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ORIGINAL RESEARCH ARTICLE

Prevalence and Surveillance of Influenza Viruses in Hospitalized Patients with Respiratory Infections in and Around Chennai, Southern Part of India

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Abstract The COVID-19 pandemic has heightened concerns about increasing cases of human influenza virus, which pose a significant public health threat to the population in and around Chennai. Therefore, this study analyzed the prevalence of influenza viruses, including influenza A (InfA) and its subtypes H1N1 and H3N2, influenza B (InfB), and respiratory syncytial virus (RSV), in the population of Chennai and surrounding areas. The study included 444 nasopharyngeal and oropharyngeal samples collected between September 2022 and March 2023 from hospitalized patients with severe respiratory symptoms. The sample group consisted of 249 men and 195 women between the ages of 1 and 100 years. The collected samples were processed for detection of InfA, H1N1, H3N2, InfB, and RSV according to the guidelines of the Indian Council of Medical Research (ICMR) and the Department of Public Health, Government of Tamil Nadu. InfA and H3N2 were found to be the most prevalent, with prevalence rates of 11.4%, and 5.1%, respectively. They were followed by H1N1 (3.1%), InfB (2.02%), and RSV (0.5%). H3N2 was more frequently observed in adults and elderly aged groups (4.5%). The most common symptoms associated with InfA infection were fever, body aches, cough, and headache. In addition, RSV, H1N1, and H3N2 viral positivity (11.4%, 5.1%, and 0.5%, respectively) were higher in children aged 1–5 years. This study shows that H1N1 and H3N2 viruses were the predominant strains during the study period in Chennai and provides valuable information for public awareness and preventive measures against early influenza virus infections.

Keywords Infections · Viral RNA · PCR · Influenza · Prevention

Introduction

Influenza is a respiratory illness caused by the influenza virus, primarily type influenza A and B. The novel H1N1 virus, commonly known as "swine flu," is a new influenza strain affecting people worldwide, leading to a pandemic. The symptoms include fever, cough, sore throat, runny or stuffy nose, body aches, headache, chills, fatigue, and, in some cases, diarrhea and vomiting. Individuals at high risk of serious complications include children under the age of five, pregnant women, and people with chronic medical conditions such as diabetes, heart disease, asthma, and kidney disease. H3N2 is a subtype of influenza A and a significant cause of human influenza. Another respiratory virus called the human respiratory syncytial virus (RSV) is responsible for lower respiratory tract infections across all age groups in all Indian states. Recently, there has been an alarming outbreak of H3N2 influenza cases throughout India, causing concern among the population. Hospitals across the country have reported thousands of H3N2 cases and two deaths over the past few months. So far, 3038 H3N2 influenza cases have been confirmed in the country, according to the Indian Council of Medical Research (ICMR).

Data from the Integrated Disease Surveillance Program and Integrated Health Information Platform (IDSP-IHIP) indicates that in January 2023, a total of 397,814 cases of acute respiratory illness (ARI) or influenza-like illness (ILI) were reported in the country. This number slightly

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increased to 436,523 in February 2023, and within the first nine days of March 2023, there were 133,412 cases. In February, a total of 955 H1N1 cases were reported, with the majority of cases coming from Tamil Nadu (545). This suggests that it is not unusual to see an increase in flu cases, and H3N2 infections subtype are more common. Therefore, Government of India has issued the guidelines to control the increase in flu cases.

Influenza-like infections and severe acute respiratory infections, including COVID-19, H3N2, H1N1, and adenovirus, have significantly affected the population in India since January of this year. In early January, during the transition from winter to summer, both government and privately-run hospitals observed a rise in flu-like cases among people of all age groups. Acute respiratory tract infections are a major cause of morbidity and mortality worldwide [1]. Influenza A and B are the leading causes of upper and lower respiratory tract diseases in all age groups, particularly infants, young children, elderly individuals, immunocompromised patients, and adults [2]. Influenza can occur regionally as an endemic or globally as a pandemic, posing a serious threat to public health. The annual outbreaks of influenza incur significant economic costs, amounting to approximately 6–14 billion pounds in Europe and $87.1 billion in the USA [3]. Reducing the public health burden of acute respiratory infections in children remains a major priority and an immense challenge [4]. Acute respiratory infections result in 1.9 million childhood deaths each year in developing countries, with 20% of these deaths occurring in India. The role of viruses in the etiology of acute respiratory infections in developing countries like India is not well-studied. Among children hospitalized with acute lower respiratory infections (ALRI) in India, half of the infections have a viral etiology [5].

