



## Laboratory Medicine

## A SURGE OF H3N2 INFLUENZA CASES IN PATIENTS PRESENTING WITH FLU-LIKE SYMPTOMS: AN EXPERIENCE FROM A TERTIARY CARE HOSPITAL

<b>Dr Melody Baruah</b>	Consultant Microbiologist, Department Of Laboratory Medicine, Health City Hospital, Guwahati, 781022, Assam.
<b>Dr Himadri Dutta</b>	Consultant Microbiologist, Department Of Laboratory Medicine, Health City Hospital, Guwahati, 781022, Assam.
<b>Dr Deep Jyoti Kalita*</b>	Consultant Pathologist, Department of Laboratory Medicine, Health City hospital, Guwahati 781022, Assam. *Corresponding Author

**ABSTRACT** **Introduction:** Seasonal influenza remains a considerable cause of excess morbidity, mortality, and healthcare-system burden. Over the time, many variants of influenza viruses have evolved and created panic situations, especially in the vulnerable population. **Aims and objectives:** Present study was conducted to observe the epidemiological profile, clinical presentations, associated comorbidities, laboratory parameters and outcome of the patients presenting with respiratory symptoms following a surge of Influenza A cases in a tertiary care super specialty hospital of North East India. **Materials and Methods:** This observational retrospective study was done during the period of March 2023 to August 2023. All the suspected cases for influenza like illness attending our hospital and tested for SarsCoV -2, H1N1 and H3N2 were included. Viral nucleic acid was extracted and detection was done using Truenat COVID-19 and Trunat (H1N1+H3N2 combo) micro PCR assay. The epidemiological data, clinical profile, co-morbidities, laboratory parameters and outcome of these patients were recorded and statistical analysis was done using Chi square and Z test for obtaining p value. **Results:** A total of 350 cases were included in the study. 66 confirmed cases of Influenza A (3 H1N1 and 63 H3N2) were detected during the study period and none tested positive for COVID-19. Majority of patients belonged to the age group of >60 years. Both genders were equally affected with slight male preponderance. 15 patients required Intensive Care Unit (ICU) admission while 15 patients were managed in ward. The predominant symptoms at presentation were fever (84.57%), cough (46.28%) and Rhinitis (42.8%). Most common comorbid conditions associated were diabetes mellitus (10.28%), hypertension (7.42%), coronary artery disease (4.86%) and chronic obstructive pulmonary disease (COPD) (3.42%). **Conclusion:** From this study, it can be hypothesized that the prevalence of Influenza A H3N2 is high in the elderly population, and is more common during the monsoon season with most patients presenting with symptoms resembling flu. Vaccination, early recognition of the disease, and prompt initiation of treatment seem to be the only way to reduce the disease progression and associated mortality. TruenatPCR helps in the rapid and early diagnosis of Influenza A cases.

**KEYWORDS :** H1N1, H3N2, Trunat PCR, Flu-like illness, Influenza

### INTRODUCTION:

Seasonal influenza remains a considerable cause of excess morbidity, mortality, and healthcare-system burden. Over the time, many variants of influenza viruses have evolved and created panic situations by increasing morbidity and mortality, especially in the vulnerable population. (1) During the first pandemic of H1N1 influenza, H3N2 cases were also isolated. (2) As per the Indian Council of Medical Research (ICMR), the H3N2 virus has been responsible for a significant increase in the number of flu cases in India over the past few months. (3) The H3N2 virus is highly contagious and spreads through respiratory droplets when an infected person coughs or sneezes. The symptoms of H3N2 infection are similar to seasonal flu and include fever, sore throat, cough, fatigue, and body aches. While most people recover from H3N2 infection within a week, in some cases it can lead to severe complications such as pneumonia, bronchitis, and even death. (4) ICMR stated that in the current outbreak of H3N2 about 10% of the patients suffering from severe acute respiratory illness (SARI) caused by the virus required oxygen support and 7% required an ICU care. (5) Present study was conducted to observe the epidemiological profile, clinical presentations, associated comorbidities, laboratory parameters and outcome of the patients presenting with respiratory symptoms following a surge of Influenza A cases during the 6 months study period in a tertiary care super specialty hospital of North East India.

