Research Article

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Study of Pandemic 2009 Influenza A (H1N1) Virus by Cellular and Molecular Profiling in Uttarakhand population: Clinical influence and epidemiology.

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Abstract:

Influenza is one of the most serious and dread full disease spread throughout the world and caused by the Influenza virus. Immunologic 4 types of influenza viruses are known i.e., Category A, B, C and D, mostly affect different types of cattle and cause illness in humans. Cellular and Molecular Profiling of Influenza virus was done from the patients suffer with Influenza like illness in different parts of Uttarakhand state. During this study, 97 samples were collected from the patients for an interval of 8 month (September, 2017 to April, 2018). Firstly, RNA was isolated from the collected sample and then extracted RNA was utilized further for Real Time PCR amplification of suspected cases of Influenza A and Influenza A H1N1. 143 bp region of the influenza virus A genome and 94 bp region of the influenza virus B genome, 80 bp region of influenza virus H1 (2009 H1N1 virus) genome were targeted during amplification. After molecular analysis out of 97 samples 20.61% sample were positive for Influenza A virus, 9.27 % samples was positive for Influenza A (H1N1) and 63.91% samples were found negative for the target used. In this study, we analyzed that the highly affected population was in the age group of 41-60 (42.85%) and lowest was in 0-20 age group i.e., 12.5%. From this study it has been also observed that female populations were more susceptible to influenza virus as compared to the male. Category A virus show higher consequence of symptoms as compared to the category B, C. Highest positive case was recorded in October and September month and lowest in winter months. The virus seems to have reached a peak in September/October and has been on the decline since then.

Keywords: RT-PCR, Influenza virus, Molecular profiling, Uttarakhand

Introduction

The Orthomyxoviridae (Influenza viruses) are a major determinant of morbidity and mortality caused by respiratory disease, and outbreaks of infection sometimes occur in worldwide epidemics [1].

The first case of P-09-H1N1 positive in India was reported on 16thMay, 2009, from 23 year old passenger, who takes his trip from USA arriving at Hyderabad airport [2]. After that, the virus soon became endemic and spread to almost all major cities in India [3]. Mortality associated with Influenza virus in India was 981 in 2009, 1763 in 2010, 75 in 2011, 405 in 2012, 629 in 2013, 265 in 2016 and 1100 in 2017 in different states of the Country [4]. Mutability and high frequency of genetic reassortment and resultant antigenic changes in the viral surface glycoproteins make influenza viruses formidable challenges for control efforts. Immunologic 4 types of influenza viruses are documented so far i.e., A, B, C and D type. Influenza type A is antigenically highly variable therefore responsible for most of the cases of epidemic influenza. Influenza type B may exhibit antigenic changes and sometimes causes epidemics. Influenza type C is antigenically stable and causes only mild illness in immunocompetent individuals [5]. Influenza type D primarily affects cattle and are not known to infect or cause illness in humans [6]. Some molecular and cellular diagnostic reports on influenza virus were published from different parts of country [7-12]. Few attempts on vaccine development and prevention of influenza virus was done by some works i.e., [13-21]. For the proper

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diagnosis and management of the disease, now a days newer molecular tools and techniques are used. Influenza virus detection by RT-PCR is much more sensitive than traditional culture methods, it has a faster turnaround time and the advantages over real time RT-PCR. During epidemics of the swine flu Real Time PCR is a method of choice, which is highly sensitive, specific with fast turnaround time and can be very useful for the cases, which are hospitalized so that they can be isolated from the normal patients. Fast turnaround time also serve for the proper containment of this highly contagious virus in normal population.

Thus, the current study was carried out to study the Cellular and Molecular Profiling of Influenza Virus and to study the epidemiology with respect to hilly areas, which includes the correlation of the different parameters (season, gender, age group, temperature, humidity, pressure and wind speed) with respect to Influenza Virus and its Clinical Relevance to study its epidemiology.

