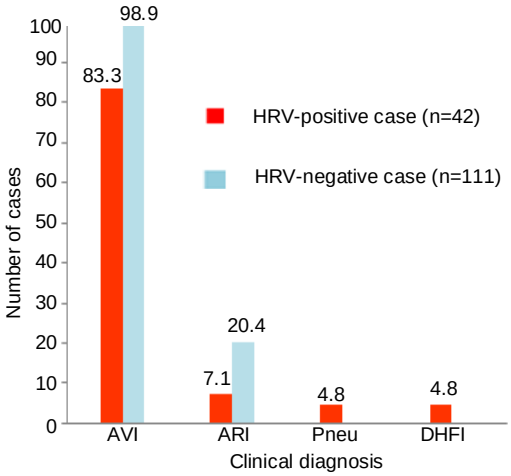
Fig. 2. Gel image showing PCR bands of HRV gene

HRV-positive cases mainly presented with fever, cough and rhinorrhoea that accounted for 100%, 100% and 81%, respectively. Gastrointestinal symptoms such as diarrhoea and vomiting were observed in 7.1% and 2.4% of HRV-positive cases and fast

breathing was observed in 2 HRV cases (4.8%). There was no HRV-positive case that presented with tightness of chest (Table 2).

Table 2. Clinical presentations of influenza-like illness (ILI) cases

	HRV cases (%)	
	Positive	Negative
Fever	42(100)	111(100)
Cough	42(100)	111(100)
Rhinorrhoea	34(81)	101(91)
Diarrhoea	3(7.1)	5(4.5)
Vomiting	1(2.4)	16(14.4)
Tightness of chest	0(0)	0(0)
Fast breathing	2(4.8)	0(0)



AVI=Acute viral infection
ARI=Acute respiratory infection
Pneu=Pneumonia
DHFI=Dengue haemorrhagic fever infection

Fig. 3. Clinical diagnosis of influenza-like illness (ILI) cases

Most of the HRV-positive cases had clinical diagnosis of acute viral infection (AVI) accounting for 83.3%. A few cases had been diagnosed as acute respiratory infection (ARI), pneumonia and dengue haemorrhagic fever grade 1 (DHF I) accounting for 83.3%, 7.1%, 4.8% and 4.8%, respectively (Fig. 3).

DISCUSSION

Acute respiratory infection (ARI) including ILI is a common cause of frequent visit to Out-Patient Setting of Yangon Children's Hospital.[20]A total of 153 children who attended the hospital due to ILI were recruited in the study. When their NP swab specimens were tested for HRV gene, 26 cases (27.5%) showed HRV positive. In other studies, HRV was found to be responsible for 16% of ILI cases aged up to 25 years and 26% of ILI cases aged less than 5 years.[20, 21] This study included the children population aged less than 13 years and prevalence of HRV was found to be 27.5%. It was observed that the prevalence of HRV depends on the age range of ILI cases. According to the previous studies conducted at YCH, influenza virus was detected in 6-9% of children with ILI attending the hospital.[7-9]

Therefore, the proportion of HRV cases was found to be larger than that of influenza cases among ILI cases attending YCH. The remaining 72.5% ILI cases may be due to other respiratory viruses such as influenza virus, adenoviruses, respiratory syncytial virus, human enterovirus, human meta-pneumo virus, parainfluenza viruses and human corona virus 229E or OC43 that were not tested in this study.[2, 3]

Among HRV-positive children, 23 children (54.8%) were males and 19 children (45.2%) were females. The ratio of male to female was 1.2:1. In agreement with this study, Miller and scientists have also found that males were slightly more affected by HRV than the females.[21] In this study, a large number of HRV cases were detected in children under 5 years of age and the detection rate was found to decrease in the older age groups. A study in New York also stated that HRV detection rate increased with the decreasing age of children.[22] In a study in Latin America, overall HRV positivity rate was greater in the children under 5 years of age than children above 5 years which is in agreement with this study.[21]

Seasonal pattern of HRV varies geo-graphically. Peak of HRV infection is seen in early fall and spring in template regions. In tropical regions, HRV showed distinct seasonality in rainy season.[23] However, HRV infection cases were detected through-out the year in Trinidad which is a country

with tropical weather.[24] This study in Myanmar demonstrated that HRV cases were detected in both rainy season and winter season especially in June, November and December highlighting the timing for preventive measures of ILI cases due to HRV infection. Regarding the clinical presentations of HRV, fever, cough and rhinorrhoea were found as the main presenting features of children with HRV. In previous studies conducted in YCH, laboratory-confirmed influenza cases were also found to present with these symptoms as their main symptoms.[7-9]

Therefore, these findings highlight the importance of laboratory confirmation for definite diagnosis finding of viral respiratory infection. However, fever presented by HRV cases is usually lower than influenza cases.[25] Similar finding was also observed by this study in which most HRV-positive children (172) presented with low grade fever (mean=100.7°C, SD ±0.85). A few HRV cases (4.8%) in this study were clinically diagnosed as dengue infection. This may be due to the constitutional symptoms such as headache, myalgia and vomiting that can be found in both HRV infection and dengue infection.[26, 27]

Apart from such cases, HRV cases were mostly diagnosed in the hospital setting as acute viral infection. So, HRV should also be considered in the management of AVI cases especially presenting with ILI.

Conclusion

This study provided baseline information about ILI cases due to human rhinovirus that would be useful for the assessment of HRV outbreak and management of children with influenza-like illness.

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