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Research Article

An Observational Study on Influenza- An Infectious Disease in India

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ABSTRACT

Influenza viruses constantly circulate in many animal hosts, such as humans, birds, horses, dogs and pigs. Seasonal Influenza virus infections in humans cause annual epidemics that result in millions of human infections worldwide and have significant health and economic burdens. Influenza pandemics can also have devastating effects globally, resulting in millions of deaths. Influenza is a globally important respiratory pathogen that continues to pose a significant public health problem. Influenza infects 10 to 20% of the world's population annually and is one of the leading causes of infectious respiratory disease today. Seasonal infections result in 3 to 5 million cases of severe disease worldwide (World Health Organization WHO factsheet 211: Influenza; 2003) and between 17,000 to 51,000 deaths in the United States every year. The annual economic burden associated with these recurrent infections and hospitalizations is estimated to be a staggering $87 billion, with a majority of this burden borne by young children and individuals over the age of 65. In temperate zones, annual epidemics tend to peak during winter, while in tropical regions, infections can occur throughout the year. The exact cause for this seasonality is not clear.

Keywords: Influenza, Biosafety cabinets, Influenza subtypes, H1N1, Real-time PCR.

INTRODUCTION

In India Influenza viruses have the potential to cause contagious respiratory illness ranging from mild flu to severe respiratory illness resulting in death. In April 2009, a new Influenza A virus (H1N1) emerged abruptly and within a few weeks there was a global spread of this virus leading to more than 4500 deaths by October 2009. The first case of pandemic Influenza in India was reported from Hyderabad in May 2009,[1] immediately after which Influenza surveillance system was put in place with the support of National Center for Disease Control, New Delhi and World Health Organization.[2]

Thirteen regional laboratories were set up across India under Integrated Disease Surveillance Project network with the aim to provide information regarding national and local Influenza activity and control efforts.

The first death was a 14-year-old girl in Pune, Maharashtra on 8th and on 9 August a 43-year-old man in Ahmedabad, Gujarat, a 42-year-old teacher in Pune and a 53-year-old woman in Mumbai died. On August 10, a 53-year-old doctor in Pune and a 4-year-old in Chennai died.[3] On August 11 a 7-year-old girl in Vadodara, Gujarat died. On August 13, a 26-year-old woman became Bangalore's first victim of swine flu.[4] An eleven-month-old boy, a 75-year-old woman and a 37-year-old woman died taking the toll in Pune, severely hit by the virus, to 15 and across the country, to 24. A lady having a young daughter of 5 yrs died near Mumbai in Khopoli on August 14. On August 13, three people died at different hospitals in Bangalore, according to the reports.[5]

A (H1N1) Influenza outbreaks during 2013 in India: The pandemic virus continues to circulate and cause waves of infections leading to hospitalization and complications in different parts of India despite the fact that the pandemic stage of the H1N1 virus had ended in August 2010. Once a pandemic has occurred, it is expected to have sporadic outbreaks of smaller magnitude in subsequent few years.[6] Northern India had an unusual heightened activity of A (H1N1) Influenza in first quarter of 2013 that led to 261 deaths till February 28, 2013. different parts of India despite the fact that the pandemic stage of the H1N1 virus had ended in August 2010. Once a pandemic has occurred, it is expected to have sporadic outbreaks of smaller magnitude in subsequent few years. Northern India had an unusual heightened activity of A (H1N1) Influenza in first quarter of 2013 that led to 261 deaths till February 28, 2013.[7]

History of Influenza

Since 1932, when the Influenza virus was first isolated in the laboratory, the history of this infection can be recorded and confirmed by laboratory diagnosis. In the two centuries before this time, infections can be identified by the known signs and symptoms of disease and the explosive nature of outbreaks.[8] Thus, although

sharing many symptoms with other respiratory infections, Influenza presents in addition as a sudden onset of three-day fever, with muscle pain and a degree of prostration out of all proportion with the severity of other symptoms. Secondly, epidemics usually occur suddenly without warning, infecting a large percentage of people, and disappear after a few weeks or months.[9]

Influenza Epidemics

Epidemics of Influenza occur in most countries in some years and in some countries in most years: for many they are common annual events, unpredictable in time and severity. However, history indicates certain features which make Influenza epidemics more likely. Firstly, epidemics tend to occur in winter months when cold, crowding of people and higher humidities are a feature: indeed, in areas where continuous high humidity is a characteristic, infections may occur throughout the year.[10] Secondly, epidemics are more likely to occur when a variant virus appears which shows antigenic changes from previous strains, and cross-reacting antibody, acquired by previous infection, is low in both percentage positive and titre.[11]

