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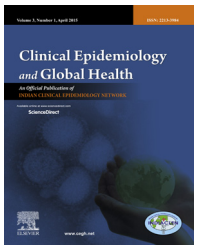

Original Article

Seropositivity to influenza A(H1N1)pdm09 and influenza A (H3N2) and risk of infection in health care workers

G. Nandhini, S. Sujatha *

Department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, 605006, India

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ABSTRACT

Background: Health care workers are at an increased risk of infection during influenza outbreaks. This study on seropositivity of influenza A viruses provided an opportunity to evaluate this risk.

Methods: This cross-sectional study was conducted during August–October 2013. Health care workers in a tertiary care center, Pondicherry completed a detailed questionnaire including sociodemographic and professional details, vaccination status, known history of swine or seasonal flu infection and contact with flu infected patients. Serum was collected and hemagglutination inhibition (HI) assaywas performed using reference antigens -influenza A (H1N1) A/California/07/2009, influenza A (H3N2) A/Victoria/361/2011 according to World Health Organization protocol. HI titres ≥40 were considered protective.

Results: Serum samples were received from 138 health care workers. Seropositivity to seasonal influenza A (H3N2) and pandemic influenza A(H1N1)pdm09 was 100% and 83% respectively. There was no association of any sociodemographic factor with seropositivity to influenza A viruses.

Conclusion: Regardless of sociodemographic, professional and vaccination status, most health care workers had seroprotective antibody titers against influenza A viruses, indicating that they are not at an increased risk of infection.

1. Introduction

The pandemic strain of influenza A(H1N1)pdm09 emerged in Mexico in March 2009 and the first case in India was reported from Hyderabad in May 2009. After the deadly pandemic wave of 2009, many outbreaks have been reported from different states of India, the most recent one started in December 2014, which has lead to more than 1200 deaths till March 2015.[1] During each outbreak, thousands of health care workers

* *Corresponding author*. Tel.: +91 9894058062 (mobile).
E-mail address: sujathasistla@gmail.com (S. Sujatha).

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(HCWs) are involved in primary care, sample collection and diagnosis. The propensity of HCWs to develop influenza is significant in terms of absenteeism, productivity and transmission of infection to patients.[2] Many reports have suggested that health care workers may be at a greater risk of influenza infection than non-healthcare workers since they are exposed to maximum influenza viral shedding through patient contact.[3,4] However, some studies also propose that health care workers are no more likely to experience symptomatic influenza infection than others, possibly because they develop cross protection due to increased exposure to multiple strains of influenza.[3] There are contradictory views on whether HCWs developed protective antibody titers against influenza A viruses in the post-pandemic era and the association of their sociodemographic and professional status with seropositivity.[5,6] Hence the present study was conducted to measure the seropositivity rates to influenza A viruses among health care workers in Pondicherry, India and to evaluate associations between seropositivity and participants' profession, age, vaccination status and history of known contact with influenza-infected patients.

2. Methods

2.1. Study design and sampling

Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER) is a tertiary care hospital in the Union Territory of Pondicherry, with a daily average outpatient attendance of 6200 and yearly total outpatient attendance of 18,00,000. As part of the ongoing National Influenza Surveillance Programme, JIPMER hospital has been conducting influenza-like illness surveillance (ILI) at three sentinel sites across Pondicherry from November 2011. It was observed that influenza constituted around 15% of ILI cases, with influenza A H1NI and H3N2predominating in 2012 and 2013 respectively. This cross-sectional study was conducted during August–October 2013 and participants were health care workers in JIPMER hospital who volunteered to provide blood samples for the study. Doctors, nurses, supporting staff including laboratory technicians and students were included in the study.

2.2. Data and specimen collection

Information about the participant's age, gender, occupation, influenza contact history, influenza vaccination history was recorded. Blood (2 ml) was collected by venipuncture, kept at room temperature for 30–45 min and serum was separated by centrifugation. Serum samples were stored at −80 °C until further testing.

2.3. Laboratory procedures

a. Receptor destroying enzyme (RDE) treatment

The testing followed WHO protocol involving RDE (*Vibrio cholerae* neuraminidase) treatment of serum, HA titration and hemagglutination inhibition assay. Treatment of sera with RDE was done to remove non-specific inhibitors. Lyophilized RDE was reconstituted with 20 ml physiological saline, 0.85% NaCl according to manufacturer's instructions (Denka Seiken, Japan) and stored in aliquots at −20 °C. RDE and serum was mixed in the ratio of 3:1 and this solution was incubated overnight in 37 °C water bath. The remaining RDE was inactivated by heating in 56 °C water bath for 30 min. After cooling the solution to room temperature, six volumes of physiological saline, was added and final dilution of serum was 1:10.

b. Hemagglutination (HA) titration and preparation of control antigens

Ninety-six well U-bottomed polycarbonate microtitre plates were used to titrate the WHO influenza control antigens (pandemic influenza A(H1N1) A/California/07/2009, seasonal influenza A (H3N2) A/Victoria/361/2011) with human type O RBCs. Fifty microliter of PBS (pH 7.2) was added from A2-G12 wells (1:2, 1:4, 1:8, 1:2048 dilutions of control antigens) and 100 μl control antigen was added to first well of each row. RBC control well was prepared in last row H1 by adding 100 μl PBS. Serial twofold dilutions of the antigen were done by transferring 50 μl from first well of each row from A1-G1 to next column till G12 and final 50 μl was discarded. Fifty microliters of RBC (0.75%) suspension was added to each well and mixed by manually agitating the plates thoroughly. Plates were incubated at room temperature (22 °C–25 °C) and after complete settling of RBCs in RBC control well, results were recorded. The highest dilution of control antigens that produced complete hemagglutination was considered the HA titration end point. The HA titer is the reciprocal of the dilution of control antigens in the last well with complete hemagglutination. Eight HA units/50 μl was the standard dilution used for all the control antigens, hence the antigens with higher HA titration were diluted accordingly to prepare a standard dilution of 8HA units/50 μl. Back titration was done for all the control antigens to verify the HA units. The antigens were stored at 4 °C and used on the same day of preparation.

c. Hemagglutination inhibition (HI) assay

In 96 well U-bottomed microtitre plates, 25 μl of PBS was added from B through H (B1–H12) of each numbered column. 50 μl of each serum (RDE treated; 1:10) was added to the first well of the appropriately numbered column. Serial two-fold dilutions of serum was done by transferring 25 μl from the first well of each column up to row H and final 25 μl was discarded. To all the wells with diluted serum, 25 μl of standardized control antigens (8 HA/50 μl or 4 HA/25 μl) was added. 25 μl PBS was added to serum control plate instead of antigen. Last column was allotted for RBC control where 50 μl of PBS was added. After mixing the contents by manual agitation, the plates were covered and incubated at room temperature (22 °C–25 °C) for 15 min. Fifty microliters of standardized RBCs were added to all the wells and mixed as before. Plates were incubated at room temperature (22 °C–25 °C) and after complete settling of RBCs in RBC control well, HI titers were recorded. The HI titer is the reciprocal of the highest dilution of serum that completely inhibits hemagglutination. HI antibody titers 1:≥40 were used to define seropositivity.