

Influenza virus types and subtypes among pediatric patients having influenza like illness in summer season

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Abstract: *Background:* Acute respiratory infections (ARIs) represent one of the major causes of childhood mortality and morbidity in Nepal. The Influenza virus is one of the common causes of viral ARIs and bacterial infection secondary to influenza contributes to majority of childhood death worldwide. However, the diagnosis of influenza virus infection is not routinely suggested in Nepal even for children clinically presenting with influenza like illness (ILI). *Methods:* With an aim to describe the status of viral influenza in Nepalese children with ILI, a throat swab and a nasal swab specimen were collected from each pediatric ILI patients visiting to the outpatient department of Kanti Children's Hospital (KCH). Viral influenza was diagnosed by rapid antigen detection test and further confirmed by reverse transcription-polymerase chain reaction (RT-PCR). *Results:* Among 200 ILI cases analyzed, 20 (10.0%) cases were confirmed as influenza A and 5 (2.5%) were confirmed as influenza B by rapid antigen detection test. Among 20 influenza A cases diagnosed by rapid test, 16 (80.0 %) were confirmed as A/H3 type and one was identified as pdm H1N109 by RT-PCR. Similarly, 3 out of 5 influenza B positive cases in rapid test were confirmed as influenza B by RT-PCR. *Conclusion:* Though, our study does not represent whole year data, this finding suggests routine influenza testing of all pediatric ILI cases.

Keywords: Influenza like illness, Influenza virus, Rapid test

Introduction

Influenza-like illness (ILI), also known as acute respiratory infection (ARI) or flu-like syndrome is a medical diagnosis of possible viral influenza or non-specific respiratory illness causing a set of common symptoms mimicking severe acute respiratory infection (SARI). The World Health Organization (WHO) clinically defines a case of ILI as an individual presenting with fever ($\geq 37.8^{\circ}\text{C}$) and cough, sore throat, headache or muscle ache [1].

Several pathogens are implicated as cause of ILI; including, respiratory syncytial virus (RSV), human rhinovirus (HRV), influenza virus, human meta-pneumovirus (HMPV), adenovirus, picornaviruses, parainfluenza viruses, human corona viruses (HCoV) and human boca virus (HBoV) [2]. Among these viruses, influenza virus is the major cause of viral acute respiratory infections (ARIs) [2-3]. The majority of persons

infected with influenza virus exhibit self-limited, uncomplicated, acute febrile respiratory symptoms or are asymptomatic [4-5]. Severe disease and complications due to influenza infection, including hospitalization and death, generally occur in elderly, very young, and individuals with underlying medical conditions [6].

Although influenza virus infection alone can lead to death, most of influenza-related deaths are the result of either exacerbation of underlying medical conditions or invasive co-infection with another infectious pathogen, particularly secondary bacterial infections [5]. In Nepal, ARI is most common cause of death in children less than 5 years old [7], but data are limited on mortality associated with influenza. In this study we report high rate of Influenza virus infection in Nepalese children presented with ILI.

Material and Methods

Patients and specimens: A total of 200 children with ILI were investigated during April through August 2014. A throat swab (if no running nose) or a nasal swab (in running nose) was collected from each patient after taking consent. Specimen for rapid test was tested immediately after collection and for confirmatory test was placed in viral transport media (Copan Universal Transport Media) on maintained cold packs and then transported to NIC (National Influenza Center, Teku, Kathmandu) laboratory and refrigerated at 2-8 °C before testing for influenza viruses types and sub-types by real-time RT-PCR. Patient’s demographic and clinical information were recorded using structured questionnaire.

Rapid influenza testing: The swab specimens were processed for the onsite rapid diagnosis of influenza virus infection by using Quickvue Influenza A+B test (Quidel Corporation, San Diego, USA). This is a qualitative one-step procedure for detecting group A & B nucleoprotein antigens. Standard procedures given by the manufacturer were followed for the rapid diagnosis of influenza virus.

RNA Extraction and Real Time PCR Assay: Total RNA was extracted using the QIAamp Viral RNA mini kit (QIAGEN GmbH, Hilden, Germany) according to manufacturer’s instructions. Briefly, reverse transcription at 50°C for 30 minutes, Taq inhibitor inactivation at 95°C for 10 minutes followed by 45 cycles at 95°C for 15 seconds, and 55° C for 30 seconds. Rotor-Gene 6000 Corbett, Australia was used for Real time PCR assay analysis.

