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Prevalence of Influenza Viruses A and B in Seasonal Flu : A Tertiary Care Hospital Study

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A B S T R A C T

Influenza is a public health problem and one among global threats. Influenza is viral infection leading to morbidity and mortality proving itself to be fatal during outbreaks. Understanding the pathogenesis of influenzae virus helps in framing a preventive strategy. This has become cumbersome due to the genetic mutations resulting in evolution of multiple strains. So, appropriate management of influenza is of utmost importance in curbing and combating the ill effects during any pandemic. Total 2010 appropriate swabs (nasopharyngeal and oropharyngeal swabs) were collected of the upper respiratory system from the inpatient and outpatient departments were collected from March 2023 to and Feb 2025 retrospectively. Swabs were sent in the viral transport media (VTM). Patient demographic details were collected. Concentration of extracted RNA was then measured with nanodrop software. And later was processed for RT PCR as per CDC guidelines. Microbiological reports were evaluated. Demographic details of positive patients were analysed using descriptive statistics. Out of 2010 a total of 1066 males and 944 females were included. Among which 582 patients were positive for the influenza virus with a total prevalence of n = 29.1 %; 20.2 % (n=59) of the cases were infected with H1N1 influenza virus, 19.93 % (n=58) of the cases were infected with H3N2 influenza virus. Among 2010 patients, influenza was detected in 30.3 % (n=308) females and 27.33% (n=274) males. Among the detected swabs, majority of the 14.0 % (n=141) samples were positive for influenza A which coincided with the study (19) with 3.28 % (n=33) influenzae B, 5.87% (n=59) of H1N1 and 5.77% (n=58) H3N2. A total prevalence of influenza was observed in the study with 21%.

Keywords

Influenza Viruses A and B, Seasonal Flu, RT PCR, H1N1 influenza virus, H3N2, Pandemic

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Introduction

A contagious virus that affects both the upper and lower respiratory tracts is called influenza. It is caused by a broad range of influenza viruses. While some of these viruses are unique to particular species, others can infect humans. Respiratory droplets released from the mouth and respiratory system during talking, sneezing, and coughing are how these viruses are spread. Contacting inanimate things contaminated with influenza viruses and then touching the nose or eye can spread the infection. Influenza can spread up to five to seven days after

infection and even before the patient exhibits symptoms. Most healthy people recover totally after infection in a few days, but in some high-risk groups, consequences like pneumonia and mortality are prevalent.

Pregnant women, older people, immunocompromised individuals, and small toddlers are among these categories. A high temperature, sore throat, cough, and runny nose are some of the signs and symptoms of influenza. Seasonal influenza outbreaks spread quickly and effectively. Flu outbreaks afflict a large percentage of adults and children in temperate countries every autumn and winter, however the intensity and age groups are affected differently by the season.

More deadly types of the influenza virus have developed within the last few decades. These viruses can infect animals in addition to humans. Clinicians should be aware that false-negative results are frequent and that the sensitivity of all fast tests for influenza detection is limited. The viral culture of throat secretions or the PCR test is the gold standard for diagnosis, however these techniques take several days to produce findings. Vaccination is the most important method for lowering the infection's morbidity.

Influenza replication

The lipid envelope of influenza viruses is formed from the membrane of the host cell and contains surface proteins called HA and NA, which are essential for the entry and release of the virus. Eight single-stranded RNA segments make up the genetic material of the virus. These segments encode for different proteins that are necessary for the virus's assembly and reproduction. Controlling epidemics and creating vaccines need an understanding of the molecular biology of influenza viruses, particularly their replication cycle and antigenic diversity.

In the community, widespread human influenza viruses cause life threatening, communicable upper respiratory and lower respiratory tract infections. Among these, humans can be infected by such viruses while others are confined to other species. They transmit through aerosols which are expelled from the oral cavity of the infected humans. They can easily spread by contact mechanisms as they settle over the inanimate objects from which they can be easily picked. Their incubation period usually lasts between 1 to 4 days. It is self-limiting and can be transmitted from the infected individual during the incubation period and also post recovery which usually lasts between 5 to 7 days. Post infection, healthy individuals recover completely. However, in some high-risk groups like children, geriatric age group, immunosuppressed and in pregnancy, complications like pneumonia and shock accompanying death will be evident. Clinical features include nasal discharge, cold, high-grade fever, sore throat with or without cough. It spreads aggressively and rapidly in nature leading to global epidemics during autumn and winter seasons affecting adults and children of different age groups with severity (8)(9)(10)(11).

