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Title:

High prevalence of asymptomatic carrier state with 2009 pandemic influenza virus in a remote rural South Indian community in the year 2012

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Abstract

Introduction:

There is scarcity of global data with regards to rates of asymptomatic carrier state due to 2009 pandemic H1N1 influenza virus. It will be interesting to study asymptomatic carrier state in the year 2012 when the global pandemic has been controlled. In this study we have attempted to evaluate the rates of asymptomatic carrier state due to this virus in a remote rural community from South India.

Methods:

380 consecutive, asymptomatic, community living adults without history of respiratory illness in the last 30 days were studied. Demographic and clinical profile was noted. Throat swab was obtained and tested by RT-PCR (reverse transcriptase polymerase chain reaction) for swine influenza A (HINI) as per CDC (Centers for Disease Control) protocol. Participants who were positive in this test were followed weekly by clinical evaluation for a period of 4 weeks to look for onset of respiratory symptoms.

Results:

Mean age was 42.58 yrs. Males formed 48.3% of the cohort. Mean body mass index was 23.62 kg/m2. 16.3% and 10.5% were diagnosed to have diabetes mellitus and hypertension respectively. None of the study participants had received seasonal influenza vaccine or pandemic H1N1 influenza vaccine. RT-PCR identified asymptomatic infection in 41 participants (10.8%). In uni-variate and multi-variate logistic regression analysis there were no significant associations between having asymptomatic carrier state, diabetes diagnosis, gender, age, BMI, hemoglobin A1C%. On follow-up none of the 41 positive participants developed respiratory symptoms.

Conclusion:

High prevalence of asymptomatic carrier state after 2009 H1N1 influenza virus pandemic was found in our study in the year 2012. The significance of asymptomatic infection remains unclear.

Keywords: Pandemic influenza, asymptomatic infection, H1N1 influenza.

Background:

The spectrum of clinical illness due to 2009 pandemic influenza ranges from mild illness to severe pneumonia, which may be complicated by multi-organ dysfunction and death. While preventive strategies like quarantine of infected individuals, utilizing barrier methods and contact prophylaxis are effective; the possible transmission of infection from asymptomatic individuals is a cause for concern. Meta-analysis of experimental studies on course of influenza virus infection in healthy human volunteers challenged with wild type influenza viruses indicates that 33% may have asymptomatic infection [1].

Rates of symptomatic infection due to 2009 pandemic influenza virus have come down throughout the world [2]. In the United States a recent report released in November 2011, by the Centers for Disease Control and Prevention (CDC), states that out of 2130 suspected cases of influenza only 1 was due to H1N1 virus infection [2]. Though isolated case reports of asymptomatic individuals infected with pandemic H1N1 influenza virus were published in the year 2009, there is scant data with regards to community prevalence of asymptomatic infection in healthy individuals [3, 4]. It will be interesting to study pattern of asymptomatic carrier state for H1N1 virus in the community at this time in 2011 when the pandemic is under control.

With this objective this study was conducted to identify the prevalence of asymptomatic carrier state due to pandemic H1N1 influenza virus among healthy individuals in a remote rural South Indian village called Kattanachampatty.

Materials and Methods:

Kattanachampatty is a village located 293 kms from Chennai in South India [5]. This predominantly agrarian community belongs to the Namakkal district and comprises of people belonging to low socio-economic status [5]. Our study was conducted in this village. The reason for conducting the study in this village is because this village has a stable population with few people travelling in and out of the village. Hence there was minimal risk of infection being imported into this village from elsewhere. Also, though there are small hospitals in this village, people prefer traditional healers and native treatment for their illness. Hence more or less the population here is naïve to allopathic medicine. Also, this village was the birthplace of our institution's founder. Hence, this study also served as a charity-screening program for common illnesses to commemorate the birthday of our founder.