According to the National Health Portal of India, in 2018, there were 419,962,600 cases and 3740 deaths from respiratory infections in India. The countries most severely affected included the United States, India, Brazil, France, Russia, Spain, Argentina, the UK, Colombia, and Mexico. The USA reported the largest number of cases, with 35,283,729 and over 626,668 deaths, followed by India with 31,341,507 cases and over 420,196 deaths as of July 24th, 2021 [6]. Influenza viruses undergo rapid evolution through frequent antigenic variation. Antigenic drift and shift are terms used to describe how the virus mutates and gives rise to new strains. Antigenic shift involves significant changes in the virus genome, resulting in the expression of new hemagglutinin (HA) and neuraminidase (NA) proteins. While antigenic shift occurs rarely, its role in contributing to and causing pandemics has been confirmed. Despite significant improvements in prevention, control, and management of cases, influenza infections continue to be a significant global communicable disease [7]. Therefore, the present study provides an update on the prevalence of influenza in Chennai and surrounding areas.

Methodology

As per WHO guidelines, the month wise distribution of prevalence has been divided as a rainy season (September–December 2022) and dry season (January–March 2023). Age groups have been classified as children (0–10 years), teen age (11–19 years), adult age (20–59), elderly age (60–79) and old age groups (80–100) for better understanding of the influenza strain prevalence in age wise criteria. During the period from September 2022 to March 2023, screening of flu viruses was conducted on hospitalized patients with respiratory infections using gene-based screening through the real-time PCR platform. A total of 444 oropharyngeal and nasopharyngeal swabs were collected from the hospitalized patients while following safety precautions and wearing personal protective equipment (PPE kit). The clinical samples were collected in a viral transport medium (Bio Era), packed in triple-layer packaging, and transported to the processing laboratory using a refrigerated sample transportation box. The VTM samples were processed under a biosafety cabinet level II, and the remaining samples were divided into two parts and stored at − 80 °C in a deep freezer for further confirmation.

Viral RNA was extracted from the collected swab samples using an automated nucleic acids extractor (HI media HipuraA prefilled plates for insta NX Mag 96) with a magnetic beads deep well plate kit, following the manufacturer's instructions. Reagents, enzymes, primers, probes, and nucleotides were ordered from 3D black bio biotech India limited-TruPCR. This kit targeted Inf A, H1N1, H3N2, Inf B, RSV, and the housekeeping gene RNAse P. Human RNA was used as an internal control to ensure the accuracy of the sample collection procedure. The extracted RNA was then subjected to a one-step reverse transcription multiplex PCR process using the Bio-Rad real-time PCR (CFX-96) instrument and TRU PCR kit reagents. The master mix was prepared using the kit reagents according to the manufacturer's instructions. A master mix of 15.0 µL was prepared in a PCR plate, and 10 µL of viral RNA template was added. The plate was sealed and centrifuged at 2000 rpm for 2 min. The prepared plate was placed in the Bio-Rad real-time PCR machine with thermal cycling conditions of 50 °C for 20 min, 94 °C for 10 min, followed by 40 cycles of 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s for screening of InfA, H1N1, H3N2, InfB, and RSV. After the completion of the PCR run, the results were analyzed, correlated, and presented.

