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RESEARCH ARTICLE

CO-INFECTION OF INFLUENZA A AND B IN A TERTIARY CARE HOSPITAL OF NORTHERN INDIA

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ABSTRACT:

Background: Dual infections of influenza A and influenza B are rare events that have been previously described. Such co-infections have not been widely described or characterized as a result of the previous widespread use of culture-based methods for diagnosis and the heterotypic interference generated within these models. **Objective:** To study the co-infection of influenza A and B. **Material and Methods:** A retrospective study was conducted in Department of Microbiology, SGPGIMS, Lucknow, a tertiary care hospital of Northern India, to analyze for co-infection of Seasonal Influenza A and B with severity of influenza infection in year 2012-13. Throat swab and nasal swab specimens were collected from suspected patients and RT-PCR was performed to confirm for influenza. **Results:** Out of total 551 suspected patients, 19.7% (109/551) were positive for Seasonal Influenza A, 13.8% (76/551) were positive for Influenza B, 1.45% (8/551) were positive with co-infection of Seasonal Influenza A and Influenza B. **Conclusion:** It was observed that both Seasonal influenza type A and B viruses were prevalent in Lucknow during the study period. Such surveillance data are important in the early detection of any antigenic variants that may be helpful in global influenza vaccine preparation and for any pandemic preparedness activity.
Keywords: Influenza, Co-infections, Surveillance, Diagnostics, Dual infections.

INTRODUCTION

Influenza virus is a major respiratory pathogen that causes yearly epidemics in tropical and subtropical countries, leading to majority of morbidity and mortality, situation becoming more complicated in co-morbid cases. Recurrent influenza epidemics are due to the frequent emergence of antigenic variants. With the co-circulation of two influenza A subtypes, genetic re-assortment also has an important role to play in antigenic drift [1, 2]. The outbreak of pandemic (H1N1) 2009 influenza virus was first noted in Mexico in March 2009 [3] but quickly spread worldwide. On June 11 2009, the World Health Organization declared the first influenza pandemic in >40years, triggering governments around the world to make pandemic (H1N1) 2009 a top public health priority. In this study, we present data from Lucknow, Uttar Pradesh. Pandemics in the past century occurred in 1918 (swine influenza), 1957 (Asian influenza), 1968 (Hong Kong influenza) and, to a lesser degree, in 1977 (Russian influenza). Presently, minor antigenic variant strains of influenza type A (H1N1), A (H3N2) and type B viruses are causing frequent epidemics globally. Considering the public health importance of influenza, the World Health Organization (WHO) has established a worldwide network of National Influenza Centres.

Co-circulation of multiple strains of influenza virus A in humans provides an opportunity for viral genetic re-assortment (mixing of genes from >2 viruses) [4]. Genetic re-assortment of pandemic (H1N1) 2009 virus with seasonal influenza A (H3N2) or seasonal influenza A (H1N1) viruses might thus represent a route to enhanced pathogenicity. No re-assortment events between pandemic (H1N1) 2009 and seasonal viruses have been reported in humans.

The low fidelity of the viral polymerase complex, as well as host immune selection, accounts for the accumulation of point mutations in hemagglutinin (HA) and neuraminidase (NA) genes, producing the antigenic drift on these surface glycoproteins. The antigenic drift plays an important role in the occurrence of influenza epidemics. Gene re-assortment is the other mechanism attributed to influenza virus mutation. An antigenic shift occurs in the event of a re-assortment between two viruses of different surface antigenic subtypes, leading to a novel subtype composition [5].

Dual infections of influenza A and influenza B are rare events that have been previously described [6]. We describe the use of molecular tools to characterize influenza A and influenza B co-infection in outbreak from co-circulating influenza A and influenza B. Throat swabs or Nasopharyngeal swabs were collected from suspected patients. Until the mechanisms of dual infection are understood, we believe that instances of dual infections should be taken seriously by both clinicians and public health workers. In conclusion, we believe that molecular techniques are a powerful tool that enables laboratories to extensively characterize circulating strains of influenza, including co-infections, without the use of culture-based methods.

MATERIAL AND METHODS

Specimen collection and transportation:

Throat swabs or nasopharyngeal aspirates were obtained from hospitalized patients and outpatients in SGPGIMS, Lucknow, a tertiary care centre, with symptoms of respiratory infections. Throat swabs were collected into transport medium containing 2 ml of Eagle's minimum essential medium (EMEM) (pH 7.2) with gelatin (5 mg/litre), penicillin (400 U/litre), streptomycin (400 µg/litre), gentamicin (50 µg/litre), and amphotericin B (Fungizone) (1.25 µg/litre). Specimens were placed on ice and transported to clinical virology laboratory within 24 hours after collection. All health care facilities were provided in our hospital along with the case definition of pandemic (H1N1) 2009 virus infection and reporting guidelines (revised September 8, 2009) [11]. Briefly, influenza-like illness (ILI) was defined as a patient with fever (>37.8°C), acute respiratory illness including chest pain, pneumonia, breathlessness, nasal irritation, congestion or a recent history of fever and sore throat [12].

