Original Article

Dominance of human Influenza H1N1pdm09 in flu like patients during early two months of 2020

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ABSTRACT

Introduction
Influenza is a worldwide respiratory infectious disease which affect all age groups and associated with significant number of morbidity and mortality each year. The circulating subtypes varies countrywide yearly, and it helps policy maker to get preparedness for early effective management of influenza epidemics.

Materials and methods
Nasal swabs were collected from 463 patients in January and February 2020 presenting flu-like symptoms and Rapid Influenza Diagnostics Tests (RIDTs) were performed for influenza A & B screening as a routine test. Then influenza A subtyping was done by RT-PCR followed by gel electrophoresis of 27 influenza positive samples.

Result
Among 463 cases, 106 (22.9%) were Influenza positive with huge (99.06%) dominance of Influenza A. Subtyping of randomly selected outpatient derived 27 Influenza A positive cases showed flourishing presence of seasonal Influenza A/H1N1pdm09 (21; 77.8%).

Conclusion
This small study warrants further elaborate investigation to know circulating influenza A subtyping in the country which may assist health care providers in making treatment decisions and hence, appropriate patient management.

Keywords: *Influenza Virus, H1N1pdm09, ILI, SARI, Influenza A subtype, Bangladesh.*

INTRODUCTION

Influenza is an infectious viral illness that affects the upper and lower respiratory tract. It has been considered as a major cause of flu-like illness since last century. Acute respiratory infection is a dominant public health hazard particularly in developing countries and influenza has an important impact on it[1,2]. Influenza usually presents with fever, runny nose, sore throat, muscle pain, headache, cough, fatigue, vomiting, diarrhea etc. Symptoms may range from mild to severe cases such as respiratory failure that may lead to death. Most influenza cases manifest mild illness except a few requiring hospitalizations[3,4]. Complicated cases of Influenza are more common in children below 6 months of age. However, children under 5 years and older people more than 65 years are also vulnerable. Pregnant women, and people who have chronic illness such as asthma, heart diseases, obesity, kidney disease, liver diseases and diabetes are also in high-risk group[5]. The CDC documented that seasonal influenza was responsible for 24,000-62,000 deaths during 2019-2020 season[6]. Influenza remains as the most common viral infections affecting 5%-15% of global population resulting in an estimated deaths of 250000 to 500000 each year[7,8]. In the United States, influenza causes infections of 5%-20% population resulting in an average mortality of 36000 annually[9].

Influenza is an RNA virus of Orthomyxoviridae family and categorized four types A, B, C and D. Although these subtypes are antigenically different, but they produce similar symptoms. Influenza epidemics are mostly caused by type A virus and smaller localized outbreaks caused by type B virus. Type C is the causative agent of mild respiratory symptoms in humans whereas type D usually not found in human, only seen in pigs and cattle. Influenza A is further subtyped mainly into three categories – H1N1 (H1N1pdm09 & H1N1 seasonal - two common strain of H1N1), H3N2 & H5N1 based on their presence of two surface antigens- hemagglutinin (H) and neuraminidase (N). Influenza B has two lineage- B/Yamagata and B/Victoria. Type A influenza is generally considered worse than Type-B influenza because the symptoms are often more severe in Type-A than Type-B and researchers suggest that most

adults have considerable immunity against Type-B Influenza[10].

Although, a number of rapid tests are available to detect the virus, the test may show false negative results where polymerase chain reaction (PCR) that detects the viral RNA more accurately[11] . Currently, the diagnostic tests can be done by specialized laboratories in many countries. Reverse transcriptase polymerase chain reaction (RTPCR) provides the most timely and sensitive detection[12]. Nowadays, flu subtyping Real Time PCR Kit is also available which is used for specific identification and differentiation of Influenza A subtypes in respiratory samples. RNA is extracted from specimens, amplified using RT-PCR and detected using fluorescent reporter dye probes specific for Influenza A/H1N1pdm09, seasonal H1N1 & H3N2.

Bangladesh is a tropical country in the Northern Hemisphere, and influenza is found all through the year and its annual seasonal influenza epidemic occurs in monsoon period, from May to September[13]. Laboratory diagnosis of influenza virus, especially at the beginning of a new community outbreak has important implications for case management, such as infection control procedures, consideration of antiviral treatment options and avoiding the inappropriate use of antibiotics. This study was done to identify the influenza cases along with its distribution in different age group and to explore the circulating predominant subtype in a tertiary care hospital of Dhaka, Bangladesh. As subtype specific antiviral treatment is available, we attempt to identify the predominant subtype in our community to get appropriate preparedness for proper management of mass people during an Influenza epidemic outbreak.

