RESEARCH ARTICLE

RE-EMERGENCE OF INFLUENZA A (H1N1) VIRUS AS AN EPIDEMIC AGENT AMONG DIFFERENT AGE GROUPS OF PATIENTS IN WESTERN RAJASTHAN (JODHPUR) INDIA

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ARTICLE INFO	ABSTRACT
	Background: Influenza has a major impact on public health, annually affecting 15-20% of the global population. First outbreak of influenza as a pandemic declared by WHO in June 2009. **Aim:** This study was aimed to determines the incidence of influenza A (H1N1) virus by real time reverse transcriptase polymerase chain reaction (rRT-PCR) method in different age groups of patients. **Methods:** A total of 2868 respiratory samples (throat swabs/nasopharyngeal swabs) were collected from patients suspected influenza like illness who attended OPD or admitted in Dr S. N. Medical College & Associated group of Hospitals in January 2015 to March 2015 (3 months period). The samples were collected and processed as per World Health Organization (WHO) guidelines. Viral RNA was extracted and one-step rRT-PCR was performed to detect Influenza A (H1N1) virus. **Results:** A total of 668 (23.29%) were positive for influenza A (H1N1) virus, Group specific influenza A was positive in 524 (18.27%) samples. It was observed that influenza A (H1N1) virus was prevalent in western Rajasthan (Jodhpur) during the study period. **Conclusion:** 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.

INTRODUCTION

Swine flu, which is caused by novel H1N1 virus, lead to the major pandemic in 2009 which shook the world. After the 1st description of the virus which caused the pandemic in 2009, it created a global havoc. With the declaration of the outbreak as a pandemic by the WHO and CDC in June 2009. The infection claimed over 14,000 lives and started wavering off by November with the quick decline in the no. of cases by May 2010. Even after the declaration of the end of pandemic by the director general of WHO on 10th August 2010 there have been epidemics and sporadic cases reported from many parts of India even in 2011 and 2012, which shows that the lacunae in the awareness among the people (World Health Organization, 2009; Shashwat, 2013). The disease started in India in May 2009 and the first laboratory-confirmed case was reported from Hyderabad on 16th May 2009 (Ministry of Health and Family Welfare, 2012). The state of Rajasthan, which is the largest state in India, reported its first case of H1N1 infection on 23rd July 2009 (Gupta *et al.,* 2011), Soon the disease spread to other parts of the state. Influenza viruses are known to cause frequent epidemics and periodic pandemics, and are unique with regard to their antigenic variability, seasonality and impact on general population.

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Influenza like illness (ILI) screening and surveillance programmes are critical in tracking the activity, especially of influenza viruses, across seasons. Since the critical differential diagnosis is difficult, such programmed become imperative in the control and management of respiratory viral disease outbreaks (Roy *et al.,* 2012). Influenza virus is an enveloped RNA virus of the Orthomyxoviridae family. It is endowed with an inherent capacity for genetic variation and is based on two important features; (i) the presence of a segmented genome, with eight RNA segments that are genetically independent of each other and (ii) a high rate of mutation, especially in the surface heamgglutinin (H) and neuraminidase (N) proteins (Ravi, 2009). Various study proven that pregnant women has highly susceptible to influenza infection and have been shown to be at increased risk for morbidity and death with influenza illness during seasonal epidemics and pandemics (Sonja *et al.,* 2012). The 2014-2015 H1N1 outbreak in India has reportedly led to 800 fatalities. The reported influenza hemagglutinin sequences from India indicate that these viruses contain amino acid changes linked to enhanced virulence and are potentially antigenically distinct from the current vaccine containing 2009 (Cal0709) H1N1 viral hemagglutinin (Kannan Tharakaraman *et al.,* 2015).

Aims and Objectives

1. To Study the incidence and geographical distribution of influenza A (H1N1) virus by rRT-PCR method in different age groups patients and pregnant women.

2. To study the variation in clinical presentation if any.

MATERIALS AND METHODS

Study design

The study was conducted in Microbiology laboratory of Dr. S. N. Medical College Jodhpur, Rajasthan, India from January 2015 to March 2015 (3 Months period).