The RnaseP primers/probes were used as positive internal control; positive control (PC) and negative template controls (NTC) were included in each run for the validity of experiment. The primers and probes for influenza viruses (H1N1, H3N2, H1N1 pdm09, H5N1, and influenza B) provided by US CDC and assay were carried out according to US CDC protocols [8]

Statistical analysis: Data management and analysis was performed by using SPSS (Version 16.0, SPSS Inc.). P≤0.05 was considered statistically significant.

Results

Altogether 200 pediatric ILI cases (age 3 month to 15 year, mean 4.6) were analyzed in this study, of which 68% were male. Among these ILI cases, the most common symptom was fever (100%), followed by dry cough (83.5%) and rhinorrhea (16.5%), while the least common symptom was sore throat (10%) (Table-1).

Patient characteristics	Frequency (%)
Age group (years)	
<5	114 (57.0)
≥5	86 (43.0)
Sex	
Male	136 (68.0)
Female	64 (32.0)
Clinical symptoms	
Fever(≥ 37.8°C)	200 (100)
Dry Cough	167 (83.5)
Rhinorrhea	33 (16.5)
Sore throat	20 (10.0)

Among total ILI cases, 25 (12.5%) were diagnosed as viral influenza by rapid antigen detection test (Table 2). Although no statistical difference in rate of influenza was observed among age groups ($p>0.05$), 40% of influenza cases were less than 5 year old. Among 25 influenza cases, influenza A was more common (80%) than influenza B ($p<0.05$). ILI patients who were identified as viral influenza by rapid antigen detection kit were further subjected to RT-PCR. Among 20 influenza A cases diagnosed by rapid test, 16 (80.0%) were confirmed as A/H3 type and one was identified as pdm H1N109 by RT-PCR. Similarly, 3 out of 5 influenza B positive cases in rapid test were confirmed as influenza B by RT-PCR.

Age group (yrs)	Influenza A	Influenza B	Total
<5	8 (40)	2 (40)	10 (40)
≥ 5	12 (60)	3 (60)	15 (60)
Total	20 (80)	5 (20)	25 (100)

Numbers in the parentheses indicates %

Discussion

Influenza-like illness (ILI) is a common clinical symptom in children presenting to hospital of Nepal. Although viral infections are one of the common causes of ILI, the role of viral agents in causing ARIs has not been extensively studied in Nepal. It is often difficult to diagnose respiratory viral infections in young children on the basis of clinical grounds, particularly due to the non-specific and overlapping signs and symptoms with other respiratory viral infections that also occur frequently during an influenza season. With the advent of rapid diagnostic kits, it is common practice in most developed and resourceful health facilities to diagnose ILI suspects for possible viral influenza. However, influenza testing is not currently suggested in routine diagnosis of children who are presenting with ILIs in the healthcare facilities of Nepal. In this study, we report 12.0% viral influenza cases among pediatric ILIs patients who visited in an outpatient department of a children hospital in Nepal.

Influenza A is more common cause of viral influenza than influenza B [3, 9]. We also found a higher rate of influenza A (80.0%) than influenza B (20.0%). In this study, we only tested ILI cases for possible viral influenza. Considering that the viral influenza may also occur in some ARI cases which do not meet case definition, true rate of influenza-associated morbidity among Nepalese

pediatric patients, could be even more than that is reported by this study. As children with preexisting medical conditions including chronic pulmonary, cardiovascular, renal, hepatic, neurologic, metabolic, or immune-suppressing conditions are at high risk of developing influenza-related complications [10], rate of viral influenza in these specific patients could be even higher in Nepal.

In Nepal, bacterial pneumonia is one of the most common causes of childhood deaths [7, 11]. Bacterial pneumonia and other super-infection are more common after influenza virus infection [12]. Early detection of viral influenza in a children suspected of viral ARIs may greatly help in patients care and minimize possible bacterial co-infection related morbidity and mortality [13-14].

Conclusion

Though, our study does not represent whole year data, this findings suggests routine influenza testing of all pediatric ILI cases.

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