### MATERIALS AND METHODS:

This observational retrospective study was done during the period of March 2023 to August 2023 at a tertiary care Super-specialty hospital of Guwahati, Assam, India. The study was approved and ethical clearance was obtained from the institutional ethical committee. All the suspected cases for influenza like illness attending our hospital and tested for SarsCoV -2, H1N1 and H3N2 during the study period were included. Clinical specimens like nasal or throat or nasopharyngeal swabs were collected by nylon swab and transported in the viral transportlysis medium (both supplied by the manufacturer). Viral nucleic acid detection was done by Truenat COVID-19 and Trunat (H1N1+H3N2 combo) micro PCR assay. As soon as the samples were received in the Microbiology department, viral RNA was isolated by Trueprep AUTOUniversal Cartridge Based Sample Prep Device.

Following the extraction of viral nucleic acid Six (6) µL of the purified RNA is then dispensed using the provided micropipette and tip into the microtube containing freeze dried RT-PCR reagents, including reverse transcriptase (RT) and allowed to stand for 30-60 seconds to get a clear solution. Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the Truenat H3N2+H1N1/ COVID-19 chip and the test is inserted in the Truelab Real Time Quantitative micro PCR Analyzer where the RNA is first converted into complementary DNA (cDNA) by the RT enzyme and further thermal cycling takes place. The target sequence for H1N1+H3N2 assay is the HA gene of H3N2 genome and NP and HA gene of H1N1 genome. The target sequence for COVID kit is E and Orf1a and the human RNase P gene. Detection of the human RNase P gene serves as a full process internal positive control (IPC) for proper swab collection, nucleic acid extraction and PCR. At the end of test run, results were displayed as detected or not detected. This whole procedure takes around an hour time. All the confirmed cases were notified to the concerned authorities through proper channel. Informed consent was taken from all the patients included in the study using the general consent form of our hospital. The government recommended test request forms for Sars CoV-2 and H1N1/H3N2 were used. The files of all the patients included in the study was archived from the Medical records department and their epidemiological data, clinical profile, co-morbidities present, laboratory parameters and outcome were recorded and statistical analysis was done using Chi square and Z test for obtaining p value.

### RESULTS:

A total of 350 cases presenting with flu-like symptoms at our hospital during the period of March 2023– August 2023 were included in the study. 66 confirmed cases of Influenza A (3 H1N1 and 63 H3N2) were detected during the study period. No sample was tested positive for COVID-19 RT PCR. Majority of patients belonged to the age group of >60 years (32 patients – 48.4 %). However, all the age groups seemed to be affected by the infection. (Table 1)

**Table 1: Statistical analysis of Influenza A cases–Age wise.**

Age(years)	No of cases	No of positives	No of negatives
<20	14	3	11

20-29	55	9	46
30-39	48	10	38
40-49	56	4	52
50-59	52	8	44
60 and above	125	32	93
Total	350	66	284

Age wise statistical analysis of Influenza A cases was done, Chi square value with 10 degree of freedom was found to be 9.55 where p value was 0.48 (>0.05 Not significant).

Both genders were equally affected with slight male preponderance (53.03 % – males). (Table 2).

**Table 2: Statistical analysis of Influenza A cases, gender wise.**

Gender	Total number	Positive	Negative
Male	192	35	157
Female	158	31	127
Total	350	66	284

Gender wise statistical analysis of Influenza A cases was done, Chi square value with 2 degree of freedom was found to be 0.109 where p value was 0.74 (>0.05 Not significant).

Out of the 66 diagnosed cases, 15 (22.7%) patients required Intensive Care Unit (ICU) admission while 15(22.7%) patients were managed in ward. Out of 15 patients admitted at ICU, 2 (3%) needed mechanical ventilation and 8 (12.1%) were managed by NIV (Noninvasive ventilation) and 5 (7.6%) needed O2 support. Rest of the patients were advised home isolation. There were 2 (3%) reported deaths among the patients admitted at ICU, however the cause of death was not attributed to viral Influenza. The predominant symptoms at presentation were fever (84.57%), cough (46.28%), Cold/Rhinitis/Running nose (42.8%), sore throat (30.8%), and shortness of breath (20%). Other symptoms have been listed in Table 3.