Collection of specimens

A total of 2868 clinical samples (throat swabs/nasopharyngeal swabs) were collected from OPD and IPD patients of all age group in Dr. S.N. Medical College groups of hospitals, Jodhpur Rajasthan showing Influenza like sign and symptoms. Clinical samples collected in a viral transport medium (VTM), and immediately transported to microbiology laboratory in a Styrofoam box containing ice packs or vaccine carrier box. The samples were collected with proper sterile precaution.

Data collection

The following data were collected: Patients name, age, gender, complete residential address with contact number, OPD/IPD and their clinical history.

Processing of samples and identification

As per the laboratory criteria for diagnosis of influenza specimens suggested by WHO, the RT-PCR protocol was adopted (WHO, 2009). The throat swabs in VTM samples were processed in a Biosafety level IIB (negative pressure room) Cabinet and divided into three aliquots and stored at -80°C in deep freezer. Processing of samples was done according to the CDC standard protocol (WHO, 2009). Use of appropriate biosafety measures and personal protection equipment (PPE) were as per CDC's and WHO laboratory biosafety guidelines (World Health Organization, 2004; Biosafety in microbiological and biological laboratories, 2007).

swine H1, primers and probes. A master mix of 20 μL was prepared in a 48 well PCR plate and 5 μL of RNA template was added and the plates were placed in the AB Step One Real-time PCR instrument using cycling conditions of 50°C for 30 min of reverse transcription followed by Taq inhibitor inactivation at 95°C for 10 min and PCR amplification (45 cycles) at 95°C for 15 sec and at 55°C for 30 sec. Chi-square test and two-tailed Z-test were applied to determine the associations between influenza positivity and demographic/epidemiological data.

RESULTS

In our study it was found that out of 2868 clinical samples collected, 1440 (50.2%) were male patients and 1428(49.8%) were female patients showing ILI symptoms. It was found that Out of 2868 samples collected from ILI patients 668 (23.29%) samples were positive and 74 (11%) patients died of swine H1N1 infection. Age and gender wise distribution of Influenza A (H1N1) test positive cases in urban and rural areas. 23.34% (336/1440) of influenza-positive samples were males and 23.25% (332/1428) were females. Maximum positive cases were observed in the age group of 41-60 yr (209 cases; 27.24%) of which 121(57.9%) were male and 88(42.1%) female.

The probable reason for predilection of male sex may be due to greater mobility, susceptibility and exposure to infection and also this age group consists of economically productive mobile population, travelling more for various reasons and most susceptible to exposure to infection, so they get exposed to virus easily and get infected easily. Age and gender wise distribution of swine H1N1 positive cases shown below in Table no.1. Geographically maximum patients samples 2423/2868 (84.5%) came from Dr. S. N. Medical college & Associated group of hospitals (MDMH, MGH, UH, KNCH) Jodhpur and 445/2868 (15.5%) samples came from District hospital Jodhpur division. Detail description of geographical distribution of patient's samples in Jodhpur division shown in below Table 2 and Figure 1.

Table 1. Age and gender wise distribution of swine H1N1 positive cases

Age	Total samples	Male	Female	Positive Cases
0—1yr	110	53 (48.2%)	57 (51.8%)	19 (17.27%)
1—20yr	535	295 (55.1%)	240 (44.9%)	113 (21.12%)
21—40yr	1133	513 (45.3%)	620 (54.7%)	265 (23.38%)
41—60yr	767	404 (52.7%)	363 (47.3%)	209 (27.24%)
>60yr	323	175 (54.2%)	148 (45.8%)	57 (17.64%)

RNA extraction kit (Qiagen, USA) and primers (ABI, USA) were also according to the CDC protocol (WHO, 2009). The viral RNA was extracted from clinical samples using the spin column based QAIamp® Viral RNA mini kit (Qiagen GmbH, Hilden, Germany) as per the manufacturer's instructions. Primers and probes were custom synthesized from Applied Biosystems (AB), USA, for influenza type A, (H1) Swine A, and Swine H1. Primers and probes were CDC approved. Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) was performed by using a Step One Real Time PCR instrument (AB) with a rRT-PCR kit containing 2 × PCR mix and Superscripts™ III RT/Platinum Taq Mix (Enzyme mix) from Invitrogen (CA, USA), and influenza A, Swine A and

