Assessment of influenza virus infection in rural tertiary care hospital in North India; Two years of data analysis at Dr. Rajendra Prasad Government Medical College (DRPGMC) Kangra at Tanda, Himachal Pradesh, India

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Abstract

Introduction: Influenza testing is carried out all over the world but in developing countries including India, there is paucity in timely identification of new strains/type of influenza viruses and their active surveillance.

Aim: This study aims to determine the record based analysis for the prevalence of influenza infection and circulating subtype, in and around Kangra District of Himachal Pradesh from January 2016 to December 2017.

Materials and Methods: Nasopharyngeal and throat swabs were collected in Viral Transport Medium and RNA were extracted by using commercially available kit (Qiagen). Real Time polymerase chain reaction (RTPCR) was carried out to amplify the influenza type A, H1N1/Pdm 2009, H3N2 and influenza B.

Results: Total of 281 cases for influenza infection in the year of 2016 and 2017, 133 (47.3%) cases were positives for influenza A, 43 (15.3%) positives for influenza H1N1/Pdm2009, 8 (2.84%) positives for H3N2 and 3 (1.06%) positives for influenza B. Nine deaths were reported in the year of 2017 due to H1N1/Pdm 2009 infection while no mortality was observed in year 2016 at DRPGMC Tanda.

Discussion and Conclusion: Influenza infection is more common in age group of 18 to 50 years followed by age of > 50 years. Active testing and data interpretation at all influenza testing laboratories throughout India will help the health authorities to expedite the need of influenza vaccination. Further, aggregating the data would help in understanding the circulation of influenza virus subtype at particular geographical areas.

Keywords: Influenza infection, Real time polymerase chain reaction, Data analysis.

Introduction

Influenza viruses (family *Orthomyxoviridae*) are enveloped negative strand RNA viruses. Based on the type of nucleoprotein and M-capsid protein, influenza virus can be categorized into Type A, Type B and Type C. In influenza A and influenza B viruses, 2 surface glycoproteins namely haemagglutinin and neuraminidase are present which gives the properties of cell-host interaction and release of virus from infected cells respectively.[1,2] In India, the pattern of circulating strains varied over the years: whereas influenza A/H1N1Pdm2009 and type-B co-circulated in 2009 and 2010, H3N2 was the predominant circulating strain in 2011, followed by circulation of influenza A/H1N1Pdm2009 and influenza-B in 2012 and return of A/H3N2 in 2013.[3] There have been four influenza pandemics that occurred in 1918, 1957, 1968 and 2009.[4]

Around 5 to 15 percent of world population is affected due to morbidity and mortality caused by influenza epidemics and around 250,000 to 500,000 deaths were reported by World Health Organization (WHO) due to this seasonal epidemic.[5] In developing countries including India, influenza associated mortality is difficult to predict because patients with respiratory illness admitted to the hospital may have other complications, leading to death without detection of influenza viruses.[6] Data compiled by integrated disease control programme (IDSP) till January 2017 suggested that total 11,752 cases were found positive for influenza A (H1N1/Pdm2009) leading to 561 deaths in laboratory confirmed patients in India. Similarly in Himachal Pradesh,

five deaths were reported out of 29 positives cases for influenza A (H1N1/Pdm 2009). Keeping in mind the importance of influenza virus infection, we tried to conclude the trends of influenza infection in patients visiting to tertiary care hospital at DRPGMC Tanda, Himachal Pradesh.

Materials and Methods

The samples were received from indoor patients admitted to DRPGMC Kangra at Tanda with influenza like illness (ILI) as well as severe acute respiratory illness (SARI). Suspected cases were defined with sign and symptoms as per category-C.[7] The study was conducted on the samples received from January 2016 to December 2017.

Sample Collection, Transport and RNA Isolation

The nasopharyngeal and throat swab from each patient were collected and transported in Viral Transport Medium (Himedia, India) to viral research and diagnostic laboratory (VRDL), Department of Microbiology, DRPGMC Kangra at Tanda. The samples were processed immediately for RNA isolation. RNA was extracted from both the swabs by using QIAamp Viral RNA Mini Kit, (Qiagen, Germany) as per instructions provided in the user manual.