**Table3: Clinical presentation of the cases.**

Clinical presentation	Number of cases(percentage)
Fever	296(84.57%)
Cough	162(46.28%)
Cold/Rhinitis/Running nose	150(42.8%)
Sore throat	108(30.8%)
Dyspnea (SOB)	70(20%)
Myalgia	42(12%)
Diarrhoea	31(8.85%)
Headache	14(4%)

Most common comorbid conditions associated were diabetes mellitus (10.28%), hypertension (7.42%), coronary artery disease (4.86%) and chronic obstructive pulmonary disease (COPD) (3.42%). (Table 4) The P value for all the comorbid conditions shows a significant statistical association.

**Table 4: Comorbid conditions.**

Condition	Total no of cases(n=350)	Positive cases	P value
Diabetes mellitus	86	36(10.28%)	<0.00001
Hypertension	65	26(7.42%)	<0.00001
Coronary artery disease	35	17(4.86%)	<0.00001
Bronchial asthma	21	13(3.42%)	<0.00001
Chronic kidney disease	18	8(2.28%)	<0.0004

The mean total leukocyte count (TLC) was normal (7,600) in viral influenza group of patients and high (10,029) in the non-viral influenza group of patients indicating secondary bacterial infection. P value was found to be 0.02 (<0.05, hence significant). Total leukocyte count (TLC) was normal in 78.8 % of patients, decreased in 12.1 %, and increased in 9.1%.

**Table 5: Comparison of mean values of different laboratory parameters in Viral Influenza A and non-viral Influenza A group and statistical significance of difference between both groups.**

Parameter	Disease group(Mean )	Non diseased group (Mean)	P value
CRP	57.28	95.9	0.07(>0.05)
TC	7600	10029	0.02(<0.05)
SGOT	71.90	38.82	0.15(>0.05)

SGPT	56.11	38.87	0.87(>0.05)
------	-------	-------	-------------

**DISCUSSION:**

After the 2009 pandemic, frequent outbreaks of swine flu cases have been reported in the Indian subcontinent (5,6). In the current year 2023, from January onward, multiple outbreaks of different subtypes of Influenza-A viruses have been reported in various states of India that have led to much morbidity (3038 cases till 10.3.2023) and a few mortality.(7,8) Though cases have been reported sporadically from the north-eastern states including Assam, not much of published data is available on H3N2 from these states. Due to lack of availability of a rapid, reliable and cheap diagnostic test for the detection of suspected cases in the peripheral settings, many influenza cases go undiagnosed. Truenat micro PCR (MOLBIO) system allows rapid point of care testing with less turnaround time and does not need any skilled manpower and infrastructure. It is a chip based technology where single sample can be tested for both COVID-19 and H1N1+ H3N2 combo with accurate results within one hour time.

In a study by Chadha et al. where analysis of monthly data over a five year period was done showed influenza circulation primarily from May-August for most years in Assam. (9) Our study was also done during this period when there was a surge of Influenza A cases in the community. This seasonal trend can be explained by increased rainfall and humidity which favors virus transmission. (10)

In the present study 18.85 % cases came positive for Influenza A. Our positivity rates are similar to Chadha et al.(9) who reported an overall prevalence of 14% in India and Nandhini G et al.,(5) where 12.7% of the samples were positive for influenza virus. Whereas another study done by Sona et al., from Assam found a higher positivity of 35.5% for Influenza A cases. (11) This high positivity rate could be attributed to small sample size (n=90) and the study was conducted in the pre-COVID period when there was less awareness on infection control practices.

