International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 01-04-2025, Accepted: 02-05-2025, Published: 17-05-2025
Volume 7, Issue 2, 2025, Page No. 27-30

Molecular detection and correlation of real-time PCR CT values with clinical manifestations and severity of influenza a in respiratory illness patients

Hritik Chaudhary, Siddhant Mavi, Vinod Joshi, Bennet Angel, Annette Angel
Department of Life Science, Centre of Excellence in Virology and Immunology, Sharda University, Greater Noida, Uttar Pradesh, India

Abstract
Background: Patients with respiratory like illness were considered for the study and their viral load/severity of infection and Ct (Cycle threshold) values were compared based on number and severity of clinical symptoms.
Aim & Objective: To compare the severity of infection with Ct value in both viral and bacterial-viral co-infections.
Methods and Material: Swab samples were taken and subjected to RNA Extraction along with RT-PCR using standard protocol, to determine positive and negative samples and to find out Ct values of positive cases for further analysis.
Result: Influenza a positive subjects showed more severe respiratory symptoms in comparison to the negative cases. And among positive cases, a clear trend is seen in which individuals with lower Ct values, indicative of higher viral loads exhibited more severe respiratory symptoms and patients with higher Ct values showed milder clinical presentations despite having multiple comorbidities.
Conclusion: An inverse relationship between Ct value and severity of infection/viral load is established. Co-infection with other pathogens, further exacerbated the clinical manifestation, underscoring the need for comprehensive diagnostic approaches that consider possible polymicrobial infections.

Keywords: Influenza A, RNA extraction, RT-PCR, CT value, polymicrobial infections

Introduction

Influenza virus causes contagious viral infections, resulting in small epidemics especially in winter season. Whether it will take the form of an epidemic or even larger outbreak, depends on the climatic and geographical conditions of the area. Majority of its incidences are acute infections with very less instances of severe infection resulting in fatal outcomes, people with comorbid conditions are more prone to having a severe infection [1]. Influenza virus is classified into four types A, B, C and D. Among these Influenza A, B and C causes infections in humans and some other animals such as birds, pigs etc. Whereas Influenza D virus is seen to impact only cattle and pigs [2].

The surface of the Influenza A virus is covered by spikes proteins formed by HA (Hemagglutinin) and NA (Neuraminidase). These surface proteins help in attachment and penetration in the host cell as well as its exit after amplification. M1 and M2 proteins are also present in the virus with the former being a structural scaffold and the latter being an integral membrane protein which helps in fusion of viral and endosomal membranes, triggering the release of viral RNA into the host cytoplasm for amplification. The genetic material of Influenza A virus consists of eight single stranded, negative sense RNA molecules which are present in association with Nucleoprotein (NP) and viral RNA polymerase, forming a structure known as Viral Ribonucleoproteins (vRNPs) [3, 4].

Influenza A have caused several pandemics in the past such as 1918 Spanish flu, 1957 H2N2, 1968 Hong Kong flu and 2009 H1N1 pandemic in Mexico [5]. Globally, influenza A contributes to about 3 to 5 million cases of severe illness every year, with up to 650,000 respiratory deaths according to WHO estimates. In India seasonal influenza has seen fluctuating yet substantial burden is there, especially during the monsoon and winter seasons [6].

Materials and Methods

In this study a total of 20 oropharyngeal and nasopharyngeal swab samples were collected from Sharda hospital. Clinical symptoms of the patients were recorded along with others underlying comorbidities and their vaccination status against SARS-CoV-2 infection was also noted down. The participants were made aware of the study and after signing the consent form and filling the Patient Information Sheet, the swabs samples were taken. These samples were brought to the laboratory under proper storage conditions. The samples along with VTM (viral transport medium) were transferred into centrifuge tubes under aseptic conditions and stored at -80°C till further experimentation. The samples were then briefly centrifuged, and RNA extraction was performed using QIGEN QIAamp viral RNA mini-Kit as per the manufacturer's protocol. The total RNA was subjected to RT-PCR as per the standard protocol (m/s AMbion, USA) and then Ct values of positive samples were noted down.