Influenza Pandemics

In contrast to epidemics, pandemics are rare events that occurs every 10 to 50 years. They have been documented since the 16th century (WHO 2005b), and in the last 400 years, at least 31 pandemics occurred.[12] Medical historians have used contemporary reports to identify probable Influenza epidemics and pandemics from as early as 412 BCE – and the term "Influenza" was first used in 1357 CE, describing the supposed "influence" of the stars on the disease. The first convincing report of an epidemic of the disease was from 1694, and reports of epidemics and pandemics in the 18th century increased in quality and quantity.[13]

The first pandemic that historians agree on was in 1580, this started in Asia, and spread to Africa, took in the whole of Europe in 6 months, and even got to the Americas. Subsequent pandemics with significant death rates occurred in 1729 and 1781-2; there was a major pandemic in 1880-1883 that attacked up to 25% of affected populations, and another in 1898-1900 that was probably H2N2.[14]

Influenza Virus in India

Adequate data on the prevalence and burden of Influenza in India is lacking. According to published data in India, it contributes to around 5-10% of all acute respiratory infections (ARI). The reported incidence of Influenza URI was found to be 10/ 100 child years and that of ALRI to be only 0.4/100 child years.[15] According to an Indian review, Influenza virus was responsible for about 1.5% to 14.5% of all ARIs episodes. A community-based study from north India estimated incidence of Influenza episodes among children with ARI around 180 and 178 per 1000 children per year, amongst children below 1 and 2 years, respectively. Similarly, the incidence of Influenza- associated ALRI was calculated as 33 and 44 per 1000 children per year.[16]

Swine Flu Or A (H1N1)

H1N1 pandemic in 2009-10: The pandemic of H1N1 in 2009 had several characteristics that differentiated it from seasonal flu. Globally, the illness rates were highest in children and young adults (20-40% of the population), the hospitalization rates were highest in children below one year of age, and the 'case fatality rates' (CFR) varied tremendously and were estimated to be between 0.0004-1.5%. The risk factors for severe disease and death were pregnancy, morbid obesity, asthma, children below 2; however, 25 to 30% of those who died had no underlying risk factor (16). During the 2009 pandemic, pregnant women were documented as an important risk group for severe disease across the globe.[17]

Dynamics of Seasonal Influenza Virus Circulation in India

Seasonality: In temperate regions, outbreaks consistently occur during the late autumn and winter months; in November–March in the Northern Hemisphere; and in May–September in the Southern Hemisphere.[18] In India, limited Influenza activity is usually seen throughout the year with a clear peaking during the rainy season all over the country. However, northern India has a secondary albeit a smaller peak in cooler winter months with pattern similar to temperate regions. The rainy season in the country lasts from June to August in all the regions except Tamil Nadu where it occurs from October to December.[19]

Genetic surveillance of Influenza virus circulation: In India, there is change in the genetic makeup of circulating Influenza viruses since 2009. According to Global Influenza Surveillance and Response System (GISRS) 2009-13, from second half of 2009, A(H1N1) was the most predominant Influenza virus till first quarter of 2011.[20] Second half of 2011 showed lower activity of this strain while A (H3N2) and B group viruses predominated in this half. However, from the beginning of 2012, the pandemic strain, A(H1N1) pdm 09 reappeared and co-circulated with group B and A(H3N2) viruses.[21]

Biology of Influenza Viruses

Taxonomy and structure

IAV belongs to the family Orthomyxoviridae, including five genera, Influenza A, B and C, Thogoto and Isa virus. IAVs have been isolated from a wide range of species, including humans, swine, birds, seals, cats, horses and dogs, but aquatic birds are considered the natural reservoir of IAV. Influenza B viruses have been isolated from humans and seals and Influenza C viruses have been isolated from humans and swine and usually only causes mild disease in the upper respiratory tract.[22] Influenza B viruses can cause a wide variety of disease, but generally clinical symptoms are similar to those of IAV. The IAV genome consists of a total of 13588 nucleotides and virions are enveloped and spherical or pleomorphic with a size ranging from 50-120 nm in diameter.[23] The genome of IAV is divided into eight negative sense RNA segments encoding at least 13 proteins. These proteins include polymerase basic proteins 1 and 2 (PB1 and PB2), polymerase acidic protein (PA), nucleocapsid protein (NP), surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), matrix proteins (M1 and M2) and non-structural proteins 1 and 2 (NS1 and NS2, also known as nuclear export protein; NEP).[24]

