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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) assay was 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.

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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 led 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 H1N1 and H3N2 predominating 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 $1:\geq 40$ were used to define seropositivity.

Table 1 – Participant characteristics.

Characteristics	Doctors & nurses, n (%)	Supporting staff & laboratory technicians, n (%)	Students, n (%)	Total, N (%)
Age, years				
18–30	31 (62.0)	9 (20.0)	42 (97.7)	82 (59.4)
31–40	11 (22.0)	12 (26.7)	1 (2.3)	24 (17.4)
41–50	6 (12.0)	12 (26.7)	0 (–)	18 (13.0)
51–60	2 (4.0)	9 (20.0)	0 (–)	11 (8.0)
>60	0 (–)	3 (6.6)	0 (–)	3 (2.2)
Gender				
Male	18 (36.0)	27 (60.0)	13 (30.2)	58 (42.0)
Female	32 (64.0)	18 (40.0)	30 (69.8)	80 (58.0)
Influenza vaccination				
Vaccinated	26 (52.0)	14 (31.1)	10 (23.3)	50 (36.2)
Not vaccinated	24 (48.0)	31 (68.9)	33 (76.7)	88 (63.8)
Known pandemic/seasonal flu infection or contact history				
Yes	5 (10.0)	0 (–)	1 (2.3)	6 (4.3)
No	45 (90.0)	45 (100.0)	42 (97.7)	132 (95.7)
Total	50	45	43	138

d. Statistical methods

Data obtained from the questionnaire were loaded into Microsoft Excel 2007 and then exported to SPSS version 17 for analysis. Univariate association of seropositivity with other factors was evaluated using binary logistic regression analyses. P-value of 0.05 was considered to be significant.

3. Results

Blood samples were received from 138 health care workers (median 27 years, range 18–63 years) during August–October 2013 at JIPMER hospital. Participants' characteristics are shown in Table 1. We divided the participants into 3 groups based on their occupation like doctors and nurses; supporting staff and laboratory technicians; students based on the maximum to minimum degree of frontline contact with the patients attending hospital (high to low-level exposures to influenza). The first group containing doctors and nurses were mostly from pediatric, general medicine and pulmonary medicine wards, where there are maximum chances of exposure to influenza from the patients. The second group containing supporting staff and laboratory technicians were

not in direct contact with patients, except for two participants in this group who worked in influenza laboratory (both had protective serum antibody titers against influenza A viruses). The third group containing undergraduate and research students had no contact with hospital patients and had very low exposure to influenza from the patients. Hence, the analysis of study subjects was mainly focused on their postings and level of exposure to influenza from the patients. Among the participants, 4.3% recollected their first-hand contact with patients who had laboratory-confirmed swine/seasonal influenza infection. Most of the participants had not taken influenza vaccination anytime in previous years (63.8%).

Seropositivity to influenza A (H3N2) among the participants was 100% with more than 86% of HCWs having HI titers above the borderline (≥ 80) against seasonal influenza (Fig. 1). The geometric mean titer of influenza A (H3N2) was 114.28. One healthcare worker who had protective antibody titers (1:80) against seasonal influenza A (H3N2) developed laboratory-confirmed seasonal influenza infection after three months.

Seropositivity to pandemic influenza A(H1N1)pdm09 was 83.3% (115) among the HCWs. About 56% of HCWs had HI titers above the borderline (≥ 80) (Fig. 1) and the geometric mean titer was 54.07. Details of seropositivity to pandemic influenza A

Fig. 1 – Antibody titers against influenza A(H1N1)pdm09 and influenza A (H3N2).

Table 2 – Seropositivity to pandemic influenza A(H1N1)pdm09.

	Seropositive, n (%)	Seronegative, n (%)	p-Value
Occupation			
Doctors & nurses	42 (84.0)	8 (16.0)	0.970
Supporting staff & laboratory technicians	37 (82.2)	8 (17.8)	
Students	36 (83.7)	7 (16.3)	
Age, years			
18–30	69 (84.1)	13 (15.9)	0.713
31–40	21 (87.5)	3 (12.5)	
41–50	15 (83.3)	3 (16.7)	
51–60	8 (72.7)	3 (27.3)	
>60	2 (66.7)	1 (33.3)	
Influenza vaccination			
Vaccinated	44 (88.0)	6 (12.0)	0.243
Not vaccinated	71 (80.7)	17 (19.3)	
Known pandemic flu infection/contact history			
Yes	5 (83.3)	1 (16.7)	0.819
No	110 (83.3)	22 (16.7)	

(H1N1)pdm09 and its association with participants' characteristics are shown in Table 2. Seropositivity in the younger age group was not significantly different from that in the older age group. Overall the status of vaccination did not influence the seropositivity to pandemic influenza A(H1N1)pdm09, but we observed a pattern of minor increase in seropositivity in HCWs who received vaccine in 2012, compared to those who received it in 2011 and 2010 (Table 3). No significant association was found between seropositivity and known swine flu infection/contact history.