Results and Discussion

A total of 20 samples were collected during the study period. Of these, 50% were found to be positive for Influenza a virus as mentioned in Table 1

Table 1: Detection of Influenza a virus in swab samples collected from patients with respiratory like illnesses

s. no	Sample code	Age / Sex	Locality	Ct value	Rt PCR test results (+ve or –ve)
1	MSB/25/01	M/75	Greater Noida	20.04	+ve
2	MSB/25/02	F/80	Greater Noida	17.86	+ve
3	MSB/25/03	M/75	Greater Noida	No Ct	-ve
4	MSB/25/04	M/86	Greater Noida	No Ct	-ve
5	MSB/25/05	F/47	Greater Noida	No Ct	-ve
6	MSB/25/06	M/32	Noida	21.55	+ve
7	MSB/25/07	F/90	Dankaur	No Ct	-ve
8	MSB/25/08	M/32	Greater Noida	No Ct	-ve
9	MSB/25/09	M/44	Ghaziabad	No Ct	-ve
10	MSB/25/10	F/24	Noida	26.67	+ve
11	MSB/25/11	M/33	Greater Noida	28.29	+ve
12	MSB/25/12	M/80	Greater Noida	No Ct	-ve
13	MSB/25/13	M/20	Greater Noida	27.41	+ve
14	MSB/25/14	F/70	Aligarh	No Ct	-ve
15	MSB/25/15	F/69	Agra	17.78	+ve
16	MSB/25/16	M/46	Aligarh	No Ct	-ve
17	MSB/25/17	M/70	Greater Noida	34.23	+ve
18	MSB/25/18	M/60	Delhi	24.49	+ve
19	MSB/25/19	F/81	Bulandshahr	17.65	+ve
20	MSB/25/20	F/20	Bulandshahr	No Ct	-ve

The record of the clinical conditions of each patient was also noted and is displayed in Table 2. Most patients had shortness of breath and cough as main symptoms followed by fever, loss of appetite, weakness etc. A small subset of negative cases tested positive for other infections, including one for Hepatitis B, one for Hepatitis C, and one for Typhoid, which contributed to their symptom profile including high fever with chills, swelling etc. along with the previously mentioned symptoms, but still lacked the intensity and complexity observed in Influenza A positive cases.

Two positive subjects were also having a co-infection with bacteria, *Mycobacterium tuberculosis*, and both these subjects are showing severe symptoms including high fever with chills, pneumonia, blisters in mouth, abdominal pain, constipation, loss of appetite and weight along with other symptoms of positive subjects including shortness of breath and cough with sputum. Table 3 indicates the comorbidities which the patients had along with current illness. It appeared that most of the patients (thirteen out of twenty) had taken vaccine against another respiratory virus i.e. SARS-Co-V- 2.

Table 2: Patient's clinical conditions and symptoms

	Patient code	Patient's Clinical Conditions / Symptoms			
S. no		Respiratory complaints	Cough	Breathlessness	Others
1	MSB/25/01	Shortness of Breath	Severe cough with sputum	Yes	Fever (Frequent, 1 year), Loss of Appetite, Loss of taste, Weakness, Acidity, Rashes in palms, swelling on face, lesions in mouth
2	MSB/25/02	Shortness of Breath (3 Days) - Especially while walking, Elevated heart rate	Severe cough with sputum (2 months)	Yes	Fever (seasonal), Frequent Vomiting (for few days)
3	MSB/25/03	Shortness of Breath, seasonal dust allergy	Mild cough	Yes	Fever (Frequent), HCV positive
4	MSB/25/04	Shortness of Breath (10 years)	Mild cough with sputum (became severe in last 2 days)	Yes	Hepatitis B positive, Loss of Appetite, weakness, swelling on body
5	MSB/25/05	Shortness of Breath (1 Year)	Mild cough with sputum (6 months)	Yes	Loss of Appetite and weight, Acidity, stomach pain while breathing, severe skin allergy (history of injury with metallic needle, 1 year, tetanus shot not taken) - severe pain in hands (unable to fold fingers)
6	MSB/25/06	Shortness of Breath on exertion	Dry cough	Yes	H/O TB infection, parapneumonic effusion, High fever with chills, Loss of appetite and weakness
7	MSB/25/07	Shortness of Breath (15 days)	cough and scanty sputum (15 days)	Yes	Fever (15 days), COPD, acidity
8	MSB/25/08	Shortness of Breath (started recently)	cough with scanty sputum	Yes	chest pain, body ache, overweight (110 kg), Acute respiratory distress syndrome (ARDS), pulmonary edema, Grade 2 diastolic dysfunction, Mild left ventricular hypertrophy (LHV), Rashes and redness on body
9	MSB/25/09	Shortness of Breath	cough with sputum	Yes	Fever with chills, difficulty in breathing
10	MSB/25/10	Shortness of Breath (After recent pus Abscess drainage	Dry cough	Yes	Fever, pneumonia, blisters in mouth, constipation, abdominal pain, loss of appetite