The envelope covering the virion is made of a lipid bilayer derived from the host cell membrane and contains the three viral proteins; HA, NA and M2 (Fig. 2.4). The HA protein is the most abundantly expressed of the three and constitutes approximately 80% of the surface proteins, NA constitutes approximately 17% and M2 as

the least abundantly expressed protein with just 16-20 M2 proteins per virion.[25] On the inside of the viral membrane is M1, where it makes up a matrix that holds the viral nucleoproteins (vRNPs). The vRNPs are comprised of viral RNA (vRNA) and are wrapped around NP and small amounts of NEP and at one end of the vRNPs are the polymerase proteins, PA, PB1 and PB2.[26]

Types and subtypes of influenza virus

Influenza viruses belong to the family orthomyxoviridae and are classified as A, B or C. They are negative sense single stranded RNA viruses that contain 8 separate gene segments of RNA. The segmented nature of the genome is important because it allows genetic reassortment, a process by which different Influenza viruses co-infecting the same host cell can exchange genes, resulting in progeny viruses containing genes from both parent viruses. Influenza A and B viruses are responsible for seasonal epidemics of what we generally think of as Influenza illness (Fig. 1), while Influenza C viruses cause sporadic cases of mild common cold-like illness. Influenza the large pool of genetically distinct Influenza A viruses circulating among animal species serves as a source of "novel" viruses to which humans have little or no immunity. The introduction of these viruses into human populations is responsible for periodic worldwide Influenza pandemics.[27]

Influenza type a

Influenza type A viruses can infect people, birds, pigs, horses, seals, whales, and other animals, but wild birds are the natural hosts for these viruses. Influenza type A viruses are divided into subtypes based on two proteins on the surface of the virus. These proteins are called hemagglutinin (HA) and neuraminidase (NA). There are 15 different HA subtypes and 9 different NA subtypes. Many different combinations of HA and NA proteins are possible. Only some Influenza A subtypes (i.e., H1N1, H1N2, and H3N2) are currently in general circulation among people. Other subtypes are found most commonly in other animal species. For example, H7N7 and H3N8 viruses cause illness in horses. Subtypes of Influenza A virus are named according to their HA and NA surface proteins. For example, an "H7N2 virus" designates an Influenza A subtype that has an HA 7 protein and an NA 2 protein. Similarly, an "H5N1" virus has an HA 5 protein and an NA 1 protein.[28]

Influenza type b

Influenza B viruses are normally found only in humans. Unlike Influenza A viruses, these viruses are not classified according to subtype. Although Influenza type B viruses can cause human epidemics, they have not caused pandemics.[29]

Influenza type c

Influenza type C viruses cause mild illness in humans and do not cause epidemics or pandemics. These viruses are not classified according to subtype.

Aim and Objectives

AIM

Molecular characterization of seasonal Influenza and pandemic influenza in clinical isolates- relevance for disease containment

Objectives

the current work was carried out by considering the following objectives:

- Collection of clinical samples, nasal swab and throat swab in virus transport media.
- from the suspected cases.
- Inactivation of the samples.
- RNA isolation by QIAamp® Viral RNA Mini Kit.
- Master mix preparation for Influenza A and Inf. H1N1 Artus Infl./H1 LC/RG R PCR Kit.
- Amplification by real time PCR for the differentiation of Inf. A and Inf. H1N1.

MATERIALS AND METHODS

Study Place

All the experiments were done in DNA labs. It is certified Molecular Diagnostic Laboratory. It offers a wide range of quality assured clinical laboratory test using latest technology. All the experimental work was performed in the Biosafety cabinet III [BSCIII] and all the procedures were done according to WHO guidelines. DNA Laboratory (CMRL) is an approved centre for the detection of Influenza A (Swine flu), H1N1 by National Centre for Disease Control (NCDC), New Delhi, Government of India (Table 1).