Materials and Methods

During present study a total 97 Nasal and Throat swabs samples were collected from the patients suffer with Influenza like illness (ILI), meeting criteria of the symptoms as given by National Center for Disease Control, New Delhi, India. Samples were collected from September 2017 to April 2018, in different departments of Shri Mahant Indiresh Hospital Dehradun, Uttarakhand, India which includes OPDs and IPDs for the molecular profiling of Influenza virus. RNA was isolated from collected samples with the help of QIAamp Viral RNA Mini Kit (50) Cat No. 52904, with silica column extraction method. Extracted RNA was employ further for Real Time PCR amplification of suspected cases of Influenza Virus. Master Mix was prepared for the differentiation of Influenza A and Influenza A H1N1 for all the samples. 143 bp region of the influenza virus A genome and 94 bp region of the influenza virus B genome, 80 bp region of influenza virus H1 (2009 H1N1 virus) genome was targeted for study the influenza virus in each samples. Amplification was done by utilizing Rotor gene Q Real Time PCR machine for the amplification of target gene differentiating Influenza A and Influenza A (H1N1).

Results

Out of 97 samples processed during an interval of 8 months 20 samples are positive for Influenza A virus and 09 are positive for Influenza A (H1N1) and 62 samples were found negative for both the targets. Influenza A show higher breakthrough i.e., 20.61 % case during the study in both male and female individuals. But, in number of case obtained in 8 months no sex wise tendency was found in infection of virus i.e., 51.54% in female case and 48.45% in male as shown in Table 1 and Figure 1. In this study, we scrutinized that the highly affected population was in the age group of 41-60 (42.85%) followed by 21-40 (23.68%) age group and at the age of above 60 years have (17.64%) and age group of 0-20 (12.5%) only one case as shown in Table 2 and Figure 2. The entire 97 sample was examined for influenza viruses category check and found that category A show highest consequence with respect to the symptoms 53 (54.64%) followed by category C 24 (24.74%) and category B 20 (20.61%) respectively as shown in Table 3 and Figure 3. Seasonality fluctuation in Influenza viral infection was also observed during the study and it has been seen that maximum number of suspected cases for Influenza virus were

Table 1: Gender wise distribution of Influenza A and Influenza A (H1N1).

Gender	Total cases	Positive for Influenza-A virus only	Positive for Influenza-A (H1N1) virus	Negative
Male	47	06	03	41
Female	50	12	06	38
Total	97	18	09	79

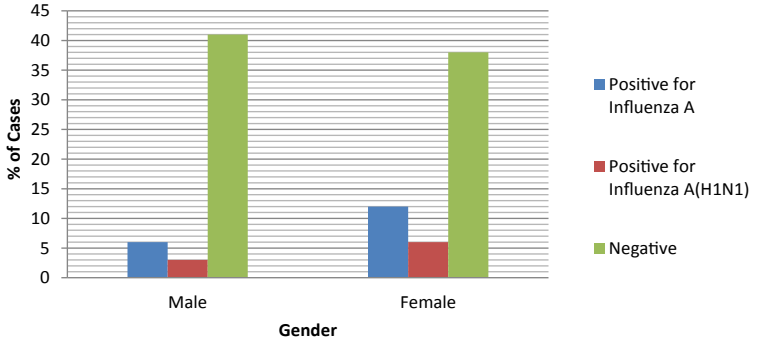


Figure 1: Percentage of Influenza A and Influenza A (H1N1) in male and female.

Table 2: Age wise distribution of Influenza A and Influenza A (H1N1).