Etiology

Typically, four classes of influenza viruses have been recognised namely A, B, C, and D types. Human infections are caused during the epidemics. Influenza A has is subcategorized into various subtypes based on the hemagglutinin (H) protein and neuraminidase (N) protein which are primarily expressed on the viral surface. Among them, 18 hemagglutinin subtypes and 11 neuraminidase subtypes (H1-18 and N1-11) were discovered. The influenza A virus can be characterized by the presence of H and N types such as H1N1 and H3N2 while influenza B virus is depicted under the lineages and the strains. The new strains develop from the lineages, influenza B Yamagata and influenza B Victoria. They are species specific as they have receptors. (12)(13)

Materials and Methods

Total 2010 appropriate swabs (nasopharyngeal and oropharyngeal swabs) were collected from the upper respiratory system from the inpatient and outpatient departments received from March 2023 to Feb 2025 retrospectively. Swabs were sent in the viral transport media (VTM). Patient demographic details were collected. Swabs were processed as per the molecular biological laboratory standard protocols. The samples, in approximately 20 minutes per sample, was aliquoted in Eppendorf tube and was processed for RNA extraction in Fully automated Qiagen machine. Concentration of extracted RNA was then measured with nanodrop software. And later was processed for RT PCR as per CDC guidelines. Microbiological reports were evaluated. Demographic details of positive patients were analysed using descriptive statistics.

Ensuring the optimal quantity and purity of RNA is

crucial for successful molecular biological experiments and for minimizing the need of sample re-processing samples. Although various kits and automated systems for nucleic acid isolation are available, there is limited data on how well they perform, especially with paediatric blood samples, which often have lower quantities.{29} In the present study, we compared the effectiveness of the automated QIAcube platform with manual Qiagen extraction kits using paediatric blood samples to evaluate their performance.

When preparing viral transport media (VTM) samples, specifically nasopharyngeal (NPS) and oropharyngeal (OPS) swabs, for RNA extraction, it's crucial to follow standardized procedures to ensure the integrity and quality of the RNA.

Results and Discussion

Out of 2010 a total of 1066 males and 944 females were included. Among which 582 patients were positive for the influenza virus with a total prevalence of n = 29.1 %; 20.2 % (n=118) of the cases were infected with H1N1 influenza virus, 19.93 % (n=116) of the cases were infected with H3N2 influenza virus.

Among 2010 patients, influenza was detected in 30.7 % (n=308) females and 27.63% (n=277) males which coincided with the study conducted by (18). This study stated that females are more prone for respiratory tract infections after puberty and prior to menopause.

Fig.1 Total sample size in males and females

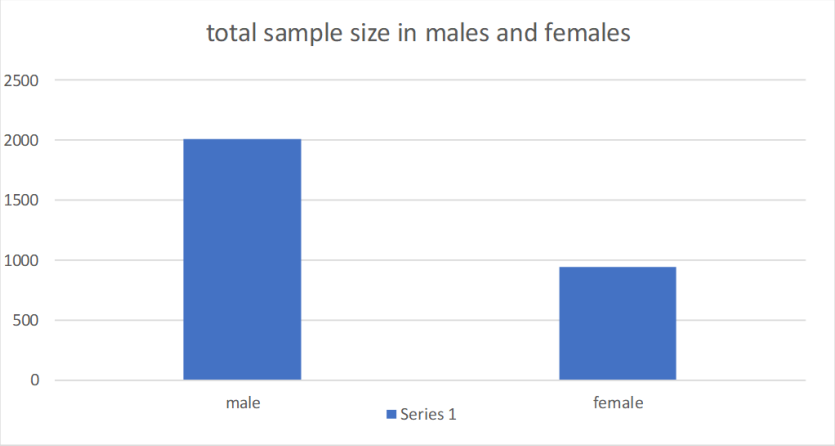
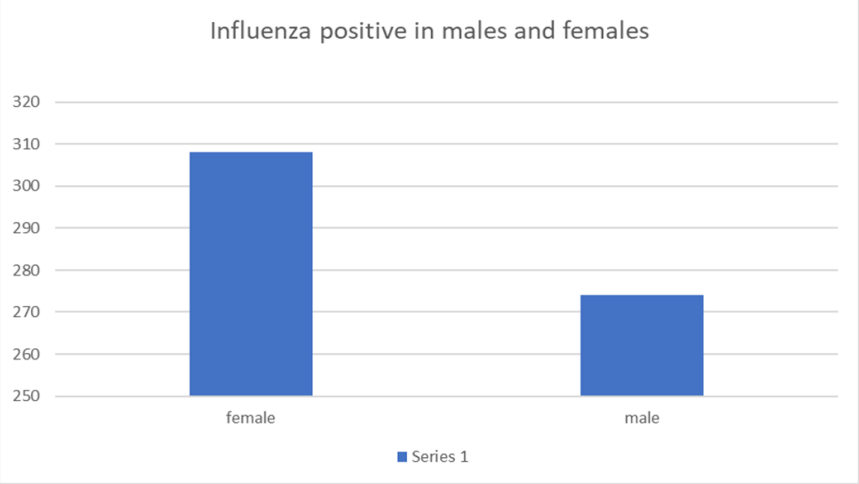