MATERIAL AND METHODS

Ethical statement

This study proposal was approved by the Research and Ethics Committee of Evercare Hospital Dhaka (ERC 25/22- 1). This study was exempt from obtaining participant's consent since only leftover specimen were used after anonymization.

Patient and clinical specimen

The data of the patients were taken from hospital information system of Evercare Hospital Dhaka, Bangladesh and the study period was January & February 2020 irrespective of their age group.

Nasal swab samples were collected from all patients presenting suspected flu like symptoms by trained medical personnel in swab stick and were transported to laboratory. Duplicate swab samples of 94 patients were collected for Influenza A subtyping. Rapid Influenza Diagnostics Tests (RIDTs) were done for Influenza A and Influenza B screening. Influenza A subtyping was done on outpatient derived randomly selected 27 samples. Clinical correlation was done among ILI (Influenza like illness) and SARI (Severe acute respiratory illness) group. Though the clinical symptoms of both groups are almost similar, ILI and SARI were separated based on WHO guideline (ILI define patients' fever with respiratory symptoms and SARI define patients' fever with respiratory symptoms who requires hospitalization).

Influenza Diagnostics Tests (RIDTs)

For RIDTs test, commercially available Standard Q Influenza A/B strip kit was used from SD Biosensor, Korea. RIDTs are colored chromatographic immunoassay for the simultaneous qualitative detection of Influenza A and Influenza B and display the result as colored bands. Nasal swab stick was inserted into an extraction buffer tube provided with kit and swab was swirled for five times. To extract the liquid from swab, the swab stick was squeezed sides of the tube while removing. The used swab stick was discarded according to the biohazard waste disposal protocol. Absorption part of sample of test strip was emerged into the extraction buffer tube, left for 12 minutes and result was observed. Negative result was declared when only control line ("C") band was observed into the strip. Influenza A or Influenza B were declared when two colored bands ("A" test line for Influenza A or "B" test line for Influenza B and "C" control line band) was observed. When three colored bands ("A" test line, "B" test line and "C" control line band) were observed the test was declared as Influenza A/B positive. Test was considered invalid when control line band ('C" band) were not visible.

Influenza A subtyping

For subtyping, specimen containing swab stick was inserted into a BD Universal Transport Medium (Becton Dickinson and Company, Franklin Lakes, NJ, USA) and viral RNA was extracted for reverse transcription polymerase

chain reaction (RT-PCR). Commercially available QIAamp® Viral RNA Mini kit (QIAGEN, Hilden, Germany) was used for viral RNA extraction from sample containing transport medium according to manufacturer's instruction. PCR was performed with a OneStep RT-PCR kit (QIAGEN) using individual primer sets for H1N1pdm09, H1N1 seasonal influenza before 2009 and seasonal H3N2. The reaction components for RT-PCR were prepared by mixing 2 µL of 5× QIAGEN OneStep RT-PCR Buffer, 0.4 µL of dNTP mix, 0.2 µL of forward primer (10 µM), 0.2 µL of reverse primer (10 µM), 0.32 µL of QIAGEN OneStep RT-PCR Enzyme Mix, 5.68 µL of Nuclease-free H2O, and 1.2 µL of Viral RNA per reaction. The PCR conditions were 50 ºC for 30 min and 94 ºC for 3 min, followed by 45 cycles of 94 ºC for 30 s, 54 ºC for 30 s, 72 ºC for 30 s, and a final extension at 72 ºC for 7 min. The PCR products were subjected to electrophoresis on a 2% agarose gel. 10 µL PCR products with 2 µL gel loading dye were mixed and loaded into well of agarose gel. Hae III DNA size marker was used as ladder and electrophoresis was run at 100 V for 30-35 minutes or until loading dye run at 80-90% of agarose gel. After that agarose gel was moved to visualize gel documentation. Influenza A H1N1pdm09 was detected when band size was found at 109 bp whereas H1N1 seasonal at 427 bp and H3N2 at 210 bp.

RESULTS

Demographics

In January & February 2020, total 463 RIDTs were performed, among them 275 (59.4%) were ILI case patients and 188 (40.6%) were SARI. Age range of these patients was 14 days to 92 years. The mean age of influenza positive patient of age group below 1 year of ILI patients was 8 months and SARI was 9 months. The mean age of 1 to 92 years ILI positive patients was 20 and SARI was 25. Predominant age group of both ILI and SARI positive cases were between 1 to 10 years. Male predominance (58%) was observed in ILI positive patients whereas female predominance (59.4%) was seen in SARI (Table -1).

