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CLINICAL SCENARIO OF INFLUENZA A PANDEMIC (pH1N1) FLU INFECTED PATIENTS AT TERTIARY REFERRAL HOSPITAL IN NORTHERN INDIA

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ABSTRACT

H1N1 subtype of pandemic 2009 (pH1N1) influenza virus lineages has raised severe concerns about its pandemic potentiality. Clinical and epidemiological factors associated and outcome for this virus remain unclear in subcontinent. We analyzed data obtained from such patients to characterize the epidemiological characteristics of H1N1 cases in India. A prospective observational study among suspected cases with 2009 influenza A (H1N1) infection in North India between 2009 to 2014. The presence of the H1N1virus was done by RT-PCR. Information of clinical and demographic characteristics were collected on proforma and Hospital information system. A total of 5090, 947(18.6%) cases found positive for pandemic flu Influenza A virus and 535 males and 412 females patients. The majority were between the age group of 6-20 years and predominantly males. The median duration of symptoms before hospitalization was 7(2-30) days. Common presenting symptoms were fever in 902 (95.23%), cough in 872 (92.06%), breathlessness in 842 (88.88%), chest pain in 706 (74.60%), diarrhea in 315(33.33%). 30 patient (3.1%) required ventilatory support. Cause of death was multi-organ failure in 10 patients (1.05%). Severe disease and complications can occur due to influenza infection that can lead to hospitalization and death. Young persons and those with medical co-morbid conditions (like asthma, diabetes, and cardiac disease) were

affected. The mainstay of treatment depends on antiviral medications. Vaccination must be employed in community for target population for preventing influenza illness and effective control of epidemics.

KEY WORDS: pH1N1; Influenza; Mortality; Respiratory illness; Pneumonia.

INTRODUCTION

Influenza virus infection is a highly contagious respiratory illness which is associated with significant morbidity and mortality worldwide. Influenza A virus is a negative single-strand RNA virus which emerges sporadically as pandemic viruses and is responsible for annual seasonal epidemics worldwide. Influenza is a serious respiratory illness of humans which leads to debilitating complications and prolonged hospitalization and death especially in the elderly.[1] Influenza A (H1N1) virus is a subtype of influenza A virus and the most common cause of influenza (flu) in humans.[2, 3] Some strains of H1N1 are endemic in human and cause a small fraction of all influenza-like illness and a small fraction of all seasonal influenza. H1N1 strains caused a few percent of all human flu infections in 2004-2005.[4] In 2009, the World Health Organization (WHO) declared the new strain of swine-origin H1N1 as a pandemic which is often referred as swine flu.[5,6]

Influenza is an acute, usually self-limited, febrile illness caused by infection with influenza type A viruses and occurs in outbreaks of varying severity almost every winter.[7] In April 2009, human infection with a new variant of influenza A (H1N1) virus were identified in the United States and Mexico and shown to cause severe illness among several patients.[8, 9] Virus spread rapidly to other parts of the world. The 2009 H1N1 virus is a triple-reassortant influenza virus containing genes from human, swine, and avian influenza viruses[10, 11] and thus has been labeled “swine flu.” Most cases of pandemic influenza H1N1 infection have been mild or sub-clinical symptoms, some patients experienced severe illness and complications from H1N1 influenza infection.[12,13,14,15,16,17,18,19] The most common cause of death is respiratory failure; other causes of death are pneumonia, high fever leading to neurological problems, and dehydration. Persons at high risk for severe disease and complications secondary to 2009 pandemic H1N1 influenza A include patients with underlying pulmonary or cardiac co-morbid conditions, immunosuppressive states, pregnancy, diabetes mellitus and obesity in children with prior neurological disabilities.[20,21,22,23] In the 2009 flu pandemic, the virus isolated from patients in the United States (U.S.) was found to be made up of genetic elements from four different flu viruses– North American swine influenza, North American

avian influenza, human influenza, and swine influenza virus typically found in Asia and Europe.[24] This new strain appears to be a result of reassortment of human influenza and swine influenza viruses, in all four different strains of subtype H1N1.[25] Preliminary genetic characterization found that the hemagglutinin (HA) gene was similar to that of swine flu viruses present in U.S. pigs since 1999, but the neuraminidase (NA) and matrix protein (M) genes resembled versions present in European swine flu isolates.[26] The six genes from American swine flu are themselves mixtures of swine flu, bird flu, and human flu viruses.[27]

