Contribution of non- influenza respiratory viruses in causation of Influenza like Illness (ILI) during influenza epidemic: A laboratory based study

Amita Jain*, Shantanu Prakash, Bhawana Jain

Virology Laboratory, Department of Microbiology, King George's Medical University, Lucknow 226003, India

ARTICLE INFO

Article history:

Received 24 April 2017

Received in revised form 10 June 2017

Accepted 20 June 2017

Available online 24 June 2017

Keywords:

Non- influenza respiratory viruses

Epidemic

Influenza like illness(ILI)

ABSTRACT

Introduction: According to CDC USA February 2016 document for outpatient illness surveillance, Influenza like illness (ILI) is defined as fever (temperature of 100 °F [37.8 °C] or greater) and a cough and/or a sore throat without a known cause other than influenza. Most of the laboratories do not test for other known causes of ILI, especially in situation like epidemics.

Objective: In the present study we detected Influenza and other respiratory viruses in the cases presenting as ILI during a flu epidemic in North India from January to April 2015.

Materials and methods: Total 200 cases, 50 from each month, that were negative for all types of influenza viruses, were randomly selected and tested by Multiplex Real- Time PCR for detection of other respiratory viruses; rhinovirus, adenovirus, Respiratory Syncytial virus (RSV), Human Bocavirus (hBoV), Para Influenza viruses 1,2,3, and 4 (PIV 1, PIV 2, PIV 3, PIV 4), measles virus and human Meta Pneumo virus (hMPV).

Results: A total of 28/200 cases (14%) showed presence of other respiratory viruses. Rhinovirus was most commonly detected, (17/200; 8.5%) followed by adenovirus (7/200, 3.5%), RSV (5/200, 2.5%), hBoV (2/200, 1%), and PIV-3 (2/200, 1%). PIV 1, 2, 4, measles and hMPV were not detected in any of the samples.

Conclusion: Even during an epidemic other respiratory viruses contribute substantially to ILI

© 2017 INDIA CLIN. Published by Elsevier, a division of RELX India, Pvt. Ltd. All rights reserved.

1. Introduction

During any influenza epidemic/outbreak, it is assumed that all influenza like illness (ILI) is caused by influenza virus; however, the contribution of non- influenza viral infections in causation of acute respiratory illness cannot be ruled out/confirmed without a comprehensive laboratory investigation. According to World Health Organization (WHO), ILI is defined as an acute respiratory infection with: measured fever of $\geq 38^{\circ}\text{C}$, and cough, with onset within the last 10 days.¹ According to CDC USA February 2016 document for outpatient illness surveillance, ILI is defined as fever (temperature of 100 °F [37.8 °C] or greater) and a cough and/or a sore throat without a known cause other than influenza.² Most of the laboratories do not test for other known causes of ILI, especially in situation like epidemics, due to either inability to handle the sample load or financial constrain. Moreover, there is also an

assumption that aetiology of ILI is already known, hence there is no advantage of laboratory testing.³

In the present study we detected Influenza and other respiratory viruses in the cases presenting as Influenza like illness (ILI) during a flu epidemic in North India from Jan to April 2015.

2. Material & methods

Patients with clinical diagnosis of influenza like illness (ILI) referred to Virology Laboratory, Department of Microbiology, King George's Medical University, Lucknow, Uttar Pradesh, India, were tested for Influenza viruses (Influenza A, Influenza A subtype H1N1, Influenza A subtype H3N2, Influenza B) during the epidemic period from January 2015 till April 2015. The WHO guidelines for ILI was followed.⁴ Nasal and throat swabs/naso-pharyngeal aspirates were collected from each patient and processed for molecular diagnosis of human influenza viruses by real-time reverse transcriptase PCR.⁵ Informed consent was taken from all participating patients/their guardians, in case of children. Institutional ethical clearance was taken.

Of total 3319 ILI cases total 200 cases, 50 from each month, that were negative for all types of influenza viruses, were randomly

* Corresponding author.