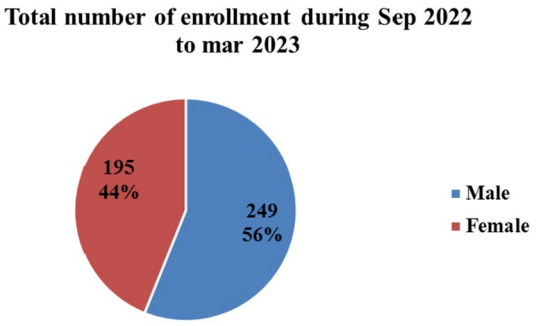


Fig. 1 Figure represent the total number of cases enrolled during study period

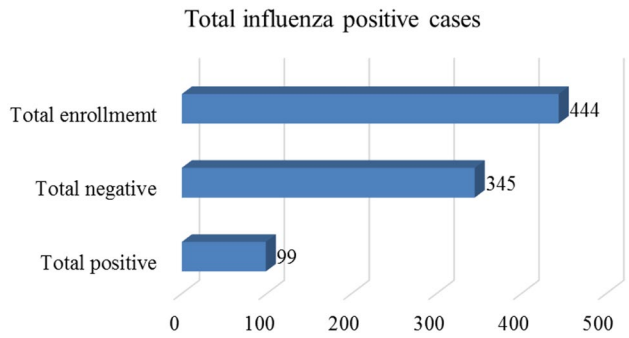


Fig. 2 Figure showing total number of influenza positive cases

Results

The RT-PCR run was analyzed based on the detection channels. In tube 1, amplification in the FAM channel indicated a positive result for InfA virus. Amplification in the VIC channel indicated a positive result for RSV, and amplification in the ROX channel indicated a positive result for Inf B virus. In tube 2, amplification in the FAM channel indicated a positive result for H1N1 pandemic virus. Amplification in the VIC channel indicated a positive result for RNAseP (Human RNA as an internal control), and amplification in the ROX channel indicated the presence of InfA H3N2 virus in the samples.

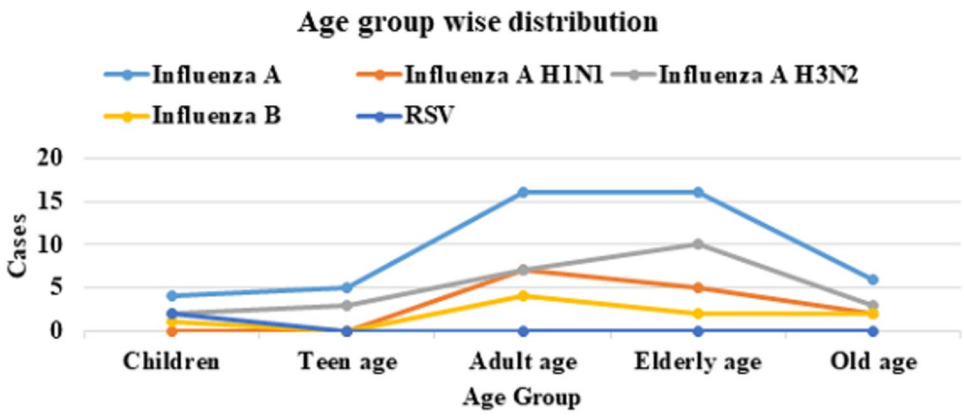
Of the 444 samples collected, 249 (56%) were male and 195 (43.9%) were female (Fig. 1). A total of 99 samples tested positive, while 345 samples were negative (Fig. 2). Most infections were found in the population elderly aged groups (60–79 years), with a prevalence of 35% (Fig. 3). Symptomatically, the following percentages were observed among the positive cases: fever (91.9%), body ache (80.8%), cough (75.7%), runny nose (61.6%), sore throat (53.5%), breathlessness (37.3%), chest pain (27.2%), diarrhea (7.07%), nausea (58.5%), vomiting (21.2%), nasal discharge (74.7%), sputum formation (81.8%), and abdominal pain (17.1%).