The villagers were informed about this charity clinic for the last 4 weeks prior to the study start through local radio advertisements, local newspapers and pamphlet distribution by social workers and village leaders. The first part of the study was conducted for 3 days (September 15th to September 17th 2012). Institutional review board of Sri Ramachandra University, Chennai, India, approved the study. The approval number is SRIRBPH10-12I. Only verbal consent was obtained from each participant in the study. Considering the low literacy level of the study participants it was considered inappropriate to get written consent from the study participants as they will be unable to read and understand the consent document. Instead a verbal consent was sought after explaining the entire study in the language that the study participants could understand and in front of the village leader; a person whom they trust. During these 3 days, 415 consecutive adults (age > 18 years) from this community who visited this charity clinic were screened. Though this charity clinic continued to screen almost 15,000 people, spread over the next 1 month for common acute and chronic illnesses, only the first 415

consecutive visitors to this charity clinic were screened for this study due to financial constraints. Of these 415 participants screened, 20 participants were excluded because they had fever or respiratory illness during the previous 30 days or at the time of visit to the charity clinic, which was determined by symptom history and physical examination and 15 more, were excluded as they refused to provide consent for testing. Though it was not in our protocol to exclude pregnant women, we did not encounter any pregnant woman in these 415 people. Hence, after exclusion, 380 asymptomatic, community living adults aged > 18 years were included in the study and formed the study cohort. It is to be noted that none of these study participants ever received seasonal influenza vaccine or H1N1 influenza vaccine, as these vaccines were not commonly available in the village.

From these 380 study cohort participants demographic data (age, gender, current smoking, history of household contact with respiratory illness in the last 30 days, any allopathic or native medication intake in the last 30 days), vaccination history (pandemic H1N1 influenza vaccine and seasonal influenza vaccine in the last 3 years), clinical data (systolic and diastolic blood pressure, height, weight), laboratory data (fasting blood sugar, hemoglobin A1C% (HbA1C %), total white blood cell count, hemoglobin, serum creatinine, serum sodium, potassium, bicarbonate, total bilirubin, serum albumin, alkaline phosphatase and creatinine phosphokinase) were obtained. BMI was calculated as per standard guidelines [6]. A diagnosis of diabetes mellitus (DM) was made as per American Diabetes Association (ADA) guidelines [7]. Diagnosis of hypertension (HTN) was made as per Joint National Commission (JNC) guidelines [8]. Throat swab (nasopharyngeal swab) was obtained from these 380 participants, stored at 2-4 degree centigrade and tested by RT-PCR for swine influenza A (HINI) as per CDC (Centers for Disease Control) protocol [9]. The study participants were tested only once. Those

who tested positive were followed by weekly visits, for 4 weeks, to the charity clinic and questioned about symptoms of respiratory illness, and evaluated with clinical examinations which included respiratory system auscultations and throat and nose examinations with a standard torch light to see redness of the pharyngeal wall and nasal mucosa. These clinical exams were conducted by the same internist (first author) for all the positive patients. No laboratory testing was conducted during these follow-up weekly visits. To ensure 100% follow-up of people who tested positive, the study physician visited participants who were positive but did not come for weekly follow-up with a social worker at their homes and the above-mentioned exams were conducted at their homes. This ensured 100% follow-up of positive cases.

Statistical analysis

Baseline characteristics of study patients were expressed in number (%) for categorical variables, and as mean ± standard deviation for continuous variables. H1N1 influenza virus infection was coded as a categorical variable. At baseline, participants were categorized as those with H1N1 virus positive and H1N1 virus infection negative. Continuous and categorical variables were compared between these two groups at baseline using students 't' test or chi-square test as appropriate. Then uni-variate and multi-variate logistic regression analysis was performed using presence of H1N1 infection as the dependent variable and age, gender, BMI, diabetes diagnosis, and HbA1C% as independent variables. Since obesity [10] and diabetes mellitus [11] are known to be poor prognostic factors with symptomatic pandemic influenza infection, we included these variables in the logistic regression model to look for their association with asymptomatic carrier state. Though we planned Cox regression on the 4 weeks follow-up data, we could not perform these as none of the participants who were positive at baseline developed respiratory disease during 4 weeks of follow-up.