Biosafety and Precaution

Biosafety

A biosafety level is a level of the biocontainment precautions required to isolate dangerous biological agents in an enclosed laboratory facility. The levels of containments range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL- 4). An agent of biological origin that has the capacity to produce deleterious effects on human. Biosafety levels: - Levels of contentment having the more the facilities grow the dangerous pathogen (BSL-2, BSL-3) example – H1N1 virus

- *BSL 1*

This level is suitable for work involving well characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. It includes several kinds of bacteria and viruses including canine hepatitis, non-pathogenic *Escherichia coli*, as well as some cell cultures and non-infectious bacteria.

- *BSL 2*

This level is similar to biosafety level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the bacteria and the viruses that causes only mild disease to humans, or are difficult to contract aerosol in lab setting, such as , human immunodeficiency virus (HIV), orthopoxviruses , Influenza A, Lyme disease, *Salmonella,* mumps, measles.

- *BSL 3*

Indigenous/ exotic agents associated with human disease with the potential for aerosol transmission. This level is applicable to clinical, diagnostic, teaching, research, or production facilities in which work

is done. It includes various bacteria, parasites and viruses that can cause severe to fatal disease in human.

- *BSL 4*

This level is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections, agents which causes server to fatal disease in humans for such as *bolivian* and argentine hemorrhagic fevers, Marburg virus, *Ebola virus*, and various other hemorrhagic diseases. This level is also use for work with agents such as smallpox that are considered contagious enough to require the additional safety measures, regardless of vaccination availability.

Materials Required

Collection of clinical samples, nasal swab and throat swab in virus transport media from the suspected cases

The Influenza like illness (ILI) can be categorised on basis of severity of the symptoms. It is categorised into 3. These are:

- *Category A*

In this category, symptoms are mild fever, cough, sore throat, headache. etc

- *Category B*

In this category, symptoms are high grade fever, severe throat & signs of category A.

- *Category C*

In this category, symptoms of A & B , drowsiness, low BP, breathlessness, chest pain etc. now the patient have to go for H1N1 testing and immediate hospitalization is required.

RESULTS

In this study, a total of 25 clinical samples were collected from the patients with Influenza like illness (ILI) symptoms, meeting criteria of the symptoms as given by National Center for Disease Control, New Delhi and CDC, Atlanta, USA (Fig.4.17). Samples were collected from medical intensive care unit (MICU), High dependency unit (HDU), Severe intensive care unit (SICU) from Shri Mahant Indiresh Hospital, Dehradun, Uttarakhand for the molecular profiling of Influenza virus and its subtypes. Samples were properly collected by wearing N-95 mask and personal protective equipment. Sample were transported to DNA Lab – A centre for applied Science at 4°C, in a sealed pack thermocol box. Further, the swabs were shifted to Biosafety Level-3 Lab for virus inactivation RNA was isolated by (QIAamp® Viral RNA Mini Kit (50) Cat. No. 52904) silica column method. Extracted RNA was utilized further for real PCR amplification.

Further master mix was prepared, for the differentiation of Influenza A and it's subtyping i.e., Influenza A H1N1 for targeting the 80 bp gene for Inf. A (H1N1) for all the samples. Amplification was done utilizing Rotor gene Q real time PCR machine for the amplification of target gene differentiating Influenza A and Influenza A(H1N1) as shown in Fig. 1.

Out 25 samples processed 3 came positive for Influenza A virus and 02 samples came positive for Influenza A (H1N1) rest of 21 samples came negative for both the targets. The case number 04 and 18 were positive for swine flu (Influenza A H1N1), case number 04 was from Saharanpur (U.P) and case number 18 was from Dehradun (U.K) with the age of 38 year female and 53 year male respectively. The samples which came positive only for Influenza A virus case number 11, 13 and case number 23.

Table 1: Instrumentation Facilities at BSL-2, BSL-3 and General Laboratory Areas

S. No.	*Name of equipment*	*Company/Make*
1.	CTM Roche TaqMan 48	Roche
2.	UV Trans-illuminator	Bangalore Genei
3.	Thermal Cycler	Bench Top
4.	Gel Electrophoretic Unit with Power Supply	Bench Top
5.	Spectrophotometer	Bench Top
6.	Micropipette	Eppendoreffe / G- Biosciences
7.	Vortexer	Bench Top
8.	PCR cabinet	Bench Top
9.	Dry Bath	Bench Top
10.	Water Bath	Bench Top
11.	Deep Freezer	Arctiko
12.	Refrigerator	Samsung
13.	Cooling Centrifuge	Thermo Scientific
14.	Micro Centrifuge	Bench Top
15.	Air Curtain	Crompton
16.	Split AC 1.5 ton for thermal cyclers	Blue Star
17.	UPS 5kw for Thermal cycler power backup	Numeric
18.	Biosafety Cabinet Class II	Esco
19.	Non- Cooling Centrifuge	Thermo Scientific
20.	Incubator	Scientific Industries
21.	Distillation Unit	Borosilicate
22.	Veriti Gradient Thermal Cycler	Applied Biosystem
23.	E-Gel ® Imager System with UV Light Base	Applied Biosystem
24.	Refrigerator (-70°C)	Mac flow engineering PVT.Ltd