Real Time Polymerase Chain Reaction (RTPCR)

The primers/ probes for influenza RTPCR were procured commercially (TaqMan® Influenza A, Applied Biosystem™ (H1N1) Assay Set, USA). Primers/probes for influenza H3N2 and Influenza B were provided by VRDL resource centre, National Institute of Virology Pune, India. Five

microlitre of extracted RNA was served as template and reaction conditions were followed according to the CDC real-time RTPCR protocol[8] (ABI Step One Plus, RTPCR instrument, Applied Biosystem, USA). The enzyme mixture (SuperScript[®] III Platinum) used for reverse transcription and amplification were procured from Invitrogen, USA. 25 µl reactions for each test sample were run on RTPCR machine and fluorescence data (FAM) were collected during the 55°C incubation step. Ribonuclease P (RnaseP) was served as internal control in all reactions.

Results

Year Wise and Seasonal Influenza Cases Distribution

A total of 96 samples were referred for influenza testing in the year of 2016 while 185 samples were tested in the year 2017. Among total of 281 cases, 133 cases were found positive for influenza-A by RTPCR. Similarly, 43 cases were positive for H1N1/Pdm 2009, 8 cases were positive for H3N2 and 3 cases were positive for influenza-B virus respectively (Fig. 1).

Further, on looking into the seasonal patterns and influenza virus infections, we observed two peaks in the year 2016 starting from January to March and again from August to November. However, in year 2017 only single peak was observed starting from May to November (Fig. 2A&B).

Influenza Cases Occurrence District Wise

Geographical spread of influenza virus infection is also an important parameter to study because the influenza viruses are more likely have chances to spread in high density population area due to droplet/contact infection. We observed that in the year 2016 (Fig. 3A) maximum cases (69) for influenza testing were from Jammu District of Jammu & Kashmir while in year 2017 the maximum cases were from Kangra District of Himachal Pradesh (137) (Fig. 3B).