Age group in years	Total no. of cases	Total Positive cases of Influenza A	Total Positive Cases of Influenza A (H1N1)	Negative
0-20	8	1	1	7
21-40	38	7	2	29
41-60	34	9	6	19
Above 60	17	3	0	14
Total	97	20	9	69

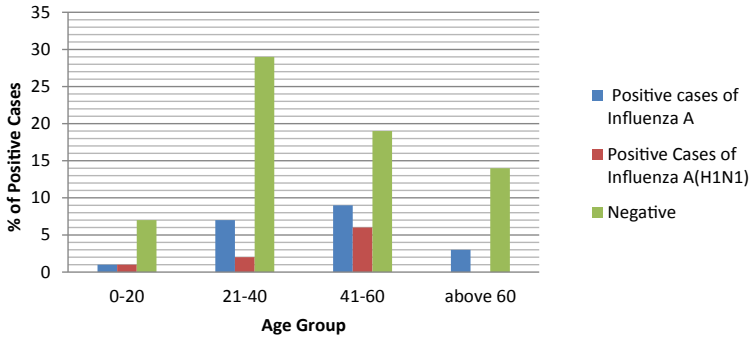


Figure 2: Percentage of Influenza A and Influenza A (H1N1) virus in different age group.

Table 3: Category wise distribution of Influenza A and Influenza A (H1N1).

Category	Total cases	Positive for Influenza A	Positive for Influenza A (H1N1)	Negative
A	53	09	06	44
B	20	06	0	14
C	24	05	03	19
Total	97	20	09	77

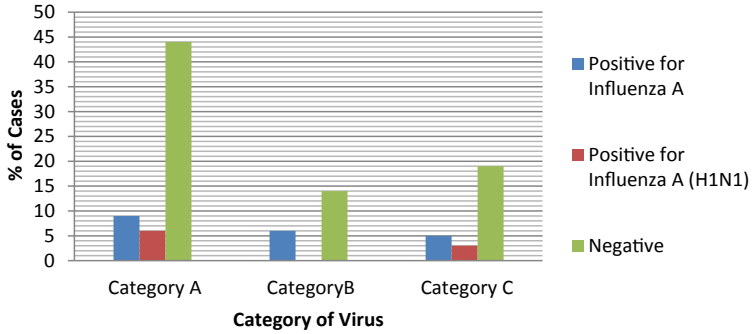


Figure 3: Percentage of Influenza-A and Influenza A (H1N1) in different category.

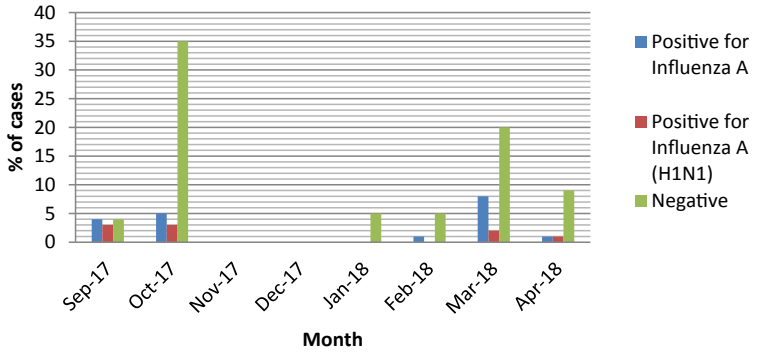
Table 4: Month wise distribution of Influenza A and Influenza A (H1N1).

Months	Total No. of Cases	Positive for Influenza A	Positive for Influenza A (H1N1)	Negative
September 2017	8	4	3	4
October 2017	40	5	3	35
November 2017	0	0	0	0
December 2017	0	0	0	0
January 2018	5	0	0	5
February 2018	6	1	0	5
March 2018	28	8	2	20
April 2018	10	1	1	9
Total	97	19	9	78

collected in the month of October, 2017 40 (41.2%) followed by March 2018, 28 (28.8%). It has been noticed that only 0-3 positive case of Influenza A H1N1 during the 8 months study. But, Influenza A virus have a favorable months are March, October and September, where we found that 8, 5 and 4 positive cases as shown in Table 4 and Figure 4 and there is no positive case of influenza was seen during the winter month of the study.