Table 2. Distribution of districts wise Positive H1N1 cases

Districts	Total cases	Positive cases
Jodhpur	2423	564 (23.27%)
Barmer	151	43 (28.5%)
Jaiselmer	120	21 (17.5%)
Jalore	79	14 (17.7%)
Sirohi	48	11 (22.9%)
Pali	47	15 (31.9%)

Symptomatically, fever (78%), chills and rigor (12%), nasal discharge (84%), ear discharge (6%), cough (88%), sore throat (38%), breathlessness (45%), headache (15%), body ache
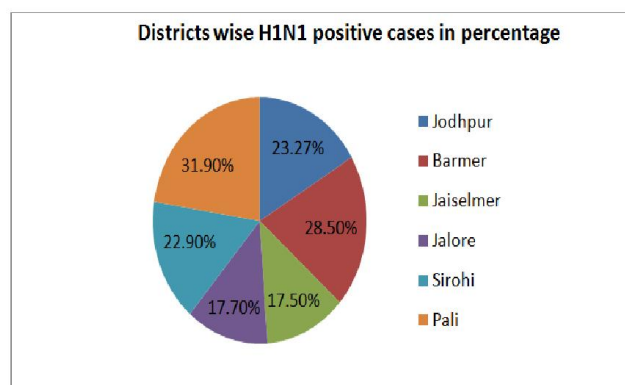
Figure 1. Distribution of districts wise Positive H1N1 cases

(27%), fatigue (10%), ARI in family (5%), vomiting (19%) and diarrhea (3%) were the symptoms observed in ILI patients. Clinical presentation of positive influenza cases, nasal discharge and cough was prominent in every patient.

(2010 to 2015). Detail description of our last 6 year data shown below in table no.3.

DISCUSSION

The clinical importance of the early identification of influenza was underscored by our recent surveillance data. Out of the total 2868 suspected influenza samples processed using rRT-PCR method, 668 (23.3%)) were positive for swine flu (H1N1) and 524 (18.27%) samples were positive for only group specific influenza A virus. The patient distribution gender wise was observed to be 50.2% males and 49.8% females, giving a male: female sex ratio approx 1:1. It was observed that gender-wise positivity for influenza virus in male and female was 23.34% (336/1440) and 23.25% (332/1428) respectively; however, statistically there was no significant difference with respect to influenza positivity in gender-wise distribution on using two-tailed test for proportion.

Table 3. Comparison of our last 6 year H1N1 data

Year	Total samples	Male	Female	Total positive	Pregnant women	Pregnant women H1N1 positive
2010	866	397 (45.8%)	469 (54.2%)	137 (15.8%)	160 (18.5%)	38 (23.7%)
2011	194	75 (38.6%)	119 (61.4%)	26 (13.4%)	38 (19.6%)	10 (26.3%)
2012	703	252 (35.8%)	451 (64.2%)	154 (21.9%)	14 (2%)	3 (21.4%)
2013	1122	470 (41.9%)	652 (58.1%)	185 (16.5%)	174 (15.5%)	45 (25.8%)
2014	130	58 (44.6%)	72 (55.4%)	2 (1.5%)	8 (6.2%)	0 (0%)
2015	2868	1440(50.2%)	1428(49.8%)	668 (23.3%)	230 (8.02%)	63 (27.4%)
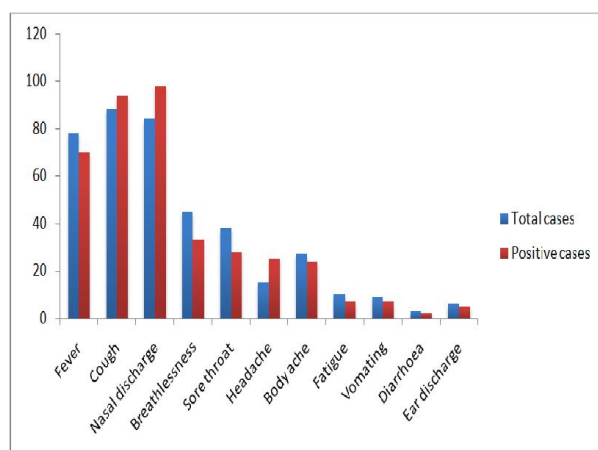
Figure 2. Clinical feature of total and positive H1N1 cases in percentage

Symptomatically, nasal discharge (98%), cough (94%), fever (70%), breathlessness (33%), sore throat (28%), headache (25%), body ache (24%), chills and rigor (9%), ear discharge (5%), fatigue (7%), vomiting (7%) were the clinical presentations observed in influenza-positive population.