In cases negative for the influenza virus, the following symptoms were observed: fever (89.8%), body ache (79.7%), cough (51.01%), runny nose (61.7%), sore throat (81.1%), breathlessness (54.7%), chest pain (28.1%), diarrhea (8.9%), nausea (48.4%), vomiting (22.6%), nasal discharge (61.7%), sputum formation (70.4%), and abdominal pain (15.6%) (Figs. 4 and 5). The most detected viruses were InfA and H3N2, with prevalence rates of 11.4% and 5.1%, respectively. They were followed by H1N1 (3.1%), Inf B (2.02%), and RSV (0.5%). H3N2 was more common in adults and elderly aged groups (4.5%). Fever, body pain, regular cough, and headache were the most common symptoms associated with flu A infection. Positivity of InfA, H1N1, H3N2, and RSV viruses were also higher in aged 1–5 years (Figs. 3 and 6). The monthly distribution showed a higher positivity rate during dry season (from January to March 2023) (Fig. 6).

Discussion

To collect detailed information on the prevalence and impact of influenza virus in Chennai and surrounding areas, we conducted a study on hospitalized individuals of different age groups from 1 to 100 years with severe respiratory syndrome from September 2022 to March 2023. Of the 444 respiratory

Fig. 3 Figure represent the age group wise distribution of influenza viruses



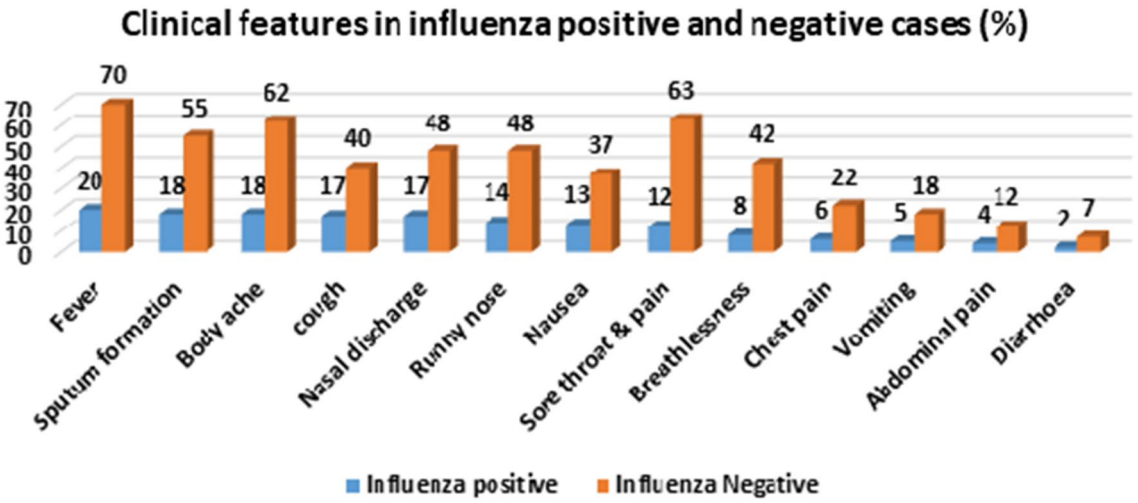


Fig. 4 Participants clinical features in influenza positive and negative cases

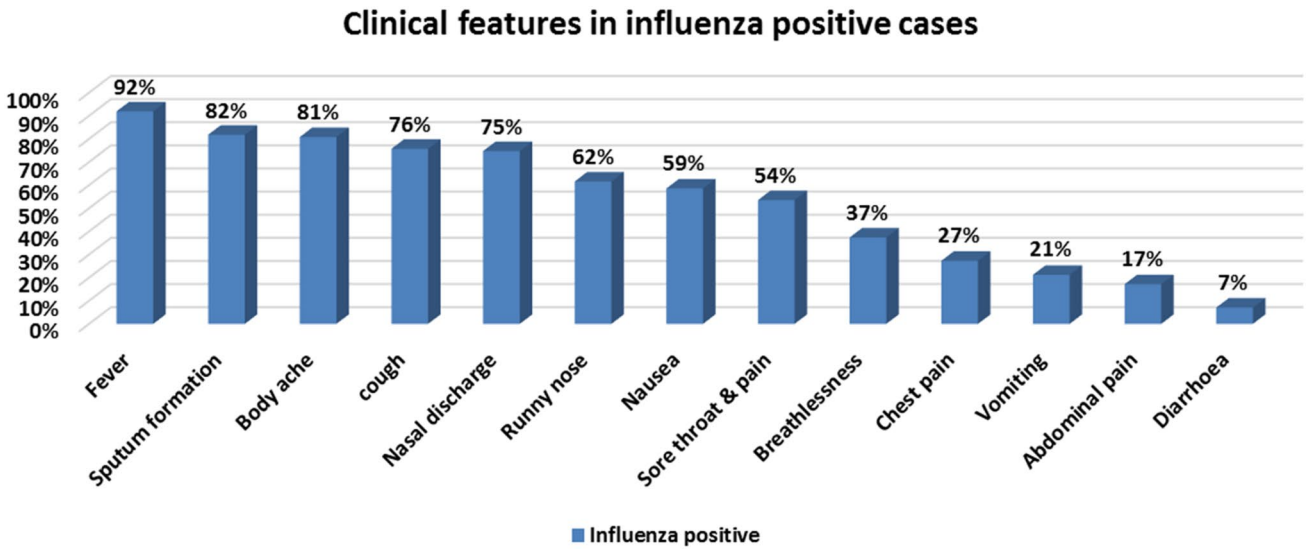


Fig. 5 Participants clinical features in influenza positive cases

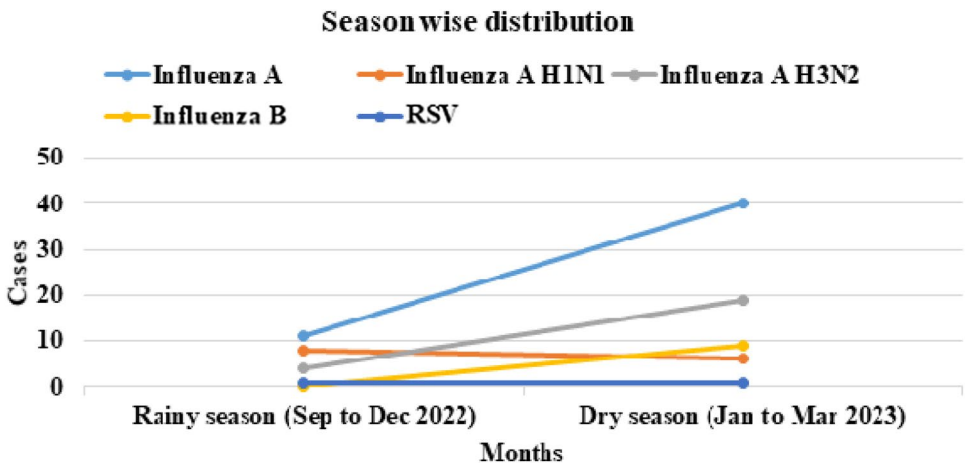