In April 2009, an outbreak of Influenza-like illness (ILI) occurred in Mexico and the USA the Centers for Disease Control and Prevention (CDC) reported seven cases of novel A/H1N1 influenza.[28,29] In 2009 it became clear that the outbreak of ILI in Mexico and the confirmed cases of novel influenza A in the southwest US were related.[30] The disease then spread rapidly, with the number of confirmed cases rising to 2,099 in mid year of 2009, despite aggressive measures taken by the Mexican government to curb the spread of the disease.[31] On 11th June, 2009, the WHO declared an H1N1 pandemic, moving the alert level to Phase 6, marking the first global pandemic since the 1968 HongKong flu.[32] On 29th November, 2009 worldwide update by the WHO stated that 207 countries and overseas territories/communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009[33], including at least 8,768 deaths. In 2010 worldwide update by the WHO stated that 208 countries and overseas territories or communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including at least 13,554 deaths.[34] In India, after declaration of pandemic (phase 6) by World Health Organization (WHO) on 11 June[35], an active surveillance was started for detection of influenza cases in persons with travel history to influenza positive countries. All suspected cases of pandemic H1N1 were detained and hospitalized. Only confirmed cases were provided with antiviral treatment. In Pune, the first pandemic H1N1 positive case was detected on 22 June 2009 in a traveler coming from USA. This was followed by 1 more case in June and 8 cases up to 14 July. All these cases were either persons with foreign travel history or the contacts of such persons. There was a progressive increase in the number of swine flu cases all over the subcontinent. The spectrum of illness ranged from mildly symptomatic patients to severe illness. The first death due to pandemic H1N1 was reported on 3 August 2009. Thereafter, the active surveillance in community was started by screening all suspected cases of influenza.

MATERIAL AND METHODS

Ethics Statement

The study was approved by the Institutional Ethics Committee (IEC) of Sanjay Gandhi Postgraduate Institute of Medical Sciences Lucknow. All participants gave written informed consent before their samples were collected and processed in the laboratory.

Sample collection and processing

The throat swab of patients suspected with influenza inpatients and outpatients clinic were collected in VTM (Viral Transport Media) at time of severity were collected from the Pulmonary Medicine Department and General Hospital at Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow. The throat swab samples were used for virus identification by real time PCR. As per the Ministry of Health & Family Welfare (MOHFW) guidelines on categorization of H1N1 cases[8], only category C patients were subjected to testing for H1N1. Although all patients included in the study were categorized as category C, (Symptoms included breathlessness, chest pain, drowsiness, fall in blood pressure; sputum mixed with blood, bluish discoloration of nails; children with red flag signs like somnolence, high and persistent fever, inability to feed well, convulsions, shortness of breath, difficulty in breathing; worsening of underlying chronic conditions). This study was carried out during the period of June 2009 to February 2014.

This study was a prospective analysis of the clinical epidemiological information consisting of clinical presentations, history of contact among H1N1 positive persons and positivity rate of different types of influenza viruses in these samples. A total of 5090 throat swabs and nasal swabs/ nasopharyngeal swabs were collected by clinicians in viral transport medium (VTM) from category C patients(Acc. to CDC guideline), Samples were accompanied with duly filled Proforma indicating demographic characteristics, date of onset of symptoms, co-morbidities, travel/contact history, antiviral treatment, etc. These samples were processed in Bio Safety level 3 (BSL3) laboratory within 2-3 h of receipt of sample.

RNA extraction and real-time PCR

Viral RNA was extracted from all samples by using the QIAamp Viral RNA mini kit (Qiagen) according to the manufacturer's guidelines. Real Time-PCR of the extracts was performed by using a Agpath-ID^{TM} One-Step Real Time-PCR kit (Ambion U.S.A) according to the manufacturer's instructions, with the influenza gene primers given in Table-1. In each sample four target genes were amplified; Influenza A, Swine Influenza A, Swine H1 and

RNaseP (CDC Real Time RT PCR kit). A sample was declared positive when it showed amplification in all 4 target genes.

Briefly, the 25µl reaction volume contained 5µl of 5X PCR buffer, 13µl of RNAse-free water, 1µl of 10mmol/L dNTPs, 1.5µl of 10 nmol/L reverse primer, 1.5µl of 10nmol/L forward primer, 1 µl of enzyme mix (Taq DNA polymerase and reverse transcriptase), and 2µl of viral RNA extract. Amplification was carried out in an Applied Biosystems Step One Real Time PCR with a single reverse transcription step of 50°C for 30 min, activation of hot start Taq at 95°C for 15 Sec followed by cycling step (95°C for 15 Sec. and 55°C for 30 sec).