Results:

The baseline characteristics of the study cohort are detailed in Table 1. The mean age was 42.58 years. 255 participants (67%) were < 60 years of age. Mean BMI was 23.62 kg/m2. 300 participants (79%) had their BMI < 30 kg/m2. Mean HbA1C% was 6%. Screening medical tests detected DM in 62 patients (16.3%) and HTN in 40 (10.5%) patients. Mean values of fasting blood sugar, total white blood cell count, hemoglobin, serum creatinine, serum sodium, potassium, bicarbonate, total bilirubin, serum albumin, alkaline phosphatase and creatinine phosphokinase were within normal limits.

The prevalence of asymptomatic H1N1 carrier state was 10.8% (41 of 380). In uni-variate and multi-variate logistic regression analyses age, gender, BMI, diabetes diagnosis, and HbA1C% were not significantly associated with the odds of having H1N1 influenza virus carrier state (Table 2). During the 4 weeks of follow-up none (0 out of 41) of the positive cases developed respiratory signs and symptoms of infection.

Discussion

Our study observed a significant prevalence (10.8%) of asymptomatic infection due to pandemic H1N1 influenza in a remote rural community of south India. It is interesting to note that this community represents a stable population, the village is self sustained and its inhabitants are predominantly agricultural workers and hence don't move out of the village often. Also, this remote rural village is not regularly visited by people from other areas. Hence, the probability of infection being imported from other areas is minimal. Also, it can be noted that none of the study participants had respiratory illness or had household contact with respiratory illness in the last 30 days (Table 1). Hence, we can infer that the community as a whole had a low prevalence of symptomatic respiratory disease. Also, from Table 1 we understand that none of the study

participants had received any medication for any illness in the last 30 days. Also, none of the study participants had received pandemic H1N1 influenza or seasonal influenza vaccines. Also, the fact that 16.3% and 10.5% of the study cohort had a diagnosis of incident DM and incident HTN (Table 1) proves that this community had been far from the influence of modern medicine. This being the case it is not clear as to why the prevalence of asymptomatic infection due to pandemic H1N1 influenza be so high.

Further, factors such as diabetes [11], obesity [10] and age [12] which are poor prognostic indicators in symptomatic pandemic H1N1 influenza infection were not associated with asymptomatic carrier state in our study (Table 2). In fact none of the patient characteristics were significantly associated with having asymptomatic carrier state (Table 2).

Annual symptomatic seasonal influenza rates and symptomatic pandemic H1N1 influenza rates during the epidemic and the period after that for this village and the surrounding areas has not been reported. Further, similar to the scarcity of data the world over for asymptomatic pandemic H1N1 influenza rates, this data is not known for this village and the surrounding areas.

The relevance of asymptomatic pandemic H1N1 influenza virus carrier state in this population is not clear. None of the 41 positive participants developed symptomatic illness in the 4 weeks of follow-up. It is hard to comment if this observation represents the way an epidemic dies down in the community or it represents the beginning of a new epidemic. Since there are no comparison studies it is hard to arrive at an inference.

Limitations:

Small sample size is an important limitation of our study. This affects precision of our estimates and limits our ability to analyze effect modification due to patient characteristics. Also, we could

not measure antibody titers for pandemic H1N1 influenza virus infection in this population due to financial constraints. This data would have helped us understand immune status and previous infection status in this population. Similarly, we could not measure seasonal influenza antigen and anti-body levels in this population. This data would have helped us compare asymptomatic infection with seasonal influenza and pandemic H1N1 influenza virus infection in this population. Also, it would have been interesting to find out as to how many of the positive cases remained positive at the end of 4 weeks follow-up. This would have shed light on the viral shedding pattern in the community. We could not do this due to financial constraints. Also, we enrolled the first 380 consecutive eligible participants who visited the charity clinic for this study. We might have introduced a selection bias as only the most motivated, health conscious, and healthy individuals are likely to visit the charity clinic first. Also, technical aspects such as single throat swab assessment for H1N1 infection, only clinical assessment during 4 weeks follow-up period to assess development of clinical disease in positive participants, no laboratory confirmation and single observer evaluation (first author) without assessment for reliability might have created a situation where some symptomatic cases might have been missed.