		surgery)			and weakness, H/O tuberculosis infection
11	MSB/25/11	Shortness of Breath	cough with sputum	Yes	Fever, Fatty liver, kidney infection
12	MSB/25/12	Shortness of Breath	cough with sputum	Yes	Fever, COPD
13	MSB/25/13	Shortness of Breath, chest pain	cough with scanty sputum(10 days)	Yes	Fever (10 days), Pleural effusion, weight loss
14	MSB/25/14	Shortness of Breath (2 days), chest pain	cough (2 months)	Yes	Fever (2 months), COPD, LRTI (Lower respiratory tract infection)
15	MSB/25/15	Shortness of Breath	cough with sputum	Yes	Fever, constipation, COPD (Chronic obstructive pulmonary disease) APD (Acute pulmonary disease)
16	MSB/25/16	Shortness of Breath (1.5 year)	cough with yellow sputum (2 days)	Yes	Fever (2 days), Typhoid positive,
17	MSB/25/17	Shortness of Breath with mild chest pain	cough with runny nose and white sputum	Yes	COPD, Grade 1 fatty liver, Grade 1 prostatomegaly, old treated hydropneumothorax
18	MSB/25/18	Shortness of Breath (2 years)	Cough with green sputum (4 years) Severely increased since 15 days	Yes	COPD/APD, fever and cough since 1 week (increased at night), chest pain while coughing, acidity, cholilithiasis, chronic cholecystitis, grade 1 fatty liver
19	MSB/25/19	Shortness of Breath (3 years)	Cough with sputum (3 years)	Yes	Abdominal pain, acidity, COPD, left pleural effusion, vomiting, seizures, loss of consciousness
20	MSB/25/20	Shortness of Breath (2 months)	Cough (2 months) with expectoration	Yes	Fever (on/off 2 months), pleural effusion, headache and weakness

Table 3: Details of comorbidities existing and vaccination status against SARS-CoV-2 in the study patients

S. no.	Patient code	COMORBIDITIES			Vaccination status (SARS Cov-2)
		Hypertension (H/O)	Diabetes (H/O)	Covid 19 (H/O)	
1	MSB/25/01	No	No	No	Yes (3 doses)
2	MSB/25/02	No	No	No	Yes (3 doses)
3	MSB/25/03	No	Yes	No	Yes (3 doses)
4	MSB/25/04	Yes (1 year)	No	No	Yes (3 doses)
5	MSB/25/05	No	No	No	Yes (3 doses)
6	MSB/25/06	No	No	No	Yes (3 doses)
7	MSB/25/07	Yes (1 year)	No	No	No
8	MSB/25/08	Yes (controlled by medication)	No	No	No
9	MSB/25/09	Yes	No	No	Yes (2 doses)
10	MSB/25/10	low BP	No	No	No
11	MSB/25/11	No	No	No	No
12	MSB/25/12	No	No	No	No
13	MSB/25/13	No	No	No	Yes (3 doses)
14	MSB/25/14	Yes (on medication)	No	No	Yes (2 doses)
15	MSB/25/15	Yes (on medication)	No	No	No
16	MSB/25/16	No	No	No	Yes (3 doses)
17	MSB/25/17	low BP	Yes	No	Yes (3 doses)
18	MSB/25/18	No	No	No	Yes (3 doses)
19	MSB/25/19	No	No	No	Yes (3 doses)
20	MSB/25/20	No	No	No	No

The present study aimed to investigate the current respiratory virus circulating in the NCR region. We also aimed to investigate whether severity to infection is due to presence of multiple infection by Respiratory viruses or whether there is co-infection by any bacterial species. The correlation between Influenza A virus infection, symptom severity, and RT-PCR Ct (Cycle Threshold) values was also done. The swab samples when analyzed by RT-PCR indicated 50% presence of Influenza A virus in the hospitalized patients indicating the present season to be positive for the virus. All positive cases displayed a visible amplification peak on RT-PCR with specific Ct values, whereas negative samples exhibited no amplification and consequently no Ct value.