Fig.2 Influenza positive in Males and Females



Among the detected swabs, majority of the 28.0 % (n=282) samples were positive for influenza A which coincided with the study (19) with 6.68 % (n=66) influenzae B, 11.87% (n=118) of H1N1 and 16.77% (n=116) H3N2. Pie charts above represents the overall positivity of influenza A which was majorly seen in both the genders (males and females) which coincided with a study published by (30).

Majority of the samples were received in the month of October 2023 which can be due to the cold climatic conditions which is favourable for the viral replication in the anterior nasal nares which was stated in the study (20)(21). In this study the relationship between weather and influenza with mortality was discussed. They have drawn a conclusion stating that the infection of the respiratory system has risen due to the seasonal variation during cool temperatures with less humidity specifically. Seasonality variation among the pattern of influenza mortality remains unclear. However, latest study conducted in relation with cold/dry weather along with humidity (22), stated that relations existed between the environmental factors and virus characteristics (23)(24) It such as the physiological drying of nasal nares,(25) increased aerosol transmission, (26) and human factors (27) etc.

Fig.3 Influenza Positive in Males

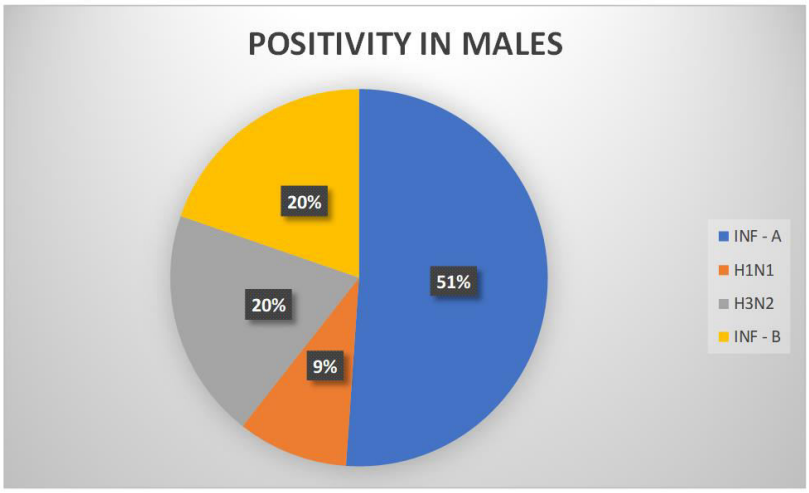
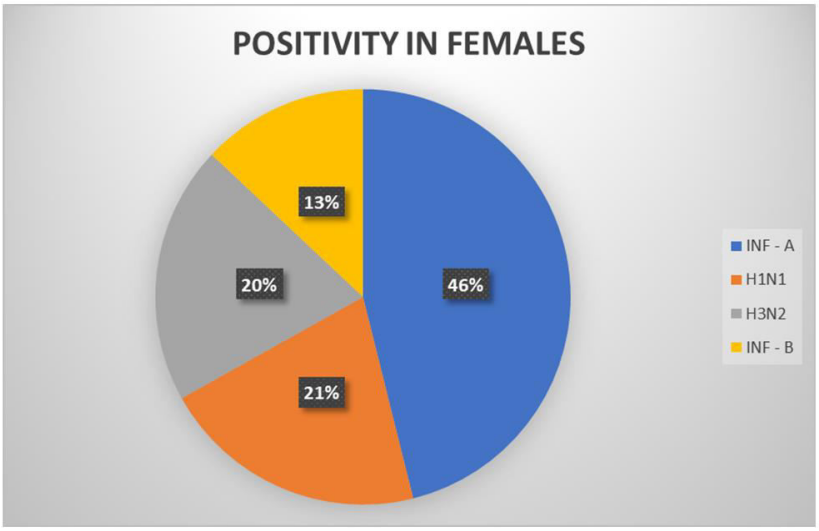


Fig.4 Influenza Positive in Females



In this study, both the nasopharyngeal swabs and oropharyngeal swabs detected the majority of the viruses which coincided with the study (28). This study stated that the nasal swabs and oral swabs collectively together are the ideal approach for flu virus detection.