RNA extraction and PCR:

RNA was extracted from throat swab/ nasopharyngeal swab by using commercially available QIAGEN viral RNA extraction kit. (Qiagen, Santa Clara, CA). Reverse Transcriptase Real Time PCR (ABI-7500) procedure was performed using commercially available applied kit according to Van *et al*. Primer and probes were used as per CDC guidelines. A confirmed case of pandemic H1N1 and seasonal influenza A was an ILI case with laboratory confirmation of pandemic H1N1 or seasonal influenza A virus by real time reverse transcriptase polymerase chain reaction (RT-

PCR) [13], and also the cases who were tested but were negative for either influenza A or Swine flu were grouped into the ILI category for statistical analysis.

Data was analysed using SPSS software version 16; Inc, Chicago, ILI, USA). One-way analysis of variance was used to compare differences in mean age between the 3 groups (ILI, confirmed pandemic [H1N1] infections risk factors) and the $\chi 2$ test (2-sided) to compare gender. Only confirmed cases were provided with antiviral treatment and a retrospective study was performed to know the trend and characteristic of influenza infection.

RESULTS

Out of total 551 suspected patients, 19.7% (109/551) were positive for Seasonal Influenza A, 13.8% (76/551) were positive for Influenza B, 1.45% (8/551) were positive with co-infection of Seasonal Influenza A and Influenza B.

DISCUSSION

Dual infections of influenza A and B viruses appear to be a rare event and only few publications have reported simultaneous infection by two different types of influenza viruses in humans [3-7]. Thus, the factors that may be responsible for such events are not clear yet, even though the host immune system and the virus properties have been suggested [3-7]. It is interesting to note that the eight co-infected patients reported in this study represented individuals with a weak immune system; indeed, in all publications clinical manifestations in co-infected individuals were identical to those observed in single infections, with classical symptoms, without any clinical complication. However, it has to be mentioned that both these latter observations may be related to the specific cohort (hospitalized patients) taken into consideration in the current study. Thus a clear correlation between dual infection and immunodeficiency/maturation, or dual infection and severity of the disease cannot be absolutely concluded by these data. On the other hand, all patients fully recovered.

Finally, in contrast with previous literature data [4], in our cases we did not observe significant differences in the RNA levels of the two viruses in the initial sample, a part for a slight higher amount of influenza B virus in the case of the child (roughly 10 times). However, in the case of the adult patients, for whom three sequential samples were analysed, we observed a different kinetic in the viral clearance, with the influenza B virus becoming undetectable more rapidly than the influenza A virus. This finding may be related to a different in vivo replication efficiency of two viruses. In fact, influenza A infections are usually more severe than those related to influenza B. However, further studies would be needed to support this hypothesis. Such surveillance data are important in the early detection of any antigenic variants that may be helpful in global influenza vaccine preparation and for any pandemic preparedness activity.

Samples positive for Seasonal influenza A, were subtyped and all were H3N2 influenza A. All the samples were from Lucknow region, six female and two males were reported to be positive with dual infection of Influenza A and Influenza B. All patients were immuno-compromised, were admitted in our tertiary care hospital.

REFRENCES

1. Falchi A, Arena C, Andreoletti L, Jacques J, Leveque N, Blanchon T, et al. Dual infections by influenza A/H3N2 and B viruses and by influenza A/H3N2 and A/H1N1 viruses during winter 2007, Corsica Island, France. *J Clin Virol*. 2008;41:148–151.
2. Buonagurio DA, Nakada S, Parvin JD, Krystal M, Palese P, Fitch WM. Evolution of human influenza A viruses over 50 years: rapid, uniform rate of change in NS gene. Science. 1986 May 23;232(4753):980-2.
3. Lin YP, Gregory V, Bennett M, Hay A. Recent changes among human influenza viruses. Virus Res. 2004 Jul;103(1-2):47-52.
4. Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009 Jun 18;360(25):2605-15.
5. Lowen AC, Steel J, Mubareka S, Palese P. High temperature (30 degrees C) blocks aerosol but not contact transmission of influenza virus. J Virol. 2008 Jun;82(11):5650-2.
6. Yasuda, J., D. J. Bucher, and A. Ishihama. 1994. Growth control of influenza A virus by M1 protein: analysis of transfectant viruses carrying the chimeric M gene. J. Virol. 68:8141–8146.
7. Fonseca K, Tarrant M, Lam S, Li Y: Dual infection with influenza A and B viruses. Pediatr Infect Dis J 2002, 21:795-796.
8. Shimada S, Sadamasu K, Shinkai T, Kakuta O, Kikuchi Y, Shinohara M, Uchida K, Doi R, Kohmoto K, Shimizu M, Nakajima S: Virological analysis of a case of dual infection by influenza A (H3N2) and B viruses. Jpn J Infect Dis 2006, 59:67-68.
9. Toda S, Okamoto R, Nishida T, Nakao T, Yoshikawa M, Suzuki E, Miyamura S: Isolation of influenza A/H3 and B viruses from an influenza patient: confirmation of co-infection by two influenza viruses. Jpn J Infect Dis 2006, 59:142-143.
10. Eshaghi A, Blair J, Burton L, Choi KW, De Lima C, Duncan C, Guyard C, Higgins R, Lombos E, Low DE, Mazzulli T, Drews SJ: Characterization of an influenza A and influenza B co-infection of a patient in a long-term care facility with co-circulating influenza A and influenza B. Int J Infect Dis 2009, 13:127-128.
11. http://www.who.int/csr/disease/avian_influenza/guidelines/en/
12. www.who.int/csr/disease/swineflu/
13. http://www.who.int/csr/resources