Majority of patients belonged to the age group of >60 years (48.4 %) followed by 30-39 years (15.2%) in our study and both genders were equally affected with slight male preponderance (53.03 %). However, most of the other studies done on H1N1 reported maximum number of cases in younger population (20-40 years by J Vijayalakshmi et al. [12] and 27.3 years by Siddharth, et al.[13]). However E. Rothman et al., reported that patients with Community acquired Influenza (CAI) H3N2 were older than patients with CAI H1N1 (median age 78 and 64 years, respectively) which is similar to our study. (14) This finding can be attributed to age related comorbidities present in the elderly population.

In our study 22.7% required ICU admission and all these patients needed O2 support which is much higher than ICMR data (10% required oxygen support and 7% required ICU care).(4) This high rate could be explained by the fact that H1N1+H3N2 PCR testing is not available in other private hospitals and most of these diagnosed patients were referred from the periphery with aggravated symptoms to our Laboratory. While most people recover from H3N2 infection within a week, in some cases it can lead to severe complications such as pneumonia, bronchitis, and even death. (3) Among the 2 reported deaths in our study, one patient died of sepsis and another patient was immunocompromised and had lymphopenia which is an established risk factor for poor outcome in viral Influenza patients. (15)

The symptoms of H3N2 infection are similar to seasonal flu and include fever, sore throat, cough, fatigue, and body aches. (Boppana et al.) (3) Even our study reported similar symptoms like fever, cough, Rhinitis, sore throat, and shortness of breath.

Most common associated comorbid conditions in our study were diabetes mellitus (10.28%), hypertension (7.42%), coronary artery disease (4.86%), chronic obstructive pulmonary disease (COPD) (3.42%), Bronchial asthma (3.42%) and Chronic kidney disease (2.28%) which is similar to studies done elsewhere.(11, 12, 13) Minchole et al. also reported more frequent association with cerebrovascular disease, and chronic heart disease. (16)

In our study the mean total leukocyte count (TLC) was normal (7,600) in viral influenza group of patients and high (10,029) in the non-viral influenza group and the P value was found to be 0.02 (<0.05, hence significant). H3N2 is more severe than H1N1 in terms of leucopenia as per other studies. (17) Prasad et al. reported that low TLC was more

suggestive of Influenza A infection in the presence of appropriate clinical signs while higher TLC indicated secondary bacterial infection. In their study total leukocyte count (TLC) was normal in 49% of patients, decreased in 22.37%, and increased in 13.16% which is similar to our findings. (18)

Since none of our patients were vaccinated for Influenza vaccine hence comparison between vaccinated and non-vaccinated patients could not be done.

### CONCLUSION:

On the basis of the findings of this study, it can be hypothesized that the prevalence of Influenza A H3N2 is high in the elderly population, and is more common during the monsoon season with most patients presenting with symptoms resembling flu. However the patients requiring hospital admission and O2 support could be quite higher than other Influenza A viruses. Hence the H3N2 outbreak in India is a significant concern that requires immediate attention and action from both the government and individuals. General population with increased susceptibility to influenza virus should be encouraged to get the vaccine. Vaccination, early recognition of the disease, and prompt initiation of treatment seem to be the only way to reduce the disease progression and associated mortality. Truenat H1N1, a chip based molecular test for detection of swine flu influenza H1N1 and H3N2 helps in the rapid diagnosis and better management of the influenza cases. User friendliness of the instrument allows less expertise which can be utilized even in the peripheral settings.

**Conflict of interest:** None declared.

**Acknowledgement:** We would like to thank Molbio for technical and financial support and all the staff of Department of Laboratory Medicine and Medical records department, Health City Hospital.