Result Interpretation For Influenza A and Influenza H1N1 (Detection of CT Value by Real Time PCR)

After the run was finished, we analysed the data. The following results were possible (Table 2).

If signal was detected in fluorescence channel cycling green (Fig. 2). The result of the analysis was positive: the sample contains Influenza RNA or, for the Influenza H1 PCR, Influenza H1 RNA.

- In this case, the detection of a signal in the cycling orange channel was dispensable, since high initial concentrations of Influenza RNA (positive signal in the cycling green channel) can lead to a reduced or absent fluorescence signal of the internal control in the cycling orange channel (competition) (Fig. 3).
- If in fluorescence channel cycling green no signal was detected.

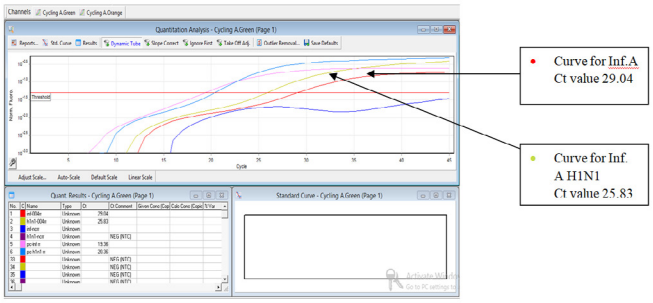

Fig. 1: Positive results for both Inf. A and. Inf. A H1N1

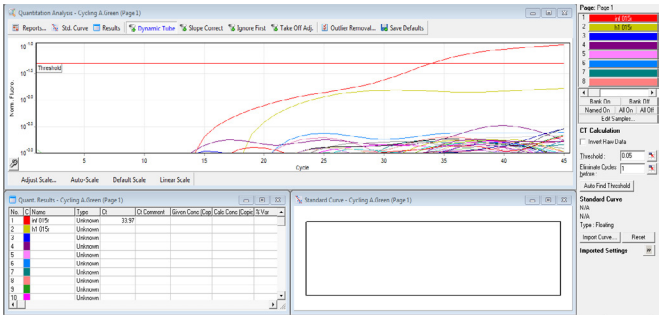

Fig. 2: Positive results for Inf. A only (Ct value 33.97) in cycling green channel

at the same time, a signal from the Internal control appears in the cycling orange channel. In the sample no Influenza RNA was detectable. It can be considered negative.

In the case of a negative Influenza PCR, the detected signal of the internal control rules out the possibility of PCR inhibition.

Graphical Representation of Data

Above data show us that the age group between 20 to 40 and 40 to 60 age were positive for Influenza virus and Inf. A/H1N1 (Fig. 4). The gender-wise distribution of Influenza virus shows us that females are more infected to Influenza virus than males (Fig. 5 and Table 3).

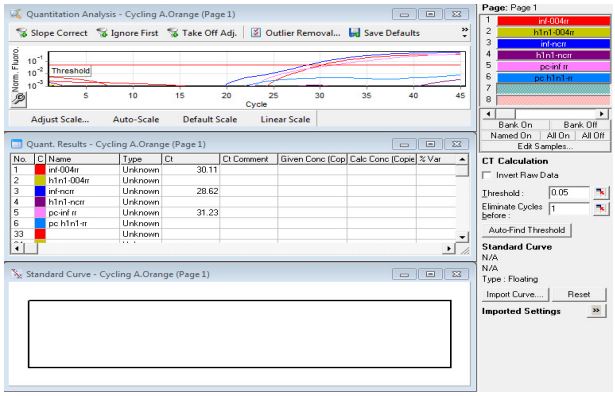

Fig. 3: Internal control for Influenza A virus in cycling orange channel

DISCUSSION

H1N1 Influenza or swine flu is a contagious disease that is caused by the Influenza virus. Infection with the H1N1 Influenza virus can result in severe illness and life- threatening complications. Symptoms of H1N1 flu are similar to those of the common flu and scientists are actively studying the situation to better understand its range of symptoms and how it is spread. For healthy people, resting and drinking plenty of fluids usually allows infected people to recover from the flu. For people at high risk of developing flu complications, medications and hospitalization may be needed. The flu can be prevented by avoiding close contact with sick people and by washing your hands frequently. If you have the flu, you can help stop the spread of this infectious disease by staying home while you are sick and by covering your mouth and nose as you cough or sneeze.[30]