E-mail addresses: amita602002@yahoo.com, mita60@gmail.com (A. Jain), shantanukgmu@gmail.com (S. Prakash), drbhawana_kgmu@hotmail.com (B. Jain).

Fig. 1. Flow diagram showing enrollment and processing of patients/samples.

selected (permuted block randomization) and tested by Multiplex Real- Time PCR for detection of other respiratory viruses as per previously reported method.⁶ The detailed flowchart is given in Fig. 1. Viruses tested were; rhinovirus, adenovirus, Respiratory Syncytial virus (RSV), Human Boca virus (hBoV), Para Influenza viruses 1,2,3, and 4 (PIV 1, PIV 2, PIV 3, PIV 4), measles virus and human Meta Pneumo virus (hMPV).

3. Results and discussion

During January 25th to April 30th 2015, total 3319 cases of ILI were referred to the laboratory, of which 815 (24.6%) were positive for H1N1, 6 (0.2%) for H3N2 and 17 (0.51%) for influenza B. Of total selected 200 cases, 28 (14%) showed presence of other respiratory viruses. Rhinovirus was most commonly detected, (17/200; 8.5%) followed by adenovirus (7/200, 3.5%), RSV (5/200, 2.5%), hBoV (2/200, 1%), and PIV-3 (2/200, 1%). PIV 1, 2, 4, measles and hMPV were not detected in any of the samples (Table 1). Total 5/28 (17.86%) cases showed coinfections; Adenovirus and Rhinovirus (2), ADV and RSV B (1), ADV and hBoV (1), Rhinovirus and hBoV (1) were combinations detected together. The RSV strains (5) were subtyped in subtype B (4/5) and subtype A (1/5).

Month wise distribution of respiratory viruses was analyzed; rhinovirus showed its presence throughout the flu season, and hBoV being present as co-infection in the month of January. Common symptoms remain fever (100%), cough (96.4%), coryza (57.1%) and breathlessness (35.7%) followed by headache (25.0%), body ache (25.0%), vomiting (17.85%), and diarrhea (17.85%). The clinical presentations of ILI patients testing positive for Non influenza respiratory viruses and those testing negative for all tested respiratory viruses were compared and no significant difference was noted.

The definition of Influenza like illness (ILI) is often used for influenza surveillance⁷ where influenza has been accounted for only 10% to 53% of ILI cases.^{8–10} Wide range of viral pathogens may be associated with ILI cases with overlapping respiratory symptoms which is important for better understanding of their contribution to overall morbidity. Influenza virus was prevalent etiological agent of ILI cases during this outbreak, however, a number of respiratory viruses accounted with low proportion (14%) in upper respiratory tract infections. This is contrast to other studies where non-influenza viral pathogens contributed significant proportion (45%–65%) in ILI cases.^{11,12} These studies were done during non epidemic period, while present study is done during a period when an epidemic of pH1N1 was already declared.

Rhinovirus was prevalent over other noninfluenza viral pathogens as it is most frequent cause of upper respiratory infection across all age group individuals.¹³ A coinfection of more than two non influenza viruses in ILI patients was also observed in this study which has been reported in other studies also but with varying prevalence.^{9–11}

Even during an epidemic other respiratory viruses contribute substantially to ILI.

Conflict of interest

None.

Acknowledgements

The authors acknowledge the Indian Council of Medical Research, New Delhi, India for financial support [5/8/7/14/2009-ECD-1(Vol.II)] and infrastructure [5/8/7/14/2010-ECD-1]. We are grateful to the staff of Virology Diagnostic Laboratory (VDL) for

Table 1
Non influenza viral pathogens detected during 2015 flu outbreak.