Fig. 6 Season wise distribution of influenza viruses during the study period

samples collected from these patients, 56.08% were male and 43.9% were female. The prevalence of influenza virus positivity was 22.25%, while the negative rate was 77.7%. However, no statistically significant difference was found in influenza positivity according to gender.

A study in Kenya (2011) found a prevalence of severe acute respiratory infections of 12.4 cases per 100 persons, with a higher rate of 30.4 cases per 100 in children. The study also found higher positivity for RSV and influenza cases [4]. Present study found lower positivity rate of RSV (0.5%) prevalence in Chennai. Another study conducted in Toronto, Canada, (August 2014) found that influenza positivity rates ranged from 11 to 56%. Higher positivity was observed in children aged 3–5 years compared with unvaccinated children younger than 2 years [8]. In a study conducted in Kuwait between August 1993 and June 1994, 523 children under 13 years of age participated in a prospective study of acute lower respiratory infections (ALRI). Among them, 62 children tested positive for various viruses. RSV was the most frequently identified virus, with 31 of 62 children testing positive. It was followed by influenza A, parainfluenza, Mycoplasma pneumonia, and adenovirus [9]. During 2013–2017 in Russia, 1560 samples from hospitalized children were analyzed, and 1128 samples (72.3%) tested positive for influenza viruses. The most frequently detected viruses were RSV (358/1560, 23.0%), influenza virus (344/1560, 22.1%), and rhinovirus (235/1560, 15.1%) [10].