A comprehensive analysis of the clinical presentation of the Influenza A positive subjects revealed a clear trend of more severe respiratory symptoms in comparison to the negative cases, symptoms include shortness of breath in all the subjects, mild to severe chest pain both while resting or during exertion in all the subjects, dry cough or cough with sputum(white or green colored) in all the subjects along with runny nose in some, out of 10 subjects we found high fever (on/off for a few days to weeks/months) in 8 subjects with frequent vomiting in some, loss of appetite and weight is also observed among some of them and other respiratory conditions such as COPD(Chronic Obstructive Pulmonary disorder), APD(Acute Pulmonary disorder), ARDS(Acute Respiratory Distress Syndrome) and pneumonia were observed in 7 out of 10 subjects, indicating a more advanced stage of respiratory compromise, some of them had other comorbidities such as fatty liver(Grade 1), prostatomegaly, kidney infection, cholilithiasis and chronic cholecystitis.

Two positive subjects were also having a co-infection with bacteria, *Mycobacterium tuberculosis*, and both these subjects are showing severe symptoms including high fever with chills, pneumonia, blisters in mouth, abdominal pain,

constipation, loss of appetite and weight along with other symptoms of positive subjects including shortness of breath and cough with sputum.

Hi On the contrary, most of the subjects who tested negative for influenza A virus, have fewer symptoms as compared to the positive ones, including shortness of breath, mild cough with scanty sputum, and other respiratory disorders such as COPD, APD, ARDS are observed only in 4 subjects. A small subset of negative cases tested positive for other infections, including Hepatitis B, Hepatitis C, and Typhoid, which contributed to their symptom profile but still lacked the intensity and complexity observed in Influenza A positive case. The major difference between influenza A positive and negative cases is in the severity of symptoms, with positive ones having more number and more severity of symptoms.

From these observations, we infer that the subjects with lower Ct values, indicative of higher viral loads, exhibited the most severe clinical symptoms, despite having fewer or no underlying comorbid conditions. Conversely, those with higher Ct values, representing lower viral loads presented with relatively milder symptoms, even in the presence of multiple comorbidities. These findings underscore the importance of Ct value as a potential indicator of viral load and disease severity in Influenza A infections. They also highlight the necessity of considering co-infections and underlying health conditions when evaluating patient prognosis and treatment strategies.

Conclusion

Influenza A virus, a member of the Orthomyxoviridae family, remains a significant public health concern due to its high transmissibility and genetic variability. Accurate and timely diagnosis is essential in controlling the spread and severity of the disease.

A detailed clinical analysis revealed that individuals with lower Ct values, indicative of higher viral loads exhibited more severe respiratory symptoms, such as high-grade fever, persistent cough with sputum, breathlessness, and, in some cases, pneumonia. In contrast, patients with higher Ct values showed milder clinical presentations despite having multiple comorbidities such as hypertension, fatty liver, and chronic respiratory issues. Co-infection with other pathogens, such as Mycobacterium tuberculosis, further exacerbated the clinical manifestation, underscoring the need for comprehensive diagnostic approaches that consider possible polymicrobial infections.

Thus, if an understanding of the coinfections be made, the diagnostic readiness and understanding the clinical spectrum associated with viral load, healthcare systems can better prepare for and respond to seasonal and pandemic influenza threats.

References

1. Peteranderl C, Herold S, Schmoldt C. Human Influenza Virus Infections. Semin Respir Crit Care Med,2016:37(4):487-500. doi:10.1055/s-0036-1584801
2. Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger». Influenza Virus. Transfus Med Hemothe,2009:36(1):32-39. doi:10.1159/000197314
3. Dou D, Revol R, Östbye H, Wang H, Daniels R. Influenza A Virus Cell Entry, Replication, Virion Assembly and Movement. Front Immunol,2018:9:1581. Published 2018 Jul 20. doi:10.3389/fimmu.2018.01581
4. Dadonaite B, Gilbertson B, Knight ML, *et al*. The structure of the influenza A virus genome. Nat Microbiol,2019:4(11):1781-1789. doi:10.1038/s41564-019-0513-7
5. Saunders-Hastings PR, Krewski D. Reviewing the History of Pandemic Influenza: Understanding Patterns of Emergence and Transmission. Pathogens,2016:5(4):66. Published 2016 Dec 6. doi:10.3390/pathogens5040066
6. World Health Organization. Available from: Influenza (seasonal)