Age Distribution and Influenza Cases

Age distribution is another important parameter in influenza infection study. Data from two years study was compiled and showed that age group of 18-50 years was found to be more prone to influenza infections. As shown in Table 1, 37.8% of influenza-A and 67.79% H1N1/Pdm 2009 positive cases were observed in age group 18-50 years followed by 28.89% of influenza-A and 34.88% of H1N1/Pdm 2009 in the age group of > 50.
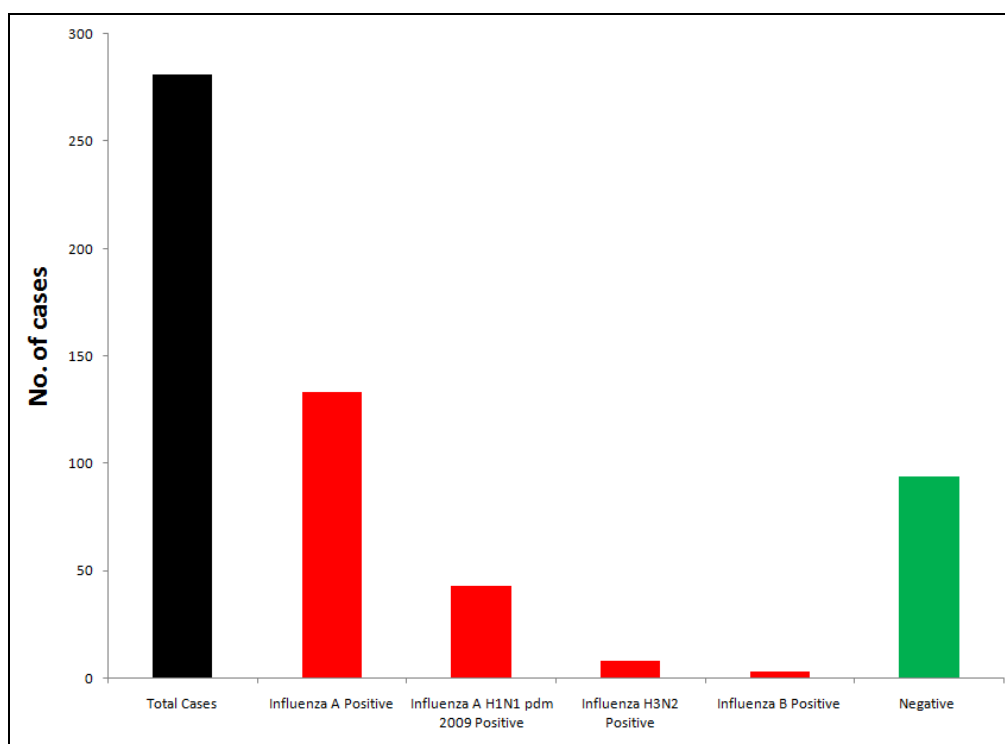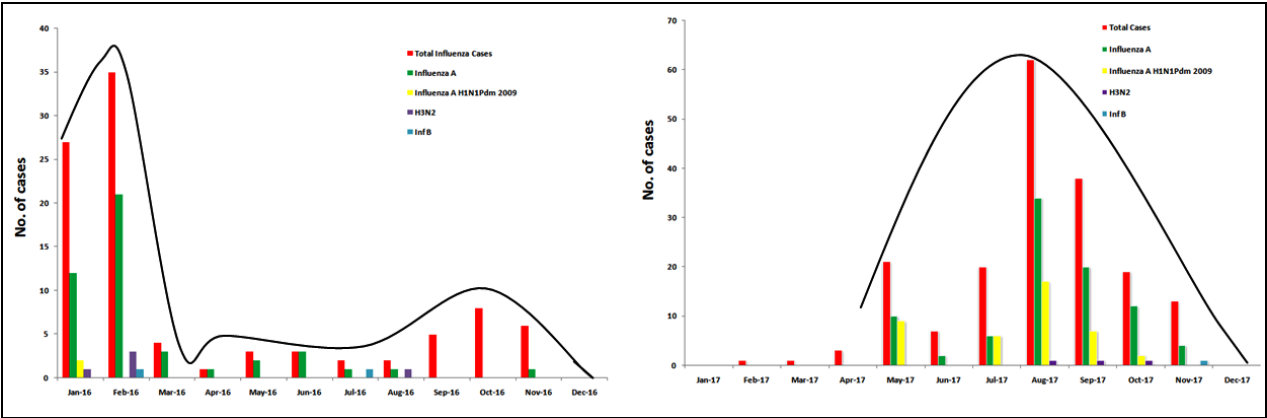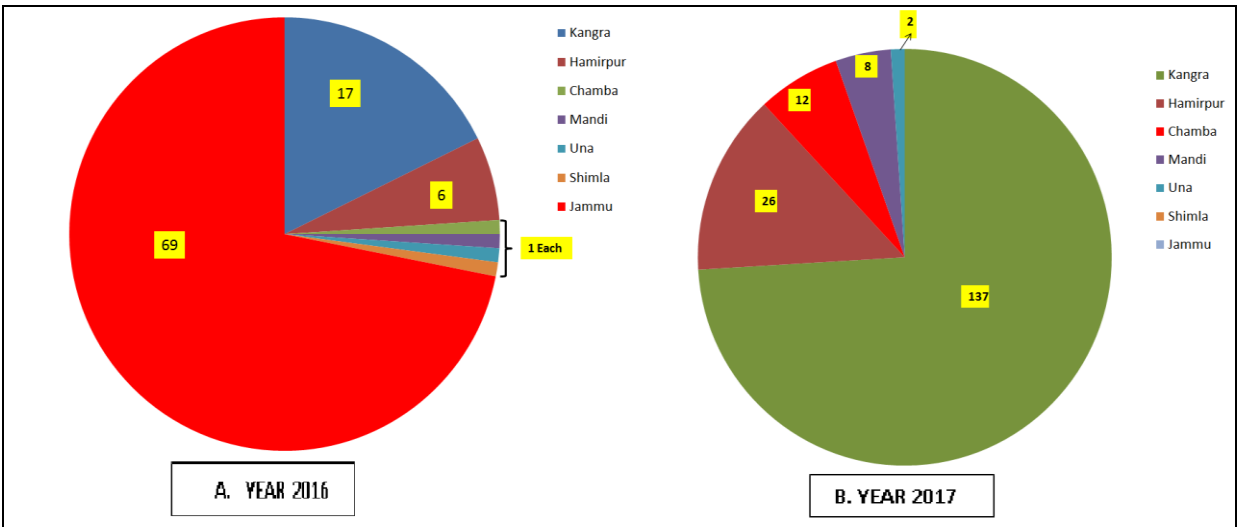