RESULTS

Demographic and clinical characteristics of the patients are described in table 2. A total of 5090 patients with clinically suspected influenza belonging to age group 0-60 years and both sexes were tested from 17 districts of Uttar Pradesh during June 2009 to February 2014. There were (947/5090) 18.60% cases found positive for pandemic flu Influenza A virus, The sex distribution showed that there were 535 males and 412 females patients among the 947 swine influenza A/H1N1 positive cases between 2009-2014.

In 2009 there were 1975 clinically suspected cases, 400 (20.25%) cases were reported positive for swine influenza A/H1N1 infection by real time PCR detection and characterization as per the protocol of CDC. The sex distribution showed that there were 218 males and 182 female patients (Fig.1) among the 400 swine influenza A/H1N1 positive cases. In 2010 there were 1692 clinically suspected cases of influenza and 355 (20.98%) cases were confirmed positive for swine influenza A/H1N1 infection. Among these patients there were 210 males and 145 females showing an overall male predominance. In 2011 from 551 clinically suspected cases 50(9.07%) cases were reported positive for swine influenza A/H1N1 infection with 34 males and 25 females patients. In 2012 out of 742 clinically suspected cases there were 120(16.17%) cases which were reported positive for swine influenza A/H1N1 infection with 65 males and 55 females patients. In 2013 there were 130 clinically suspected cases only 13 (10%) cases were reported positive for swine influenza A/H1N1 infection with 8 males and 5 females patients. There were none cases found positive for pandemic Influenza A in February 2014. The majority of infected individuals were between the age group of 6-20 years and predominantly males were affected (Figure 2). The median age was 23(11-40) years. History of travel or contact with a swine flu patient was

present in 18(28.57%) patients. The median duration of symptoms before hospitalization was 7(2-30) days. Common presenting symptoms were fever in 902 (95.23%), cough in 872 (92.06%), breathlessness in 842 (88.88%), chest pain in706 (74.60%), vomiting in 150 (15.85%), throat pain in 917 (96.82%), body ache in 75 (7.92%), pregnancy in 30(3.1%), multi-organ failure in 10(1.05%), dyspnea in 705(75%), diarrhea in 315(33.33%) and myalgia in 30(3.1%). We have 60(6.34%) pneumonia patients and chest X-ray findings i.e. bilateral pulmonary infiltrates found in 195(20.63%) patients. None of the patient had hemoptysis. Patients haemoglobin ranged between 6.5-13.8 g/dl and high pulse rate between 96-128/min and lower respiratory rate between 20-28/min as compared to healthy individuals. Various co-morbid conditions were observed like asthma in 105 (11.09%), obesity in 45(4.7%), Type1 or 2 diabetes in 30(3.1%), and heart disease in 34 (3.5%).

The overall mortality was seen in 15 cases during this period. The mortality rate was higher among population of high-risk groups such as children, the elderly, health care workers, and people who had chronic illnesses such as asthma, diabetes, heart disease, or are immuno-compromised . None of the patient had received any prior vaccination for influenza illness.30 patient (26.98%) required ventilatory support, of them 7 patients were given non-invasive ventilator support and 23 were given invasive ventilator support. 842 total patients (88.88%) were cured and discharged from the hospital, 15 (1.58%) patients died, and 90 patients took discharge from hospital against medical advice or shifted to other hospitals. The median duration of hospitalization was 7(2-30) days. Cause of death was multi-organ failure in 10 patients (1.05%) and sepsis with adult respiratory distress syndrome in eight patients.

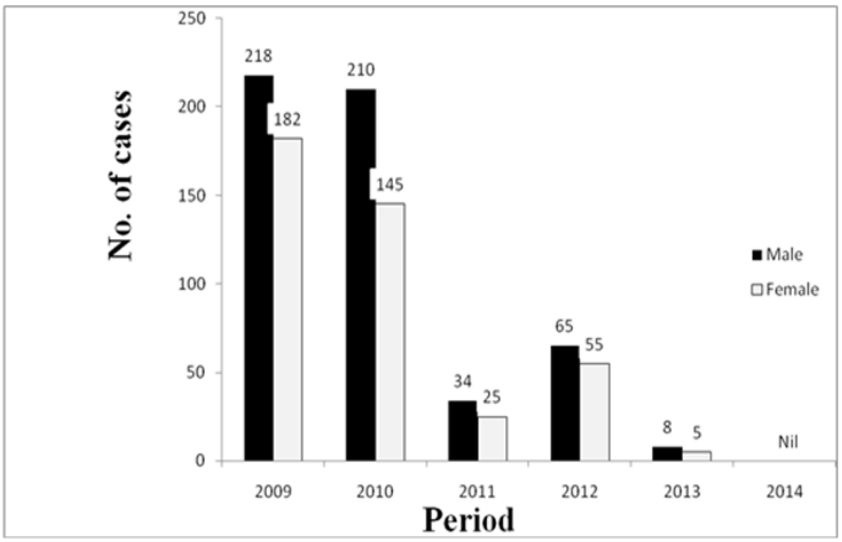

Figure 1: The year-wise trend of pandemic Influenza AH1N1 reported from June 2009-February 2014.