Clinical presentations:

Patients present with influenza like symptoms demonstrated invariably with fever along with cough, sore throat, running nose, headache, difficulty in breathing, body ache. Gastrointestinal features were also very common, such as diarrhea and / or vomiting. Fever was essential inclusion criteria and presented in all ILI and SARI cases.

Table-1: Age & Sex distribution of Influenza positive population

	ILI (n = 69)		SARI (n =37)	
AGE(Y)	**M (%)**	**F(%)**	**M(%)**	**F(%)**
<1	3(4.3)	1(1.5)	2(5.4)	0
1-10	20(29)	8(11.6)	7(18.9)	9(24.3)
11-20	5(7.2)	2(2.9)	0	4(10.8)
21-30	2(2.9)	5(7.2)	1(2.7)	0
31-40	2(2.9)	9(13)	3(8.1)	3(8.1)
41-50	4(5.8)	2(2.9)	0	4(10.8)
51-60	2(2.9)	2(2.9)	0	0
61-70	1(1.5)	0	2(5.4)	2(5.4)
>70	1(1.5)	0	0	0
Total	40(58)	29(42)	15(40.5)	22(59.4)

Table-2: Clinical presentation of Influenza and Non-Influenza cases

	ILI		SARI	
Symptoms	**Inf +ve (%)**	**Inf -ve (%)**	**Inf +ve (%)**	**Inf -ve (%)**
Fever	69 (100)	206 (100)	37 (100)	151 (100)
Cough	65 (94.2)	206 (100)	37 (100)	144 (95.4)
Cold/Runny nose	53 (76.8)	109 (52.9)	19 (51.4)	57 (37.7)
Sore throat	7 (10.1)	28 (13.6)	4 (10.8)	8 (5.3)
Breating difficulty	2 (2.9)	15 (7.3)	15 (40.5)	33 (21.9)
Headache	9 (13.0)	26 (12.6)	4 (10.8)	1 (0.7)
Diarrhoea	1 (1.4)	3 (1.5)	0	6 (3.9)
Vomiting	6 (8.7)	10 (4.9)	5 (13.5)	16 (10.6)
Bodyache	15 (21.7)	35 (16.9)	9 (24.3)	9 (5.9)

Among influenza cases of ILI patients, majority of influenza positive patients had cough (94.2%) and running nose (76.8%) followed by other features. Among SARI Influenza positive cases, breathing difficulty (40.5%) was noticeable feature along with cough (100%) and runny nose (51.4%) (Table-2).

Sub-type determination

Total 275 patients presented with influenza like illness (ILI), among them 69 (25.1%) were Influenza positive by RIDT. Influenza positivity was 37(19.7%) among 188 cases of severe acute respiratory illness (SARI).

All ILI positive cases were Influenza A positive, whereas single case of Influenza B was reported among 37 SARI positive cases. Due to shortage of reagents and lack of availability of duplicate samples from SARI patients we could subtyped 27 Influenza A positive cases from ILI patients to determine the strain of H1N1pdm09, H1N1 seasonal influenza before 2009 and seasonal H3N2. We found 21(77.8%) cases of H1N1pdm09 strain, 6 (22.2%) were unsubtyped and none of the cases were Inf A/H1N1 seasonal and Inf A/H3N2 (Table-3).

Table-3: Distribution of study population among ILI & SARI and their association with Influenza Virus

ILI (n = 275)		**SARI (n = 188)**		**TOTAL (n = 463)**	
Inf -ve (%)	Inf +ve (%)	Inf -ve (%)	Inf +ve (%)	Inf -ve (%)	Inf +ve (%)
206 (74.9)	69 (25.1)	151 (80.3)	37 (19.7)	357 (77.1)	106 (22.9)
Types (n = 106)					
Inf A	69 (65)		36 (34)		
Inf B	0		1 (1)		
Subtypes (n=27)					
Inf A/H1N1pdm09		21 (77.8)			
Inf A/H1N1 seasonal		0			
Inf A/H3N2		0			
Inf A other than 3 subtypes		6 (22.2)			

DISCUSSION

Infectious diseases are most common in Bangladesh and different viral infections are considered as prime etiological agent of flu-like symptoms in community. Just before COVID-19 pandemic situation started in Bangladesh, we screened total of 463 patients presented with flu-like symptoms during short period of two months, among them 106 (22.9%) cases were found Influenza positive. Hospital-based influenza surveillance (HBIS) in Bangladesh 2020, an average of 8% influenza positive cases were reported over the entire year which is less than previous years and lower than our study (22.9%)[14]. The possible cause of this difference may be our study period is short and just before the beginning of COVID-19 pandemic in our community.