In an earlier prevalence study conducted in Chennai in 2002, of 240 children with respiratory syndromes, 12.5% (30/240) tested positive for influenza virus. Of these, 10% (24/240) were positive for influenza A H3N2 virus and 1.66% (4/240) were positive for influenza B virus [11]. Another study conducted in Delhi between 2005 and 2007, 301 children were studied, of whom 3% (9/301) tested positive for influenza A virus [12]. In a study conducted in Kerala from 2010 to 2016, 43% of 2154 swab samples tested positive for influenza. Of these, 26.7% were H3N2, 6.3% were influenza B, and 10% were H1N1 [13]. Present study found 11.4% positive for influenza A and 5.1% positive for H3N2 virus which revealed that, decreasing trend in influenza virus prevalence in Chennai.]. A previous study in India concluded that influenza poses a significant burden to children under 5 years of age, highlighting the need for public health interventions for prevention and control [14]. Our study also found higher positivity rate of nfluenza A virus infection in children age groups (11.4%).

Influenza affects nearly 10% of the world population annually, resulting in approximately half a million deaths per year. Thus, the effectiveness of influenza vaccination is the most efficient method to prevent influenza infection and its complications [7]. A 2003 study revealed that frequent epidemics that occurred after pandemics and attributed them to minor antigenic variations in the pandemic virus strain. The study pointed to the worldwide circulation of minor antigenic variants of influenza virus types A H1N1, H3N2, and type B that cause frequent epidemics. It has been proposed to expand surveillance of influenza viruses in India [15]. Another study found that from August 2009 to July 2017, 855 children aged 3 months to 15 years became ill with H1N1 influenza, of whom 310 were hospitalized and 29 died. It was found that from 2009 to 2012, 76.5% of patients were admitted in August to October, while from 2015 to 2017, 89.3% came in January to March. The percentage of children under five years of age increased from 54.0% in 2009–2010 to 77.7% in 2015–2017 [16]. This study revealed that increasing positivity rate of influenza infection during dry season (January to March) rather than rainy season.

Prevalence of respiratory viruses in India from 1970 to 2020 and reported that most of the Indian states have higher rate of RSV, influenza and parainfluenza infections [6]. A communicable disease control plan and emphasized the need to strengthen global surveillance, national and international infrastructure, prevention and control measures, and support for communicable disease control research [16]. Influenza-associated mortality is high in children younger than 5 years and in persons older than 65 years and these estimates can serve as a basis for influenza prevention and control strategies in India, including vaccine introduction [17]. From 2017 to 2018 in the state of Andhra Pradesh, 1286 samples were analyzed. 109 samples were positive for influenza A, 356 samples for H1N1pdm, 38 samples for H3N2, and 19 samples for influenza B. The study also found no significant difference in positivity between genders [18] and we also found no significant difference in gender wise positivity.

A respiratory virus survey was conducted in four villages near Calcutta. Of the 4171 respiratory samples analyzed, 483 samples were positive for parainfluenza virus types 1, 2, 3, and 4B, RSV, and adenovirus. Infections with parainfluenza virus types 1, 2, 3, and RSV were common in village areas [19]. In Kashmir, India, 194 samples were analyzed, and 21 patients tested positive for influenza viruses. Among them, 13 patients were positive for H1N1pdm2009, 6 patients for H3N2, and 2 patients for influenza B, all of whom presented with febrile acute respiratory infections [20]. Recently, the re-emergence of monkeypox virus in humans in India has raised fears of another pandemic [21]. During 2007 and 2009 in the pediatric population of Mumbai, 100 samples were analyzed and 11 samples (11%) tested positive for influenza viruses. Among them, influenza A, H1N1, H3N2, and influenza B were detected at rates of 1%, 5%, 5%, and 5%, respectively [22].