Sr. No.	Viruses	Total Positive	Jan 2015 (n = 50)	Feb 2015 (n = 50)	March 2015 (n = 50)	April 2015 (n = 50)
1	Rhinovirus	17 (51.51%)	5	4	4	4
2	Adenovirus	7 (21.21%)	3		2	2
3	Respiratory syncytial virus	5 (15.15%)	–		3	2
4	Human Boca virus	2 (6.06%)	2		–	
5	Parainfluenza 3	2 (6.06%)	–		1	1
Total		33	8 ^a	4	8 ^a	8 ^a

Parainfluenza 1–4, Measles and human Meta Pneumo viruses not detected.

^a Co infections found in 5 cases.

their contribution in diagnosis and investigation of outbreak cases during 2015.

References

1. WHO. *Global Epidemiological Surveillance Standards for Influenza*. Available from: http://www.who.int/influenza/resources/documents/WHO_Epidemiological_Influenza_Surveillance_Standards_2014.pdf.
2. CDC. *Overview of Influenza surveillance in United states*. USA: Department of Health and Human services, Center for Disease Control; 2016 Available from: <https://www.cdc.gov/flu/weekly/overview.htm>. (Accessed on 18 February 2016).
3. Ministry of Health & Family Welfare-Government of India. *Laboratory confirmed Cases and Deaths caused by Pandemic Influenza A H1N1: State/UT-wise*. USA: Department of Health and Human services, Center for Disease Control; 2015 Available from: <http://www.mohfw.nic.in/showfile.php?lid=2614>. (Accessed on 8 March 2015).
4. WHO. *CDC protocol of real-time RTPCR for swine influenza A (H1N1)*. Available from: http://www.who.int/csr/resources/publications/swineflu/CDCRealtimeRTPCR_SwineH1Assay-2009_20090430.pdf. (Accessed on 15 March 2011).
5. Centers for Disease Control and Prevention (CDC). *nterim guidance on case definitions to be used for investigations of novel influenza A (H1N1) cases*. Available on: <http://www.cdc.gov/h1n1flu/casedef.htm>. (Accessed on 13 October 2011).
6. Singh AK, Jain A, Jain B, Singh KP, Dangi T, Mohan M, et al. Viral aetiology of acute lower respiratory tract illness in hospitalised paediatric patients of a tertiary hospital: one year prospective study. *Indian J Med Microbiol*. 2014;32:13–18.
7. Ortiz JR, Sotomayor V, Uez OC, Oliva O, Bettels D, McCarron M, et al. Strategy to enhance influenza surveillance worldwide. *Emerg Infect Dis*. 2009;15:1271–1278.
8. Bellei N, Carraro E, Perosa A, Watanabe A, Arruda E, Granato C. Acute respiratory infection and influenza-like illness viral etiologies in Brazilian adults. *J Med Virol*. 2008;80:1824–1827.
9. Laguna-Torres VA, Sanchez-Largaespada JF, Lorenzana I, Forshey B, et al. Influenza and other respiratory viruses in three Central American countries. *Influenza Other Respir Virus*. 2011;5:123–134.
10. Razanajatovo NH, Richard V, Hoffmann J, Reynes JM, Razafitrimo GM, Randremanana RV, et al. Viral aetiology of influenza-like illness in Belgium during the influenza A(H1N1)2009 pandemic. *Eur J Clin Microbiol Infect Dis*. 2012;31:999–1007.
11. Njouom R, Yekwa E, Cappy P, Vabret A, Boisier P, Rousset D. Viral Etiology of Influenza-Like Illnesses in Cameroon, January–December 2009. *J Infect Dis*. 2012;206:S29–S35.
12. Tan XQ, Zhao X, Lee VJ, Loh JP, Tan BH, et al. Respiratory viral pathogens among Singapore military servicemen 2009–2012: epidemiology and clinical characteristics. *BMC Infect Dis*. 2014;14:204 Available on: <http://www.biomedcentral.com/1471-2334/14/204>.
13. Denny Jr. FWJr. The clinical impact of human respiratory virusinfections. *Am J Respir Crit Care Med*. 1995;152:S4–12.