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Full Length Research Paper

Prevalence and epidemiology of pandemic H1N1 strains in hospitals of Eastern India

Anupam Mukherjee[1], Tapasi Roy[1], Anurodh S Agrawal[1], Mehuli Sarkar[1], Renu Lal[2], Sekhar Chakrabarti[1] and Mamta Chawla-Sarkar[1]*

[1]National Institute of Cholera and Enteric Diseases, Beliaghata, Kolkata, India.
[2]CDC Influenza Coordinator, Global Disease Detection Program, New Delhi, India.

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A novel Influenza A (pH1N1) virus which emerged in April, 2009, spread rapidly across the continents to become pandemic by June, 2009. In Eastern India, testing for influenza was initiated in June, 2009 and continued through July, 2010 to determine prevalence and epidemiological character of circulating pandemic H1N1 strain. Real time PCR was done on nasal and throat swab samples of patients with influenza like symptoms of those who sought medical care in local government hospitals. Of 2971 patients tested, 382 (12.86%) were positive for influenza A and 103 (3.47%) for influenza B. Of 382 influenza A positives, 284 (74.35%) and 98 (25.65%) were subtyped as pandemic H1N1 (pH1N1) and seasonal H1N1 and H3N2, respectively. The novel pH1N1 virus followed typical influenza seasonality in Eastern India and revealed a unimodal peak in July and August, correlating with the rainy season. Most of the positive cases presented with mild ILI symptoms with minimal serious complications. Though four deaths were attributed to pH1N1 infection in 2010, all four had underlying serious medical complications. Infection rate was highest in age group of >55 years followed by 5 - <18 years of age group.

Key words: Pandemic, H1N1, influenza.

INTRODUCTION

Pandemic H1N1 (pH1N1/2009) virus appeared in first quarter of 2009 in the west coastal region of North America and spread very rapidly to other countries through April to June, 2009 (Dawood et al., 2009; WHO-Influenza pandemic, 2009) . The World Health Organization (WHO) formally declared the pandemic (Phase 6) on 11th of June, 2009 (WHO -Influenza pandemic, 2009). As of 1st August 2010, more than 214 countries and overseas territories or communities have reported laboratory confirmed cases of pH1N1/2009 virus, including over 18449 deaths worldwide (WHO-Pandemic (H1N1) 2009 - Update 112). This virus is unique compared to previous pandemics since it had high transmission ability but low virulence compared to previous pandemic viruses of this century (CDC-Outbreak of swine-origin influenza A, 2009). The first case of P-09-H1N1 positive in India was reported on 16th May, 2009, from a 23 year old passenger who traveled from USA arriving at Hyderabad airport (John and Moorthy, 2009). After that the virus soon became endemic and spread to almost all major cities in India.

Here we report circulation and prevalence of pH1N1/2009 along with the seasonal influenza A and influenza B strains in Eastern India states during June 2009 to July 2010. Suspect case samples from both outpatients (OPD) and in-patients (IPD) departments were collected in hospitals by the state health officers and sent to National Institute of Cholera and Enteric Diseases (NICED), for laboratory testing. First positive case in Eastern India was identified on June 23rd, 2009 from a traveler from Australia. This report provides relevant clinical and epidemiological features of circulating pH1N1 strains identified from 2971 patients.

*Corresponding author. E-mail: chawlam70@gmail.com. Tel: +91-33-2353-7470. Fax: +91-33-2370-5066.

Table 1. State wise distribution of pH1N1 and seasonal influenza tested at NICED, Kolkata.

States	Sample tested	No. positive of pH1N1/2009-10	No. positive of seasonal influenza
West Bengal	2780	265	192
Jharkhand	31	2	1
Orissa	46	3	2
Meghalaya	28	7	2
Mizoram	76	4	4
Sikkim	3	0	0
Tripura	1	0	0
Andaman and Nicobor	6	3	0
Total	2971	284	201

THE STUDY

Patients reporting to hospitals and international travelers with ILI symptoms who received medical care as outpatients or inpatients in the hospitals throughout the epidemic wave in seven states (West Bengal, Jharkhand, Orissa, Meghalaya, Mizoram, Sikkim and Tripura) and one Union territory (Andaman and Nicobar Islands) were referred to NICED, Kolkata for Influenza testing (Table 1). Nasal/Throat swabs were taken in VTM after collecting relevant clinical information and epidemiological information such as age, gender, underlying disease, travel history, close contact with confirmed case etc from the patient. RNA was extracted using QIAamp viral RNA Mini Kit (Qiagen GmbH, Germany) and Real time RT-PCR was used for clinical confirmation of the presence of pH1N1, and Influenza A viruses in the collected samples using specific probes and primers (TaqMan® Universal PCR Master Mix and H1N1 influenza A MGB Assay [Set 1 and 2], Applied Biosystems, USA) on a ABI 7500 cycler (Applied Biosystems, USA). The Influenza A positive, but pH1N1/2009 (specific HA gene primer) negative samples were further subtyped for seasonal H1N1 or H3N2 strains. The Influenza A negative samples were processed with Influenza B matrix gene specific primers.