A study focused on the effectiveness of neuraminidase inhibitors in reducing mortality in patients hospitalised with influenza A H1N1 pdm2009 virus infection. The analysis included data on 29,234 patients from 78 trials conducted

between Jan. 2, 2009, and March 14, 2011 and this study advocated early investigation of neuraminidase inhibitor treatment in adults hospitalised with suspected or confirmed influenza infection [23]. In October 2016, highly pathogenic avian influenza viruses (H5N8) were detected in waterfowl at two zoos in India. These viruses were different reassortments of H5N8 viruses previously isolated from wild birds in the Russian Federation and China in May 2016. This suggests that the virus spread during the southward winter migration of birds [24].

During April 2016–August 2018 enrolled 343 children aged 45 days–17 years with acute respiratory infections (ARI). Among the 343 samples analyzed, RSV and influenza viruses were detected most frequently, with prevalence rates of 17.1% and 10.9%, respectively. They were followed by human parainfluenza virus type 3 (HPIV 3) with 4.2%, human metapneumovirus (hMPV) with 4%, and human rhinovirus (HRV) with 3.7% [25]. A study on the treatment and prevention of the pandemic H1N1 flu virus and suggested that antiviral drugs are most effective when used within 48 h of the onset of the first clinical signs [26]. A serologic survey conducted in Uttar Pradesh between 2009 and 2016 found that the seroprevalence rate of H1N1 virus, based on the hemagglutination inhibition (H1) test, ranged from 5.2% in 2009 to 36.3% in 2011 [27]. In June 2008, an outbreak of equine influenza (EI) virus was reported in India after a hiatus of 2 decades. The outbreak initially started in Jammu and Kashmir, a northern state of India, then spread to other parts of the country and affected horses in 11 states. The virus isolated from clinical cases was identified as H3N8 at various locations throughout the country [28]. In the twenty-first century, there have been several outbreaks of highly pathogenic and contagious viruses of zoonotic origin. These include severe acute respiratory syndrome coronavirus (SARS-CoV-2), Ebola virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and Nipah virus [29, 30].

Present study revealed that InfA and H3N2 strains prevalence rates is higher compared with Inf B and RSV and H3N2 was more common in adults and elderly aged groups. Fever, body pain, regular cough, and headache were the most common symptoms associated with flu A infection. Positivity of InfA, H1N1, H3N2, and RSV viruses were also higher in aged 1–5 years. The monthly distribution showed a higher positivity rate during dry season (from January to March 2023) rather than rainy season.

Conclusion

In conclusion, this study sheds light on the analysis of the prevalence of influenza viruses, including influenza InfA, H1N1, H3N2, InfB, and RSV, in the population of Chennai and its surrounding areas. The study included 444 samples collected from hospitalized patients with severe respiratory symptoms, and results showed that InfA and H3N2 were the most prevalent strains. H3N2 was more commonly observed in adults aged 51–90 years, while RSV, H1N1, and H3N2 had higher positivity rates in children aged 1–5 years. Fever, body aches, cough, and headache were the most common symptoms associated with InfA infection. The prevalence of influenza virus varied among age groups, with the highest infection rates observed in the population aged between 60 and 79 years. The data obtained from this study will contribute to the early detection of influenza viruses and provide valuable information for public awareness and preventive measures against influenza virus infections in Chennai and its surrounding areas.

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Author Contributions CNS: conceived and designed the study. SSK prepared the materials and performed experiments. CNS and SSK analyzed the data and wrote the manuscript. AK reviewed the manuscript. All authors read and approved the final manuscript.

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Code Availability Not Applicable.

Declarations

Conflict of interest Authors declared no conflict of interest.

Ethical Approval Not Applicable.

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