Comparisons in Clinical features of total patients and swine flu (H1N1) positive patients samples tested in our laboratory is shown below In Figure no.2. After 2009 pandemic of influenza A (H1N1) Virus we compare the last 6 years (from 2010 to 2015) data of our institution. We compare total positive for H1N1, total male patients, total female patients, total pregnant women samples and positive H1N1 pregnant women from total samples process in Microbiology laboratory Dr. S. N. Medical College Jodhpur Rajasthan by RT-PCR Method in last 6 years

We compare our last 6 years records (from 2010 to 2015) and analyses that maximum sample (2868) and maximum positive 668(23.29%) for swine H1N1 was came in year 2015 while minimum total sample 130 and minimum positive 2(1.5%) for swine H1N1 was came in year 2014. We also found that female population was more (>50%) than male from 2010 to 2014 but in year 2015 male population was more (>50%) than female. Maximum positive cases (27.4%) for influenza H1N1 in pregnant women was found in year 2015.We also observe that total number suspected patients samples was gradually increases from the year 2011 to 2015 except 2014. There is considerable evidence that pregnant women are at increased risk for more severe illness from influenza infection. Cardiopulmonary adaptive changes occurring in pregnancy, such as increased cardiac rate and stroke volume and reduced pulmonary residual capacity, may increase the risk of hypoxemia and contribute to the observed increased severity of influenza (Elisabeth Sappenfield *et al.,* 2013). In our study a total of 230 pregnant women sample tested out of which 63 (27.4%) were positive for H1N1 and 18 (28.57%) women died due to influenza A (H1N1) infection. Asmita et al shown Pregnant women with H1N1 constituted 33% of total mortality (Asmita *et al.,* 2013). Influenza virus infection in infants is generally more frequent among those aged 6 to 12 months than in the first 6 months of life, potentially owing to the protection conferred by maternal influenza antibodies acquired transplacentally or through breastfeeding (GlezenWPTaber *et al.,* 2009). In present study 110 suspected infant patients samples tested out of which 19 (17.27%) were positive for influenza A (H1N1) virus. Out of 110 infants samples 65 (59%) were 6 to 12 months of age and remaining 45 (41%) were less than 6 month of age groups. Various study proven that inactivated influenza vaccine given

to all pregnant women during flu season (November-March) that have been shown to have substantial benefits for both mother and baby (Macdonald *et al.,* 2009). Various surveillance studies have demonstrated that relative humidity, rainfall and differences in temperatures influence the outbreaks of Influenza. In countries with temperate climate, influenza outbreaks occur in winter (Nalini *et al.,* 2005; Zaman *et al.,* 2009; Agrawal *et al.,* 2009). In our present 3 months study period maximum influenza positive cases reported in month of February than positive cases gradually decreases. We had not observed any correlation between temperature variation and influenza outbreaks.

Conclusion

The present study revealed that proportion of swine flu positive cases were higher in 41-60 years of age group, with approx equal (1:1) male, female ratio. Pregnant female was highly susceptible for infection. Nasal discharge and cough was most common symptoms found. The risk of death was seen more in cases of pregnant female and infants. We emphasize on monitoring of influenza in different age groups, different gender and different geographical locations, which will be helpful in patient management who are at risk for influenza complications. In all available measures influenza A (swine flu) still not controlled therefore it required more funding for surveillance, public health efforts, diagnosis and vaccine development needs to be enhanced.

Summary

Early case detection can reduce the burden of disease, so the health system should be strengthened and voluntary early reporting of cases should be encouraged through various health campaigns. Continuous monitoring would be required for early detection of any antigenic variants to understand the seasonality and analyses factors such as temperature, rainfall and humidity in the transmission of influenza viruses.

Acknowledgement

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