RESULTS AND DISCUSSION

During June 2009 to July 2010, a total of 2971 samples were tested. These represent majority of specimens from West Bengal (n = 2780), followed by Mizoram (n = 76), Orissa (n = 46), Jharkhand (n = 31), Meghalaya (n = 28), Andaman and Nicobar (n = 6), Sikkim (n = 3) and Tripura (n = 1) (Table 1). Real time PCR analysis revealed that 382 of 2971 (12.86%) samples were Influenza A positives, out of which 284 (74.35%) samples confirmed for pH1N1/2009. Among seasonal Influenza A positive samples [n = 98 (25.65%)], 56 subtyped as H1N1 and 42 as H3N2. In addition, 103 (3.47%) samples were found to be positive for seasonal Influenza B. Over all among 2971 patients tested, only 9.56% (n=284) pH1N1/2009 incidence was observed including four deaths during the fourteen month study period (Figure 1). Month wise analysis of the cases revealed a major peak during the rainy season in this part of India. More than 44% of all pH1N1/2009 cases (n = 125) were detected in July, 2010 followed by 41.5% (n = 118) in August, 2009, however the number of patients reporting with Influenza like symptoms were also high during these months (Table 2). Only one peak was observed in this region, which correlates with seasonality of influenza infection reported earlier from this part of the country (Agrawal et al., 2009). During the first half of 2010 (Feb to June), Influenza B was prevalent, which is consistent with our previous report (Agrawal et al., 2009).

Age-wise analysis of positive cases per total number of samples screened revealed that, maximum positivity for pH1N1 viruses belonged to the age group of >55 yrs (14%) followed by 5 - <18 years (13%) (Figure 1) . In parallel, it also revealed seasonal Influenza A (H1N1/H3N2) predominance in 5 – 18 years age group and Influenza B in less than 5 years age group (Figure 1). Of 2971 patients screened, 1872 were male and 1099 were females (ratio 2:1), however no gender specificity of infection was observed as percent infection (no. of positives/ no. enrolled X 100) among genders (16.67%: 15.74%) were similar (data not shown).

In India, Influenza virus had been generally ignored in public health and in healthcare. Etiology-specific diagnosis requires laboratory tests that are not widely available in here. Therefore what we know about epidemiology and clinical features are entirely from research studies only. Unlike temperate climate countries where seasonal influenza incidence reaches epidemic proportions during winter months (John and Moorthy, 2009), in tropical countries like India year round circulation of strains has been reported though the infection peaks during rainy season (June-September) (Agrawal et al., 2009, 2010). However in northern India with very cold winter season (December-February), two peaks of infection are observed, one in rainy season and one during winters (unpublished observation). In India, except in few metro cities, etiology-specific diagnostic tests are not widely available. The National Institute of Virology (NIV) started limited influenza surveillance in Pune in 1976, which expanded in 2004, to multisite network of influenza virus surveillance laboratories at

