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Influenza Viruses in Children Attending Yangon Children Hospital, Myanmar during Influenza Season in 2013

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Abstract

Globally, circulating subtypes of human and avian H5 influenza viruses occasionally cause epidemics. To determine the burden of influenza virus among children with influenza-like illness (ILI) who visited a hospital in Yangon, a cross-sectional study was conducted in Yangon Children Hospital from June to August 2013. Nasal swabs were taken from 100 children with ILI and viral RNA was tested by reverse transcription polymerase chain reaction (RT-PCR). Samples positive of influenza A virus were subtyped by multiplex RT-PCR. Of 100 ILI cases, six cases (6%) revealed matrix gene of influenza A virus. Five (83.3%) out of six influenza A cases were of seasonal H3 subtype and one case (16.7%) was of pandemic H1 subtype. In 2013, majority of influenza A viruses found in ILI children was seasonal H3 subtype that was different from the previous findings during 2010-2011 when pandemic H1 subtype was predominant. The results highlighted that subtyping of influenza viruses should be continued to determine trends of predominant influenza subtype and hence, to estimate the disease outbreaks.

Keywords: influenza, children, respiratory diseases, Myanmar

Introduction

Influenza is a globally important disease that has occasionally caused epidemics and pandemics for centuries. Global burden of seasonal influenza is estimated to be 600 million cases, with 3 million cases of severe illness and 250,000-500,000 deaths per year.[1] In Myanmar, about 8-20% of influenza-like illness (ILI) cases were laboratory-confirmed to be influenza. The disease exhibits seasonality in rainy season, especially from June to August, with age prevalence of 5-9 years.[2,3]

Among many subtypes of influenza A virus, three hemagglutinin (HA), namely H1, H2 and H3, and two neuraminidase (NA), namely N1 and N2, have caused human epidemics. Due to genetic reassortment property of influenza A viruses, a new strain of a particular subtype usually emerges, resulting in influenza pandemics.[4] In 2009-2010, a new strain of H1 subtype caused pandemic, affecting about 50 million people with 10,000 deaths worldwide.[5] In Myanmar, about 90% of influenza A virus isolates in 2010 were of pandemic H1 subtype according to the data from National Health Laboratory, Myanmar.

Apart from these three HA subtypes, humans can also be infected with other subtypes of influenza A virus that mainly infect birds, which is known as avian influenza viruses. The first documented case of avian influenza H5N1 virus in human occurred in Hong Kong during 1997 while other avian influenza viruses have also caused human outbreaks in many countries.[6] Although H5N1 virus is limitedly transmitted from person to person, case fatality rate in humans is as high as 60%.[7]

During past five years, approximately 300-500 ILI cases visited Yangon Children Hospital (YCH) every year during the influenza season. It is important to determine predominant subtypes of influenza virus among people at risk in order to estimate the disease outbreaks. However, there had been limited data on subtypes of influenza virus among ILI children in Myanmar. Thus, we conducted this study to determine the predominant subtypes of influenza virus among children attending YCH in 2013. By doing so, outbreak of a particular subtype of influenza virus could be estimated and appearance of a new strain of virus could be notified timely.

Methods

The YCH is a major public children hospital in Yangon, Myanmar. A cross-sectional study was conducted at out-patient department (OPD) of YCH. We recruited all ILI OPD cases for two times per week on every Monday and Tuesday during the influenza season (June to August) in 2013.

ILI was defined as fever (oral temperature more than 100˚F) with cough or/and sore throat.[8] After a medical officer in OPD made clinical diagnosis of ILI cases, the investigators collected nasal swab specimens from them. We excluded children with respiratory distress such as severe pneumonia. During the study period, total 106 nasal swab specimens were collected from ILI children aged 6-132 months (mean age 32.7 months, SD 26.9). Among 106 samples, six specimens were excluded from the study due to inadequate amount. Thus, only 100 specimens were continued for influenza testing.

Ethical approval was obtained from Ethical Review Committee, Department of Medical Research (Lower Myanmar), Ministry of Health, Myanmar.

Transportation of Specimens

After collecting the specimens, we put them into the tubes containing viral transport media (VTM) and transported to the laboratory of Virology Research Division in Department of Medical Research (Lower Myanmar). Preparation of VTM was according to the guidelines provided by World Health Organization (WHO).[9] At the laboratory, the specimens were stored at (-70)°C until extraction of viral RNA was done.

Extraction of Viral RNA

We extracted viral RNA from nasal swab specimens by using one-step RNA extraction kit (QIAamp® viral RNA mini assay). Nasal swab specimen (140 µl), lysis buffer AVL (560 µl), absolute ethanol (560 µl), wash buffer AW1 (500 µl), wash buffer AW2 (500 µl) and elute buffer AVE (60 µl) were used according to manufacturer's instructions.