COVID-19 is a respiratory tract infection like influenza and the preventive measures, mask-wearing, hand washing, travel restrictions and social distancing are thought to have also reduced the number of Influenza cases[15]. As pandemic control measures for COVID-19 were initiated from early 2020, patients with flu-like symptoms were more focused to rule out COVID-19 instead of other viral pathogens, so overall influenza testing and detection rate were reduced later.

Among the influenza positive cases, we found predominant affected age group was 1-10 years children but after 50 years of age, the positivity rate declined. An identical study on the relative role of different age groups in influenza epidemics showed that, children aged 5–17 had the highest influenza positivity rate and while adults aged 50–64 and 65+ had the smallest relative risk[16]. Several supported studies related to the notion were also published[17-20]. The most possible explanation of more vulnerability of children as they have large volumes of virus in their nasal secretions and, after infection viruses shed for longer period than adults. They also have poorer hygiene practices (more frequent touch of mouth and nose with dirty hands), often coughing and spluttering over those closest to them. Usually, the virus spreads by coughs or sneezes but it can also be spread by touching surfaces contaminated by the virus[21]. In mass gathering such as in school, playground children can easily transmit the virus through aerosol, intermediate objects and surfaces contaminated by the virus. In addition, their immune system is weaker than adults which increases the risk of infection.

Features of Influenza are very similar to other flu-like illness such as COVID-19, Dengue, Chikungunya and other viral respiratory tract infections. Usually, the incubation period of Influenza is 1-4 days, most commonly 1-2 days. The onset of influenza is sudden and initial symptoms are nonspecific, including fever, chills, headache, feeling discomfort, loss of appetite, lack of energy and confusion. These symptoms are accompanied by respiratory symptoms such as dry cough, sore throat, runny nose, hoarse voice. In our study, among influenza cases of ILI patients, the majority had cough (94.2%) and running nose (76.8%) followed by other features which is similar with other studies[22]. Among SARI Influenza positive cases, breathing difficulty (40.5%) was noticeable feature along with cough (100%) and runny nose (51.4%).

Influenza virus subtyping has emerged as a critical tool in the diagnosis and treatment of influenza. Nowadays majority of seasonal Influenza A/H1N1 infections are resistant to antiviral drugs. In our study we found 106 Influenza positive cases, where 105 (99.06%) was Influenza A along with a single positive case of Influenza B (0.94%). Among 105 influenza A positive cases, we did subtype of 27 cases and found 21(77.78%) cases of Inf A/H1N1pdm09 and no cases of Inf A/H1N1 seasonal or Inf A/H3N2. Unidentified subtype (Inf A other than 3 subtypes) were 6 cases (22.22%). Our study supports the predominant existence of novel pandemic strain of influenza A (H1N1pdm09) which circulates worldwide[23]. National Influenza Surveillance Bangladesh (NISB) data 2020, showed similar findings of single case of Influenza-B (0.66%) among 152 Influenza positive cases. In Influenza A positive cases, predominant circulation of Inf A/H1N1pdm09 (72.73%) in Dhaka city was reported[24]. In this study, we did not include subtype of Inf A/(H5N1) which is considered as an avian disease with rare human to human transmission[25].

Annual vaccination is the primary and most effective way to prevent influenza and Influenza associated complications, especially for high-risk group. Vaccine mainly give protection towards H1N1 and H3N2 strain and one or two Influenza B strains. From CDC data it was revealed that influenza A/H1N1 viruses were oseltamivir antiviral resistant (99.5%) but sensitive to zanamivir (100%) and adamantanes (99.4%). Influenza A/H3N2 viruses were sensitive to both oseltamivir and zanamivir (100%) but were resistant to the adamantanes (100%). So, CDC recommended empirical treatment with either zanamivir alone or a combination of oseltamivir plus an adamantine, unless the results of influenza A virus subtyping are available. Thus, subtyping of Influenza virus may help for administration of sub-type specific antiviral therapy[26].

CONCLUSION

Rapid and reliable detection of newly emerging influenza viruses is important to enhance our influenza detection. Appropriate treatment of patients with respiratory tract infection depends on accurate and timely diagnosis. Early diagnosis of influenza and its subtypes can reduce the inappropriate use of antibiotics and provide the option for proper antiviral therapy. Our findings will help to give an idea about circulating dominant subtypes (Influenza H1N1pdm09) in Dhaka city.

Large scale multicentric study is recommended to guide the policy makers for better explore, prioritize strategies for Influenza prevention and control in Bangladesh.

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DISCLOSURE STATEMENT

The authors declare no conflicts of interest with this article's content.

AUTHOR CONTRIBUTIONS

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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