Fig. 1

Fig. 2

Fig. 3

Table 1: Age distribution and influenza cases

Influenza type	Age Groups (Years)		
	0-18	18-50	> 50
Influenza-A	30 (33.3%)	34 (37.8%)	26 (28.89%)
Influenza-A H1N1/Pdm2009	1 (2.32%)	27 (67.79%)	15 (34.88%)
Influenza-A/H3N2	3 (37.5%)	3 (37.5%	2 (25%)
Influenza-B	0 (0%)	1 (33.3%)	2 (66.6%)

Discussion

Influenza testing is important in every aspect because influenza may cause frequent epidemics and periodic pandemics. WHO has established national influenza laboratories all over the world to study the epidemiology and etiology of influenza infections.[9] Himachal Pradesh is a hill state located in north western Himalayas with population of 68,64,602 (Census 2011). DRPGMC Tanda is situated in Kanga District of Himachal Pradesh, which caters the patients from adjacent Districts including Chamba, Hamirpur, Mandi and Una. Influenza viral testing

in the year 2016 was also conducted from the samples received from Government medical college Jammu, a district of Jammu and Kashmir. Various studies have given different opinions regarding the influenza infection spread. Some study shown no defined season for influenza spread[10,11] while others reported the rainy season.[12-14] In our study, the maximum peaks of influenza cases were observed during winters in the year 2016 while in early monsoon to month of November in 2017. Similar types of trends are also shown by other researchers where they have observed one or two influenza seasonal peaks in a year.[15-17] Influenza surveillance data concluded from year 2004 to 2008 in India showed that in temperate regions influenza cases occurred during late autumn and winter months; while in Northern Hemisphere, it took place from November to March.[18]

Further, on looking into the relative proportion of age distribution and influenza positivity, we observed that the most of the H1N1 positive cases were found in the age group 18-50 years (67.79%) while in case of influenza H3N2 and Influenza B; positivity were insignificantly different in all age groups due to less positive cases (p <0.05). Preliminary findings suggested that age group of 5 to 30 years were at high risk of infection, whereas the age group of 30 to 50 years old were high risk of death due to

influenza infections.[19] Similarly, another reason for the high infection rate in age group of 18-50 years could be due to work exposure because most of the people in this age are working.[20] In present study no deaths due to H1N1/Pdm2009 were reported in the year 2016 from DRPGMC Kangra at Tanda, while nine deaths were reported in year 2017 due to H1N1/Pdm2009. Further, among nine mortality cases, five deaths were in the age group of 18-50 years and four deaths were from > 50 years of age were reported (IDSP).

In conclusion, our study depicted the high infection of influenza viruses in working age group (i. e. 18-50 years) while age group of > 50 years is also vulnerable. Among in and around areas of DRPGMC Kangra at Tanda, Kangra District has maximum numbers of positive cases for influenza infections during 2017. The early diagnosis of influenza virus is very important in developing countries including India because of very low vaccine compliance (below 30%)[21] due to deficiency in knowledge about the adverse effects of vaccine, misconceptions regarding the vaccine, concern about the efficacy and duration of vaccine. Hence, regular surveillance and early diagnosis will firstly leads to solve the ambiguity in conducting oseltamivir therapy and secondly it emphasizes on high compliance to the influenza vaccination in future.

Conflict of Interest: None.

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