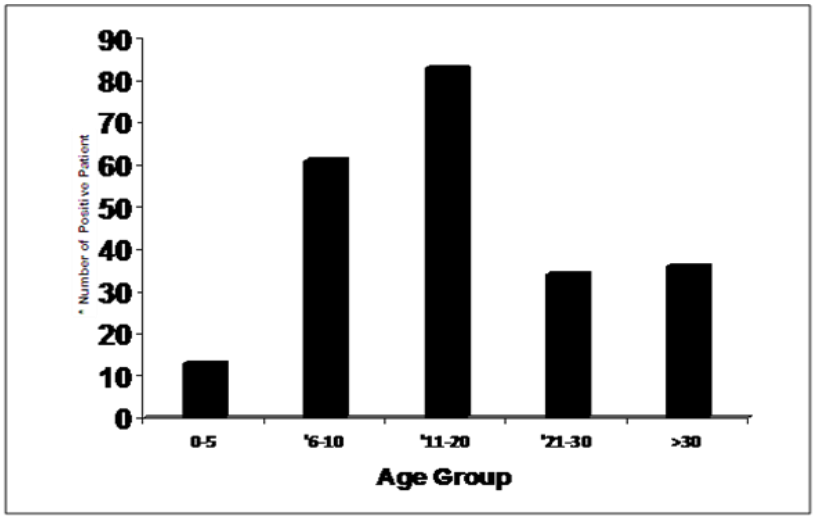

Figure 2: The age-wise distribution of positive cases for pandemic Influenza A H1N1 (n=947)

Target gene	Direction	Sequence
InfA	Forward	GAC CRA TCC TGT TAC CTC TGA C
	Reverse	AGG GCA TTY TGG ACA AAK CGT C TA
	Probe	**FAM**-TGC AGT CCT CGC TCA CTG GGC ACG-**MGB**
SW InfA	Forward	GCA CGG TCA GCA CTT A TY CTR A G
	Reverse	GTG R GC TGG GTT TTC ATT TGG TC
	Probe	**FAM**-CY A C TG C AA GCC CA"T"ACA CAC AAG C AG GCA-**MGB**
SW H1	Forward	GTG C TA TAA AC A C CA GCC TYC CA
	Reverse	CGG GAT ATT CCT TAA TCC TGT RGC
	Probe	**FAM**-CA GAA TAT ACA "T"CC RGT CAC AA T TGG A RA A-**MGB**
RnaseP	Forward	A GA TTT GGA CC T GC G A GC G
	Reverse	GAG C GG CTG TCT CC A C AA GT
	Probe	**FAM**-TTC TGA CCT GAA GGC TCT GCG CG-**MGB**

Table 1: Demographic and clinical characteristics of the patients.

Characteristics of patients	**Patients with Pandemic flu (**n=947) 18.94%
Age **(**years)	23(11-40)
Sex ratio (male/female)	535(66.49%) /412(33.51%)
Illness day on admission (day)	2 (1-5)
Steroids	586(61.90%)

Underlying medical conditions	Asthma (no.)	105 (11.09%)
	Obesity (no.)	45(4.7%)
	Heart disease (no.)	34 (3.5%)
	Type 1or 2 diabetes (no.)	30 (3.1%)
	Pulse rate	110 (96-128)/min
	Haemoglobin	10.2 (6.5-13.8)
	TLC	11,850 (17, 00- 22,000)
Clinical sign and symptoms	Fever > 38˚C (no.)	902 (95.23 %)
	Headache (no.)	928 (98%)
	Cough (no.)	872 (92.06%)
	Vomiting (no.)	150 (15.85%)
	Throat pain (no.)	917 (96.82%)
	Nasal catarrh (no.)	526 (55.55%)
	Myalgia (no.)	496 (52.38%)
	Dyspnea (no.)	705 (75%)
	Diarrhoea (no.)	315 (33.33%)
	Co-morbidity	474(50%)
	Body ache	75(7.92%)
	Pregnancy	30 (3.1%)
	Multiorgan failure	10 (1.05%)
Respiratory complication	Shortness of breath/ difficulty in breathing (no.)	842(88.88%)
	Chest pain (no.)	706 (74.60%)
	Pneumonia (no.)	60(6.34%)
Chest X-ray findings (Bilateral pulmonary infiltrates)		195(20.63%)
Ventilator		30 (3.16%)
Duration of Hospital stay		7 (2-30) days
In hospital death		15 (1.58%)