Molecular surveillance of influenza plays a crucial role to periodically assess the efficacy of the vaccine and to detect the new variants or strains with global impact on the public health. India located in the tropics; no distinct specific pattern has been observed which was similar to a study (8). Influenza viruses circulate throughout the year and no distinct seasonal patterns can be observed which probably might be because of the stable climatic conditions In the present study, a total of 2010 respiratory specimens were obtained from the patients. Out of which there were 29.1 % positive for influenza.

Fig.5 Patient data from March 2023 to Jan 2024

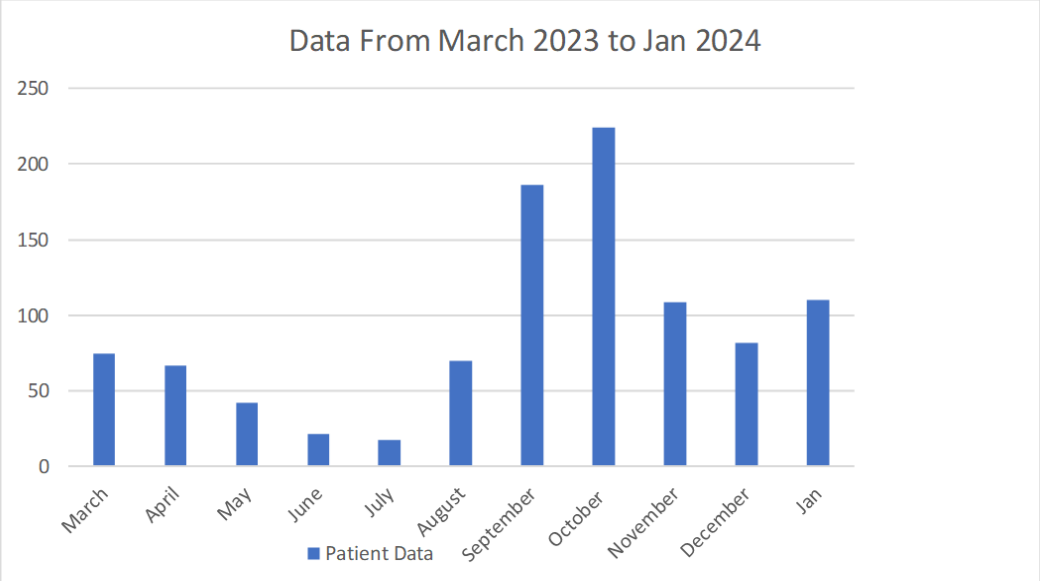
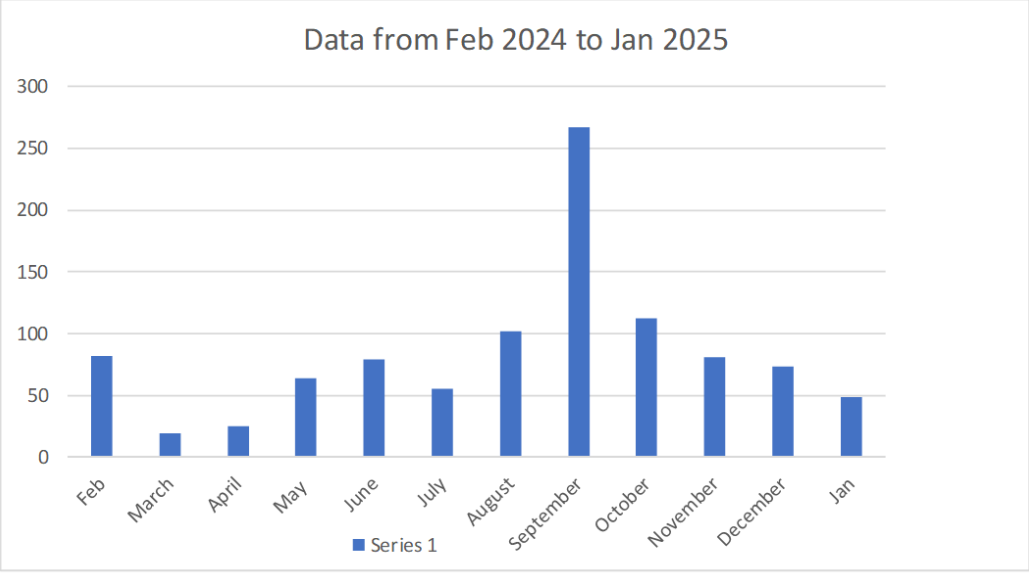


Fig.6 Patient data from Feb 2023 to Jan 2025



In the year 1933, Influenza A was isolated which was later followed by Influenza B isolation. These viruses cause epidemics during the winter season. The severity differs from one season to another season indefinitely. There is a seasonal trend which has been observed when virologic surveillance was conducted in the northern hemisphere with maximum outbreaks between October and March when compared to southern hemisphere in April and august.