Detection of Influenza Viruses by Polymerase Chain Reaction

We detected influenza viruses in specimens by reverse transcription polymerase chain reaction (RT-PCR) using primers from matrix gene of influenza A and B viruses (Table 1). Reverse transcription and PCR amplification were done by using QIAGEN one-step RT-PCR kit. Reaction mixture contained distilled water (11 µl), 5X buffer (5 µl), deoxyribonucleotide triphosphate (dNTP) solution (1 µl), sense primer of influenza A (0.5 µl), anti-sense primer of influenza A (0.5 µl), sense primer of influenza B (0.5 µl), anti-sense primer of influenza B (0.5 µl), enzyme mixture (1 µl) and template RNA (5 µl). Temperature for reverse transcription was 95˚C for 45 minutes. Cycling temperature was 94˚C for 40 seconds, 55˚C for 40 seconds and 72˚C for one minute for a total of 40 cycles. After the amplification, we mixed 9 µl of PCR mixture with loading dye. Then, the mixture was subjected to 2% agarose gel electrophoresis at 100 volts for 45 minutes and reaction bands were visualized by Molecular Imager (Gel Doc™ XR+, Bio-Rad). RNA templates from positive control specimens of influenza A and B viruses were also amplified along with RNA of testing specimens. Samples positive of influenza A virus were included in subtyping.

Subtyping of Influenza Viruses by PCR

Subtyping of influenza A virus was done by multiplex RT-PCR using primers from HA genes of pathogenic influenza A subtypes, including pandemic H1, seasonal H1, seasonal H3 and avian H5 (Table 1).

Table 1. Primer sequences applied in typing and subtyping of influenza viruses[10,11]

No.	Primer	Sequence (5' to 3')
1	Influenza A (sense)	CTT CTA ACC gAg gAA ACg
2	Influenza A (anti-sense)	Agg gCA TTT Tgg ACA AA (g/T) CgT CTA
3	Influenza B (sense)	ATg TCg CTg TTT ggA gAC ACA AT
4	Influenza B (anti-sense)	TCA gCT AgA ATC AgR CCY TTC TT
5	Seasonal H1 (sense)	CTT gTC AgA CAC CCA Agg gTg
6	Seasonal H1 (anti-sense)	CAT CCA TCT ACC ATC CCT gTC CA
7	Pandemic H1 (sense)	CTT Agg AAA CCC AgA ATg Cg
8	Pandemic H1 (anti-sense)	ACg ggT gAT gAA CAC CCC A
9	Seasonal H3 (sense)	TgC TAC TgA gCT ggT TCA gAg T
10	Seasonal H3 (anti-sense)	Agg gTA ACA gTT gCT gTR ggC
11	Avian H5 (sense)	AAC AgA TTA gTC CTT gCg ACT g
12	Avian H5 (anti-sense)	CAT CTA CCA TTC CCT gCC ATC C

Table 2. Demographic characteristics of influenza-like illness cases in Yangon Children Hospital, Myanmar, June to August 2013 (n=100)

Characteristic	Influenza positive (n=6)		Influenza negative (n=94)		P-value
	Number	Percent	Number	Percent	
Age (year)					
< 5	6	100.0	80	85.1	Not applicable
5-9	0	0	12	12.8	
10-13	0	0	2	2.1	
Sex					
Male	4	66.7	53	56.4	0.7
Female	2	33.3	41	43.6	
Residence					
Urban	3	50.0	54	54.0	1.0
Peri-urban	3	50.0	46	46.0	

As for PCR amplification, QIAGEN one-step RT-PCR kit was also used. Reaction mixture contained distilled water (9 µl), 5X buffer (5 µl), dNTP solution (1 µl), sense primer of pandemic H1 (0.5 µl), anti-sense primer of pandemic H1 (0.5 µl), sense primer of seasonal H1 (0.5 µl), anti-sense primer of seasonal H1 (0.5 µl), sense primer of seasonal H3 (0.5 µl), anti-sense primer of seasonal H3 (0.5 µl), sense primer of avian H5 (0.5 µl), anti-sense primer of avian H5 (0.5 µl), enzyme mixture (1 µl) and template RNA (5 µl). Cycling temperature was 94˚C for 40 seconds, 55˚C for 40 seconds and 72˚C for one minute for total 40 cycles. RNA templates from each control subtype of influenza A virus (seasonal H1, pandemic H1, seasonal H3 and avian H5) were also amplified along with RNA of testing specimens.

Data Analysis

EpiData software was applied for data entry and cross-tabulation of the data. Fisher's Exact test was applied to determine significance of association between the variables and association was considered as significant when P-value was less than 0.01.

Results

A total of 100 children with ILI were included in the study and total 100 nasal swab specimens were tested for matrix gene of influenza A and B viruses by PCR. Six (6%) out of 100 cases showed positive reaction for influenza A virus and 94 cases (94%) showed negative reaction. There was no case of influenza B virus.

All six influenza positive cases were under five years of age, ranging 6-44 months (mean age 22.7 months, SD 12.7). Four cases (66.7%) were males and two (33.3%) were females. However, association of sex preponderance did not exist. Half of them were from urban area while half were from peri-urban (Table 2).