### REFERENCES:

- Saxena V, Mishra A. Influenza outbreak in India: A course ahead. *Indian J. of Com. Health.* 2023;35(1):1-3.
- Park S, Kim JI, Lee I, et al. Susceptibility of human H3N2 influenza virus to oseltamivir in South Korea, 2009–2011. *J Microbiol.* 2012; 50(6):1067–1070.
- Boppana H, Kanishk K., Lakshmi V. S. Kutikup-pala et al., The recent H3N2 viral outbreak in India, 2023: Is it worrying? *Health Sci. Rep.* 2023;6:e1417, <https://doi.org/10.1002/hsr.2.1417>
- Indian Council of Medical Research. Influenza surveillance. 2021. [https://main.icmr.nic.in/sites/default/files/whats\\_new/ICMR\\_weekly\\_influenza\\_update\\_21\\_Jan\\_2021.pdf](https://main.icmr.nic.in/sites/default/files/whats_new/ICMR_weekly_influenza_update_21_Jan_2021.pdf)
- Nandhini G, Sujatha S. Epidemiology of influenza viruses from 2009 to 2013-A sentinel surveillance report from Union territory of Puducherry, India. *Asian Pacific Journal of Tropical Medicine.* 2015;8:718-23.
- Murhekar M, Mehendale S. The 2015 influenza A (H1N1) pdm09 outbreak in India. *Indian J Med Res.* 2016;143(6):821-23.
- Update on Seasonal Influenza [Internet]. Press Information Bureau. PIB; 2023 [cited 2023Mar19]. Available from: <https://pib.gov.in/PressReleasePage.aspx?PRID=1905602>
- Ministry of Health & Family Welfare-Government of India. State/UT - wise, year-wise number of cases and deaths from 2018-2023 [Internet]. Ministry of Health and Family Welfare. NCDC; 2023 [cited 2023Mar19]. Available from: <https://ncdc.mohfw.gov.in/showfile.php?lid=280>
- Chadha MS, Potdar VA, Saha S, Koul PA, Broor S, et al. Dynamics of influenza seasonality at sub-regional levels in India and implications for vaccination timing. *PLoS One.* 2015 May 4; 10(5): e0124122. Doi: 10.1371/journal.pone.0124122. PMID: 25938466; PCMid: PCM4418715
- Tamerius JD, Shaman J, Alonso WJ, Bloom-Feshbach K, Uejio CK, Comrie A, et al. Environmental predictors of seasonal influenza epidemics across temperate and tropical climates. *PLoS pathogens.* 2013; 9(3):e1003194. doi: 10.1371/journal.ppat.1003194 PubMed PMID: 23505366; PubMed Central PMCID: PMC3591336
- Sona S, Sharma A, Chamuah K, Henbi LN, Rajbongshi G. Influenza virus and its subtypes circulating during 2018–2019: A hospital-based study from Assam. *Indian J Med Microbiol.* 2022 Oct-Dec;40(4):525-530.
- J Vijayalakshmi et al., Surveillance of Swine Flu Influenza H1N1 by Chip based Real Time PCR Technology from the Clinical Specimens. *Journal of Clinical and Diagnostic Research.* 2018 Dec;12: DC01-DC04
- Siddharth, et al.: Clinical-epidemiological profile of influenza A H1N1 cases. *Indian J of Community Med.* 2012 Oct- Dec;37(4): 232-235.
- Rothman E, Olosson O, Christiansen CB, Roost M, et al. Influenza A subtype H3N2 is associated with an increased risk of hospital dissemination - an observational study over six influenza seasons. *J of Hos Infection.* 2023 Sep; 139: 134-140.
- Asmita A. Mehta , V.Anil Kumar , Suresh G. Nair , Fini K Joseph , Gireesh Kumar , Sanjeev K. Singh. Clinical Profile of Patients Admitted with Swine-Origin Influenza A (H1N1) Virus Infection: An Experience from A Tertiary Care Hospital. *Journal of Clinical and Diagnostic Research.* 2013 Oct, Vol-7(10): 2227-2230
- Minchhole E, Figueredo1 AL, Omeñaca M, PanaderoC ,Royo L et al. Seasonal Influenza A H1N1pdm09 Virus and Severe Outcomes: A Reason for Broader Vaccination in Non-Elderly, At-Risk People. *PLoS One.* 2016 Nov 10; 11(11): e0165711.
- Kazi M, Watanabe A, Aizawa H. Differences in clinical features between influenza A H1N1, A H3N2, and B in adult patients. *Respirology.* 2003; 8(2): 231-233.
- Prasad S, Indhu AJ, Margos RA, Philip S. Clinical profile and outcome of H1N1 influenza patients in a tertiary care hospital in Kochi, Kerala. *Indian J Respir Care.* 2018;7:97-101.