The yearly incidence of invasive Hib disease in children under five years old has significantly dropped since the Hib conjugate vaccine was first made available in the US in 1987 for children and in 1990 for newborns. Non-typeable H. influenzae (NTHi) is currently responsible for most invasive H. influenzae disease across all age categories in the United States. Interestingly, despite receiving the Hib vaccination, children of Alaskan Native descent have a greater incidence and prevalence of H. influenzae (incidence rate: 5.4 per 100,000) than children of other races. It mostly affects children who have not received all of their vaccinations and babies who have not finished their series of shots because the Hib conjugate vaccine is a part of the regular immunization schedule.

For children under the age of five, the incidence rates for Hib, non-b, and non-typeable H. influenzae in 2017 were 0.18, 1.7, and 1.7 per 100,000 population, respectively. The incidence of invasive non-typeable H. influenzae was 6.2 per 100,000 in persons 65 years of age and older. In about 40% of instances, NTHi is the infectious agent causing acute otitis media and sinusitis in children. It is also the reason behind otitis media recurrence in that age range. Where immunization is not widely available, healthy youngsters in underdeveloped nations cultivate H. influenzae in their throats and nasopharynx. At one year old, the carriage rate is about 20%, and by the time a child is five years old, it is over 50%.

In the present study experience reviewing RNA extraction procedures for nasopharyngeal (NPS) and oropharyngeal (OPS) swabs received in viral transport media (VTM), we discovered that optimizing the extraction protocol is key to achieving high-quality RNA for downstream molecular applications. In the present study experience with 2010 swabs processed between March 2023 and February 2025, in which swabs were obtained from both inpatient and outpatient clinics and shipped in VTM, identified a number of findings. Samples were handled within about 20 minutes of arrival, with aliquots set up in Eppendorf tubes for RNA extraction on a fully automated Qiagen machine. The process was effective for handling large volumes of samples, and the concentration of RNA extracted was quantified using nanodrop software to verify quality control.

Seasonal differences in temperature and humidity are very important in contributing to influenza spread and related deaths. In the current study, most of the samples were obtained in October 2023, which coincided with cooler climatic conditions ideal for viral growth in the anterior nasal nares. This concurs with evidence that respiratory infections rise with cooler temperatures and lower humidity due to environmental reasons such as physiological drying of nasal mucosa, increased aerosol transmission, and human behavioral responses. Seasonal influenza impact on mortality has also been much documented, with evidence of increased mortality during winter seasons relative to other seasons. Although temperature is the most significant cause of seasonality in respiratory mortality, influenza does play a key role in producing seasonality, especially during cold weather when external conditions are conducive to viral survival and transmission. These results emphasize the need for elucidating the role of environmental factors in generating influenza seasonality to guide public health interventions.

In the present study comprised of 2010 a total of 1066 males and 944 females were included. Among which 582 patients were positive for the influenza virus with a total prevalence of n = 29.1 %; 20.2 % (n=59) of the cases were infected with H1N1 influenza virus, 19.93 % (n=58) of the cases were infected with H3N2 influenza virus. Among 2010 patients, influenza was detected in 30.3 % (n=308) females and 27.33% (n=274) males. Among the detected swabs, majority of the 14.0 % (n=141) samples were positive for influenza A which coincided with the study (19) with 3.28 % (n=33) influenzae B, 5.87% (n=59) of H1N1 and 5.77% (n=58) H3N2.A total prevalence of influenza was observed in the study with 21%

In conclusion, Robust surveillance is an important measure in early diagnosis of such cases with prompt clinical acumen to combat and curb deaths due to this global hazard.

Limitations

Clinical history was not included.

Paediatric blood samples were not collected, thus effectiveness of the automated QIAcube platform with manual Qiagen extraction kits evaluation was not done.

Author Contributions

Shruti A. Satashia: Investigation, formal analysis, writing—original draft. Nileshkumar Pandya: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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