Our study was undertaken in clinical samples of patient from Uttarakhand and nearby state of Uttarakhand (Uttar Pradesh, Himachal Pradesh, and Haryana) to understand the spatial dynamics of spread and transmission of IAVs. In these cases, we observed some common symptoms of ILI e.g., Running nose, sore throat, nausea, headache, low blood pressure, fever, dehydration, etc.

Table 2: Results interpretation for the detection of Influenza A and Influenza A/H1N1 by real time PCR

Results Interpretation for Influenza virus testing (Qiagene)				
Case	*Targets*	*Channels in Real Time PCR (GREEN)*	*Channels in Real Time PCR (ORANGE)*	*RESULTS*
Case 1	INFLUENZA-A	POSITIVE		POSITIVE FOR Influenza H1N1
	H1N1	POSITIVE		
	INTERNAL CONTROL		POSITIVE	
Case 2	INFLUENZA-A	POSITIVE		POSITIVE FOR Influenza H1N1
	H1N1	POSITIVE		
	INTERNAL CONTROL		NEGATIVE	
Case 3	INFLUENZA-A	POSITIVE		POSITIVE FOR INFLUENZA A
	H1N1	NEGATIVE		
	INTERNAL CONTROL		POSITIVE	
Case 4	INFLUENZA-A	NEGATIVE		NEGATIVE FOR BOTH TARGETS
	H1N1	NEGATIVE		
	INTERNAL CONTROL		POSITIVE	
Case 5	INFLUENZA-A	NEGATIVE		
	H1N1	NEGATIVE		INVALID
	INTERNAL CONTROL		NEGATIVE	

Table 3: Results of real time PCR for the Influenza A virus and its subtype Influenza A/H1N1 for different cases.

Case no.	*Age/gender*	*Symptoms and Clinical Notes*	*Molecular Characterization of Influenza Virus*			*Results*
			Inf.A Ct value*	*Inf.A (H1N1) Ct value*	*Ct value (IC)*	
01	57/M	Breathlessness, Runny nose, cough.	-	-	35.68	Negative for Inf. A & Inf. A/H1N1
02	28/F	Breathlessness, Body ache	-	-	32.60	Negative for Inf. A/ Inf. A (H1N1)
03	50/M	Fever, cough, and dark sputum	-	-		Negative for Inf. A & Inf. A/H1N1
04	38/F	Severe patient was in ICU* on ventilator, shortness of breathing, fever, sinus 2- 3 days vomiting.	29.04	25.83	30.11	Positive for H1N1
05	76/M	Septicaemia and Right-side pneumonia, Parkinson, patient was in SICU*.	-	-	28.41	Negative for Inf. A & Inf. A/H1N1
06	50/M	Cough for past 15 days, haematolysis & breathlessness, dark coloured sputum	-	-	34.97	Negative for Inf. A & Inf. A/H1N1
07	60/M	Breathlessness	-	-	28.90	Negative for Inf. A & Inf. A/H1N1
08	20/ F	High fever, loose stool, pain abdomen from 1 day.	-	-	36.70	Negative for Inf. A & Inf. A/H1N1
09	33/M	Brancheonuemonia patient was in MICU*.	-	-	31.98	Negative for Inf. A & Inf. A/H1N1
10	35/M	Fever, Chest pain cough	-	-	27.42	Negative for Inf. A & Inf. A/H1N1
11	52/F	Fever, Breathlessness	25.65	-	24.17	Positive for Inf. A
12	30/F	Cough, runny nose, chest pain	-	-	31.56	Negative for Inf. A & Inf. A/H1N1
13	26/M	Fever cough runny nose breathlessness	26.14	-	28.25	Positive for Inf. A
14	58/M	Fever, Body ache low B.P	-	-	29.76	Negative for Inf. A & Inf. A/H1N1
15	22/F	Cough, fever, breathlessness, renal calculi	-	-	26.54	Negative for Inf. A Inf. A/H1N1
16	30/F	Fever, low B.P, dry cough, pneumonia	-	-	33.36	Negative for Inf. A & Inf. A H1N1
17	51/F	Body ache, breathlessness Headache, cough, fever	-	-	32.52	Negative for Inf. A & Inf.A/H1N1
18	22/F	Ruminant fever,	-	-	29.98	Negative for Inf. A & Inf.A/H1N1
19	53/M	Fever, Body ache, low B.P, patient was in MICU	29.12	23.79	26.18	Positive for H1N1
20	65/M	Cough, fever, breathlessness, renal calculi	-	-	26.42	Negative for Inf. A & Inf.A/H1N1
21	17/M	Fever 1-week, sore throat dry cough	-	-	28.14	Negative for Inf. A & Inf.A/H1N1
22	35/M	Head ache, fever, vomiting, sneezing,	-	-	29.56	Negative for Inf. A & Inf.A/H1N1
23	24/F	High Fever, weakness, cough , sneezing,	31.97	-	33.99	Positive for Inf. A
24	46/F	Breathlessness, high fever, head ache, dry cough	-	-	31.43	Negative for Inf. A & Inf.A/H1N1
25	49/M	Sneezing, runny nose, fever dark sputum, low B.P	-	-	28.65	Negative for Inf. A & Inf.A/H1N1