Discussion

Swine flu or H1N1 Influenza is a contagious disease



Figures 4: Seasonality wise distribution of Influenza A and Influenza A (H1N1) (2017-2018).

that is caused by the Influenza virus. Infection with the H1N1 Influenza virus can result in severe illness and life-threatening complications. Our study was undertaken in clinical samples of patients come to Shri Mahant Indiresh Hospital from Uttarakhand as well as from adjoining states which includes; Uttar Pradesh, Himachal Pradesh, and Haryana to understand the spatial dynamics of spread and transmission of Influenza Virus. Our study includes the most common symptoms in all cases were fever, cough, and breathlessness. The study showed that the season for the activation and morbidity due to this virus is in between September to April but due to some of the unknown reason, one came positive for pdm swine flu (H1N1) in the present study and she died due to this virus in the month of April 2018. Generally in post seasonal period the results do not come positive due to high temperature above 30ºC which is not favorable for Influenza virus but still we observed positive cases of Influenza A and Influenza A (H1N1), which can be possible due to some reasons like, the H1N1 virus is more prone to mutation, climate change or resistant to this temperature, as well as positive cases can be due to past exposure at different place. This study was only with 97 cases with duration 08 months. In this duration, we found that the most affected and positive cases were reported in the age group 41-60 year for both Influenza A and Influenza A (H1N1), which advocate that the maximum cases of this age group due to their occupation from one place to another and owing to the low immunity level, they got such type of virus. When studied gender wise prevalence of Influenza infection, it was seen that the females were more susceptible for Influenza A 12 (24%) cases and 06 (12%) cases were positive for Influenza A (H1N1).This may be due to the low immunity level in females or may be due to pregnancy and diabetes etc.

When studied the epidemiology of Influenza virus with respect to symptoms, most of the cases for Influenza A i.e., 16.98% and 11.32% cases for Influenza A (H1N1) were positive from Category A, followed by category C. This is very important as WHO and CDC guidelines suggest to test only category C patients.

Influenza viral infection is highly contagious. We collected the data for temperature, humidity, and pressure and wind speed of the months, in which samples for suspected cases for Swine Flu were collected. As the favorable temperature for swine flu virus infection is in between 25ºC to 30ºC, thus in this study in the month of September, October, the temperature was 26ºC and 23ºC, respectively on an average, which is the most favorable incubating temperature for swine flu virus. The humidity, pressure and wind speed in the month of September and October was 85%, 74%, pressure 1004 mbar and 1008 mbar and the wind speed was 4.7 km/h and 4.6 km/h respectively on an average for swine flu virus. From this study it has been concluded that environmental condition are the sever cause of this highly contagious viral infection and live a hygienic and healthy life. The virus is highly communicable and the people must take precautions.

Conclusion

The study although was done on 97 number of cases but was very significant as pdmH1N1 can cause death in a shorter duration of time as shown in the current report. The mutation in this virus is also of almost significance as this virus undergoes antigenic shift and antigenic drift which is responsible for epidemics and pandemics of this disease. Our study was on seasonal flu and pandemic flu in the Himalayan region which was studied for the first time. The findings were very useful as the positive turned cases were also reported to Director of General Health, Uttarakhand as well as National Centre for Disease Control, New Delhi, India. Thus, these positive cases were followed by Government Agencies which is very important for the containment of virus. So the current study is of almost relevance for the confinement of such highly contagious viral infection. The further work can be carried out on pdmH1N1 which is circulating in

the population of North India, Influenza virus is affecting the people in March and April also, either it is due to climate change or change in gene sequences of Influenza virus due to this reason Influenza virus circulating in this period and in high temperature, it is also possible that Influenza virus may be resistant to this temperature.

Phylogenetic data showed evidence of well-supported geographical clustering of highly similar pandemic 2009 H1N1 Influenza virus sequences with the majority from population of Uttarakhand and nearby state of Uttarakhand (Uttar Pradesh, Himachal Pradesh, and Haryana) suggesting some degree of transmission. Integration of molecular, epidemiological and statistical methods can help public health authorities to identify foci of this virus transmission in localized communities. Identification of transmission hot spots can lead to more targeted intervention strategies.

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