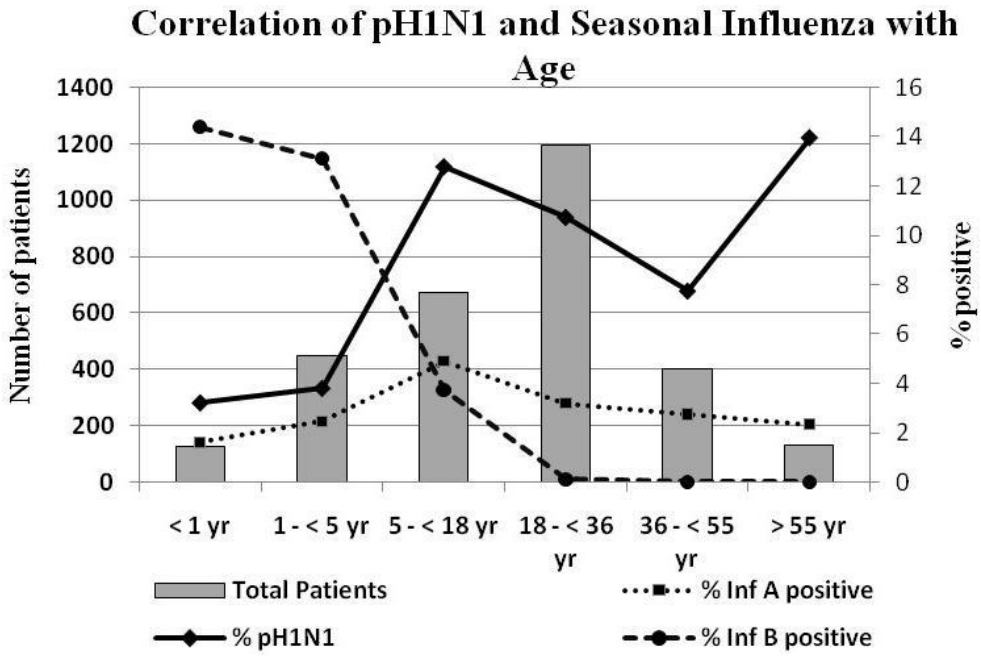

Figure 1. Agewise distribution of patients infected with pH1N1 and Seasonal Influenza viruses during June, 2009 to July, 2010.

Table 2. Monthwise distribution of pH1N1 and seasonal flu tested at NICED, Kolkata.

Month	Total no. of patients	Total no. of positive cases	Positive for pH1N1	Positive for seasonal H1N1	Positive for seasonal H3N2	Positive for seasonal Inf B
June '09	11	2	2	0	0	0
July '09	23	5	3	1	1	0
August '09	1540	208	118	49	41	0
September '09	313	21	18	2	1	0
October '09	111	10	9	1	0	0
November '09	35	3	1	1	1	0
December '09	84	6	6	0	0	0
January '10	30	1	1	0	0	0
February '10	40	7	0	0	0	7
March '10	68	11	0	0	0	11
April '10	80	6	0	0	0	6
May '10	109	38	0	0	0	38
June '10	134	42	1	0	0	41
July '10	393	125	125	0	0	0
Total	2971	485	284	56	42	103

Pune, Vellore, Chennai, Kolkata, Dibrugarh, Mumbai, Nagpur and Delhi, for detection of Influenza viruses in suspected ILI cases. The network has been generating data on influenza isolates for global vaccine selection, and seasonality in various parts of India since 2005.

Soon after introduction of pandemic H1N1 into Hyderabad, laboratory based surveillance for pH1N1 at NICED, Kolkata started from June 2009, and extended referral diagnostics to all 9 surrounding states. In general, the positive cases had typical symptoms including fever, cough, headache, running nose etc. Some cases had difficulty in breathing and required hospitalization. Only four deaths were attributed during this study period to H1N1 infection but these patients had other medical ailments like renal failure, cancer or cardiac problem. Several important observations were made during the pandemic phase of H1N1 in Eastern India. First, during 2009 (June to December) we observed low prevalence of

in eastern India (7.4%), however in June to July' 2010, virus had became endemic with almost 24% prevalence. Second important observation was co-circulation of both pandemic and seasonal Influenza during June 2009 to January 2010, which is in contrast to reports from other regions in India and worldwide, where pandemic Influenza had completely replaced seasonal influenza strains. Reason for low prevalence rates during pandemic period in this part of country is not clear; however neighboring country Bangladesh also reported low prevalence in 2009 compared to USA or Europe (WHO Bangladesh, 2009). The low prevalence may be attributed either to environmental or social factors. Alternatively, mild ILI symptoms resulting in patients not reporting to hospitals could have been one of the major reasons for comparatively low frequency of infection rates. Detailed investigations in Peru also showed that one-third infected persons were asymptomatic; one third had short febrile illness without seeking medical care and one-third were ill enough to be hospitalized (ISID, Peru, 2009). As reported from Mexico (Vargas-Parada, 2009), in India too, patients usually do not go to hospitals with mild Influenza or other respiratory infections. Trend of self medication due to easy availability of prescription drugs over the counter is another major factor in underestimation of the frequency of infection.

In Eastern India, seasonality of seasonal Influenza (Agrawal et al., 2009) correlated with seasonality of pH1N1 infection (July- Sept), whereas high rates of infection continued until December 2009 in northern or western parts of India. Majority of positive pH1N1 cases belonged to the age group of >55 years followed by 5 - <18 years, which differs from the reports of other parts of India (Ministry of Health and Family Welfare, 2010).

Compared to western countries, where pH1N1 transmission declined after February 2010, its activity increased again since May 2010 onwards in south- east Asia particularly in Bangladesh, Thailand, India etc correlating with the monsoon season (WHO -Pandemic (H1N1) 2009 - Update 112, 2010). It would be interesting to know frequency and clinical features of pH1N1/2009 in temperate countries in upcoming Influenza season starting Nov-Dec 2010. Inspite of low pathogenesis, overall the pandemic of 2009 confirmed the ease with which infection can be spread and facilitated by air and land travel and community networks and gatherings. This further necessitates the need of active Influenza surveillance and easy access to diagnostic methods, cheap vaccines and antiviral drugs in developing countries for better control and prevention of future pandemics.

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