***** Ct= cycle threshold,
* ICU= intensive care unit,
*SICU= sever intensive care unit
* MICU= medical intensive care unit.

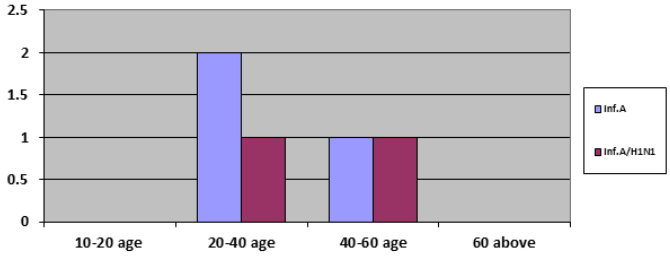

Fig. 4: Age-wise graph of Influenza virus infected patients.

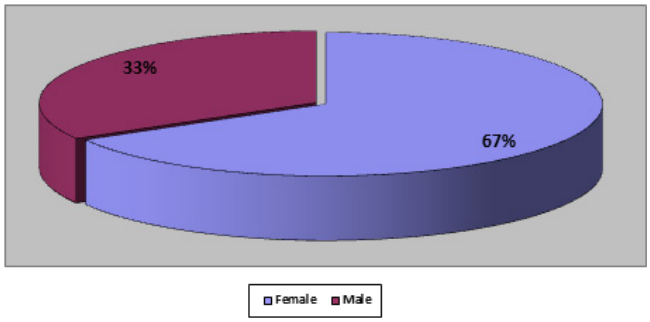

Fig. 5: Graph of gender-wise infected patients of Influenza virus

Predominance of the Inf.A(H1N1) pdm09 virus was observed in 02 sample out of 25 samples, while 02 Influenza A samples were positive. The season for the activation and morbidity of this virus is between October to March but due to some of the unknown reason. One came positive for pdm swine flu (H1N1) and died due to this virus in month of April end and one came positive in May. Seasonality variation can occur in Influenza virus as one of the cases which was H1N1 positive died and that case was from Saharanpur district. The patient died in the month of April which occasionally is not the season for this viral infection.[31]

Generally, in post seasonal period samples should not come positive due to high temperature which is not favorable for Influenza virus but still we observed positive result of Influenza A and Influenza A/ H1N1, which can be possible due to some of reasons like it may be mutation, climate change or it is also possible that Influenza virus may be resistant to this temperature. The study was limited only with 25 cases with duration spam of 5 months which was not sufficient to study properly the epidemiology of this highly contagious virus causing high rate of motility and morbidity. The current study needs large volume of samples from different region of all the state of North India.[32]

CONCLUSION

The virus is highly infectious and the people must take precautions for this Influenza virus. The study although was limited to only few numbers 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. 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. 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 transmission in localized communities. Identification of transmission hot spots can lead to more targeted intervention strategies. 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 April and May 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.

CONFLICT OF INTEREST

Nil.

SOURCE OF SUPPORT

None.

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