DISCUSSION

Seasonal influenza commonly starts during pre-winter period and ends as summer sets in, that is during the months of August to March in South Asian region. Diagnosis of influenza is confirmed by real-time PCR from throat swab[36]. Common clinical symptoms of seasonal influenza includes upper respiratory symptoms, cough, fever, bodyache, throat pain, headache, and weakness. During the 2009 epidemic, swine flu patients also demonstrated gastrointestinal symptoms- vomiting and diarrhea apart from common symptoms. Fever and cough were the most common presenting symptoms in pandemic influenza, and in our study they were seen in 95.23% (n = 902) similar to reports from USA, Japan, and Mexico[10,37,38,39] .

Vomiting and diarrhea were observed in nearly one third of patients in US series which is similar to our study [39]. Other symptoms observed in our study were vomiting 150 (15.85%), throat pain 917 (11.11%), body ache 75 (7.92%), and chest pain 706 (74.60%). Breathlessness was seen in 842(88.88%) patients in our study.

Seasonal influenza commonly affects old age people, while the 2009 H1N1 influenza significantly impacted young people. In our study, out of 947 patients, most were between 6 and 20 years of age, which suggest predilection for younger age in this 2009 H1N1 infection and 15 patients were died in our study, There was no significant difference in mortality in-patient <40 or ≥40 years of age (P = 0.583) in our study. In our study, range of TLC were 17, 00-22,000 cells/cu.mm and hemoglobin range of 6.5-13.8, were consistent with the findings of other studies [40]. Various co-morbid conditions were seen in 214 (22.39%) patients in our series. Co-morbid conditions were present in nearly 50% of hospitalized patients in USA and Mexico, which is significantly higher than our study. In our study we have only 30 (3.1%) pregnant and 45(4.7%) obese patients [39]. Six patients had more than one co-morbid condition. Most of the patients had either diabetes and/or cardiac disease as a co-morbid condition, while only one patient had immunosuppressive condition. Co-morbidities were associated with increased risk of death in pandemic influenza 2009 patients in our study (p-0.010). In series from United Kingdom, co-morbid conditions were also associated with increased risk of death, where obesity was present in significant number of patient as a co-morbidity and in another study from USA obesity was a risk factor for high mortality in H1N1 patients[41,42]. Out of 214(22.39 %) patients with co-morbid conditions, 30 required for ventilatory support. Co-morbid conditions were not associated with increased risk of ventilatory requirement (p-0.486). Influenza is known to cause myositis, renal failure and neurologic complications; however, only one patient in our study had severe myositis, renal failure and encephalitis with residual debility. Need for ventilatory care was found to be associated with significantly increased mortality (p<.0001), 10 patients out of 17 who required ventilatory support died during the course of hospital stay whereas only 4 out of 46 patients who did not require ventilatory support died eventually. In our study, in the patients who died (n=15), 12 had bilateral pulmonary opacities, while 3 had unilateral pulmonary opacities on chest radiograph on presentation. Bilateral opacities express possible adverse outcome. Any opacity on chest radiograph on presentation (unilateral or bilateral) was associated with increased mortality as compare to patient with normal chest radiograph (p- 0.071). In patients with bilateral pulmonary opacities (n=60), 30 patients required mechanical ventilator support. On

multivariate analysis ventilatory requirement, pneumonia and co-morbidities were the independent predictors of mortality controlling for age and sex.

CONCLUSION

The 2009 influenza A (H1N1) infection affected young population and those with associated co-morbidities and caused prolonged hospitalization and few required prolonged mechanical ventilation. Upgraded system of surveillance of human illness with H1N1 virus infection must be used to determine the clinical spectrum of the infection and vaccination strategies needs to be employed whenever required.

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Conflict of interest

None of the authors had any conflicts of interest.

Statistical analysis

Data were analyzed using Microsoft Excel Software and basic statistical measures like mean, median, percentage, etc. were calculated. The final multivariate model of factors associated with mortality in this study. Chi- square test, Fisher exact test, and independent sample t test were used to compare data between groups of patients when appropriate by using either SPSS for window version 16 (SPSS Inc., Chicago, IL).

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