4. Discussion

According to CDC guidelines, MMWR, 2009, serum antibody titer of ≥ 40 , as determined by hemagglutination inhibition assay, is associated with at least a 50% decrease in risk for influenza infection or disease.⁷ This serosurvey of health care workers at a tertiary care center found a seropositivity of 100% to influenza A (H3N2) and 83.3% to pandemic influenza A (H1N1)pdm09. Among the health care workers, the reported seropositivity of antibodies against pandemic influenza A (H1N1)pdm09 virus in other countries ranges from 7% to 30%,^{8–12} while we observed increased percentage of seropositivity in our study, suggesting that this group of HCWs have protective antibody titers against influenza A viruses. This observation is

similar to reports from New Zealand, India, Australia^{5,13,14} and in contrast to other studies from UK and Canada.^{3,6} This could be due to the difference in three variables; the years of study period as other reports quoted were done in 2010, 2011; geographic variations, percentage of disease burden due to pandemic influenza A(H1N1)pdm09 in these countries and the standard precautionary measures followed in respective hospital settings. We observed a single case of an HCW developing seasonal A (H3N2) infection despite having seroprotective antibody titers (1:80) against the virus. Although no conclusions can be drawn from this single case, this is further proof that the mere presence of antibodies may not ensure protection.

There was no difference in percentage of seropositivity to pandemic influenza A(H1N1)pdm09 among the professional groups, indicating that the higher possibility of patient's contact in the front-line HCWs is not a factor for increased seropositivity. This is in agreement with report from UK⁶ and contrary to observations from studies conducted in India and Taiwan, where they showed a varying percentage of seropositivity among front-line and non front-line categories of health care workers.^{9,10}

We observed that age differences did not show any marked variation in seroprevalence among HCWs. A recent study from India also showed that age could not be associated with seropositivity in health care personnel.¹⁵ Moreover, influenza vaccination status had no association with seropositivity, which might be because only 2% of the health care workers had annual influenza vaccination in the study year, i.e., 2012.

Seropositivity rates were similar in HCWs who reported a history of contact with patients with proven pandemic influenza A(H1N1)pdm09 infection (83%) and those who did not report any history of contact (83%). Similar observations have been reported from another study from Vellore, India.¹³ It is likely that some participants may have had mild infection or have been in contact with patients with subclinical infection, which they could not recall at the time of sampling; however such cases might be very minimal due to mass panic and awareness that exists regarding swine influenza pandemic.

The study has a few limitations as it relied on a collection of data that could be subject to recall bias like history of contact with patients with swine or seasonal influenza infection and previous influenza infection among HCWs. We did not have baseline serotiters of HCWs to determine the accurate post-pandemic increase in antibody titers.

We conclude that the seropositivity to the pandemic and seasonal influenza A viruses was high in health care workers during the post-pandemic period. Seropositivity was not influenced by sociodemographic and professional status. Regardless of vaccination status and year of vaccination, health care workers had good seroprotective antibody titers against pandemic and seasonal influenza A viruses and are not at increased risk of influenza infection. This was reflected in the respective year's influenza surveillance reports where we encountered very little influenza activity with only a single case of confirmed influenza in health care workers. With different influenza strains circulating each year, similar seroprevalence studies have to be frequently conducted with the relevant antigens to understand and implement infection control measures in health care workers in order to limit influenza transmission in hospital settings.

Table 3 – Year of influenza vaccination and seropositivity.

	Seropositive to influenza A(H1N1)pdm09, n (%)	Seronegative to influenza A(H1N1)pdm09, n (%)
Total Vaccinated (50)		
2010 (29)	25 (86)	4 (14)
2011 (18)	16 (89)	2 (11)
2012 (3)	3 (100)	0 (–)

Conflicts of interest

All authors have none to declare.

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