Both influenza positive and negative ILI cases presented with cough, fever and rhinorrhea. Other symptoms like vomiting, diarrhea or febrile convulsion were not seen in any ILI cases (Table 3).

Table 3. Clinical symptoms of influenza-like illness cases in Yangon Children Hospital, Myanmar, June to August 2013 (n=100)

Symptom	Number of influenza positive case (n=6)	Number of influenza negative case (n=94)
Cough	6	94
Fever	6	94
Rhinorrhea	6	94
Vomiting	0	0
Diarrhea	0	0
Convulsion	0	0

Among six cases of influenza A virus, five cases (83.3%) were of seasonal H3 subtype and one case (16.7%) was of pandemic H1 subtype. There was no case of seasonal H1 or avian H5 subtypes (Table 4).

Table 4. Subtypes of influenza virus among cases with influenza A virus in Yangon Children Hospital, Myanmar, June to August 2013 (n=6)

Subtype of influenza A virus	Number of case	Percent
Seasonal H1 subtype	0	0
Pandemic H1 subtype	1	16.7
Seasonal H3 subtype	5	83.3
Avian H5 subtype	0	0
Total	6	100.0

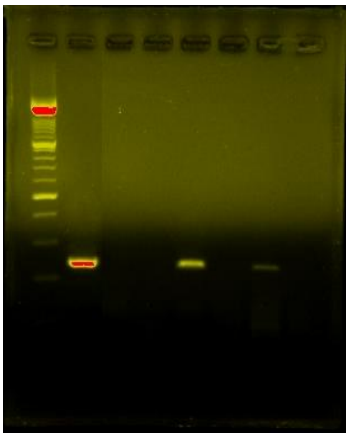

Figure 1. Gel images showing PCR bands of influenza A virus

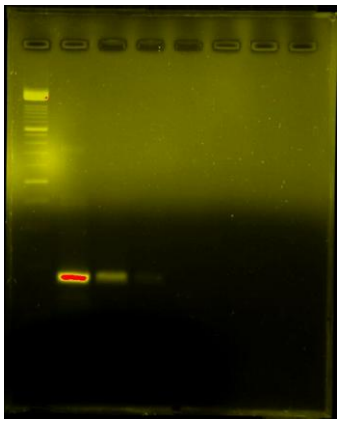

Figure 2. Gel images showing PCR bands of seasonal H3 subtype of influenza A virus

Discussion

Among 100 ILI cases, influenza viruses were detected in six cases (6%). Previous studies in Myanmar showed that influenza viruses were detected in about 8-20% of ILI cases.[2,3] This might be due to different in age prevalence of the study population. Most of the ILI cases included in this study were under five years of age (86%). There were less ILI cases in school-age which was the prevalent age of influenza.[12]

The remaining 94 influenza negative ILI cases might be due to other viral etiolgical agents such as respiratory syncytial virus, parainfluenza virus, adenovirus, rhinovirus, enteroviruses, metapneumovirus and coronavirus.[13-15] Certain bacteria species like *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae* and *Legionella pneumophila* might also be responsible for these cases although these infections are not so common in ILI cases.[14,16]

In this study, the clinical symptoms of influenza positive and negative cases were not different. Similar finding was observed in a study conducted in Myanmar during 2003-2004.[2] This proved that clinical manifestations of ILI are non-specific and accurate diagnosis of influenza needs laboratory confirmation.

All influenza positive cases in this study were due to influenza A virus and there was no case of influenza B virus. Thus, influenza virus type A could be the predominant type of influenza virus among children attending YCH in 2013. Another study conducted in Myanmar during 2003-2004 also revealed that influenza A virus was detected in majority of cases.[2] However, in a study conducted in Myanmar during 2005-2007, influenza virus type B was found to be more prevalent than type A.[3] In addition, a previous study conducted in YCH during 2010 showed that positivity rate of influenza A virus and influenza B virus were the same.[15,17] Therefore, it was noticed that prevalence of influenza virus types changed from time to time, even in the same population.

Among six cases with influenza A virus, five cases (83.3%) were of seasonal H3 subtype which could be regarded as the predominant subtype of influenza virus in the study population. Other studies conducted in Myanmar during 2003-2004 and 2005-2007 showed that seasonal H3 subtype was responsible for majority of influenza positive cases.[2,3] In agreement to these studies, a study in Alberta also found seasonal H3 subtype as the predominant subtype in January 2013 which was the peak influenza season.[18] Despite that, a previous study in YCH during 2010-2011 stated that all influenza cases were found to be pandemic H1 subtype.[19]

Limitation

Clinical presentations like myalgia and arthralgia could not be determined among ILI cases since the study population included very young children who could not complain about their illness.

Conclusion and Recommendation

In 2013, the predominant subtype of influenza A virus among children in YCH was found to be seasonal H3. The results highlighted that subtyping of influenza viruses should be continued to determine trends of predominant subtype of influenza virus and hence, to estimate the disease outbreaks.

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Suggested Citation

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