Conclusions:

We observed a significant prevalence of asymptomatic carrier state due to pandemic H1N1 influenza virus in a remote rural community of South India. The relevance of this high prevalence of asymptomatic infection at a time when the global pandemic had been controlled remains unclear.

List of abbreviations:

CDC: centers for disease control, RT-PCR: reverse transcriptase-polymerase chain reaction, HbA1c%: glycosylated hemoglobin, DM: diabetes mellitus, HTN: hypertension, ADA: American Diabetes Association, BMI: body mass index.

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Table 1: Baseline characteristics of the study population

Variables	Study cohort (n = 380)	H1N1 positive group (n = 41)	H1N1 negative group (n = 339)	P value
Age (yrs)	42.58 ± 12.53	40.44 ± 13.69	43.24 ± 12.13	0.212
Males – n (%) Females – n (%)	184 (48.3) 196 (51.7)	17 (41.5) 24 (58.5)	167 (49.2) 172 (49.6)	0.319 0.071
Household contact – n	0	0	0	-
Any medication intake	0	0	0	-
Pandemic H1N1 influenza vaccines (n)	0	0	0	-
Seasonal influenza vaccines (n)	0	0	0	-
DM– n (%)	62 (16.3)	7 (17.1)	55 (16.2)	0.875
HTN- n (%)	40 (10.5)	3 (7.3)	37 (11.0)	0.451
Smoking – n (%)	62 (16.3)	7 (17.1)	55 (16.2)	0.875
Systolic Blood pressure (mmhg)	127 ± 12.5	125 ± 14.7	127 ± 13.9	0.116
Diastolic Blood pressure (mmhg)	78 ± 5.9	77 ± 6.6	79 ± 5.1	0.218
Height (cms)	161 ± 33	159 ± 31	161 ± 37	0.148
Weight (Kgs)	60 ± 8.1	62 ± 6.5	60 ± 7.3	0.204
BMI (Kg/m2)	23.62 ± 4.15	23.24 ± 4.67	24.42 ± 3.97	0.269
Fasting blood sugar (mg/dl)	87 ± 7.6	88 ± 8.1	87 ± 8.3	0.177
HbA1C %	6 ± 0.96	6.12 ± 1.11	5.96 ± 0.91	0.358
Hemoglobin (g/dl)	14.39 ± 1.23	13.94 ± 1.44	14.53 ± 1.13	0.118
Total count (cells/mm3)	6643.75 ± 1780.66	6668.71 ± 1706.29	6635.94 ± 1809.61	0.918
Creatinine (mg/dl)	0.92 ± 0.19	0.92 ± 0.17	0.92 ± 0.19	0.888
Sodium (meq/l)	136.52 ± 3.08	136.17 ± 2.99	136.63 ± 3.11	0.404
Potassium (meq/l)	3.72 ± 0.24	3.7 ± 0.21	3.72 ± 0.25	0.642
Bicarbonate (meq/l)	23.91 ± 1.41	23.88 ± 1.69	23.92 ± 1.33	0.882
Albumin (g/dl)	4.05 ± 0.45	4.05 ± 0.43	4.05 ± 0.46	0.964
Total bilirubin (mg/dl)	0.95 ± 0.15	0.95 ± 0.15	0.95 ± 0.15	0.891
Alk. Phosphatase (IU)	107.51 ± 15.79	108.2 ± 15.65	107.3 ± 15.89	0.752
CPK (IU/l)	179.90 ± 33.23	179.41 ± 37.24	180.05 ± 32.03	0.915

BMI: Body Mass Index
HbA1C: Hemoglobin A1C
CPK: Creatinine Phospho Kinase
DM: diabetes mellitus
HTN: hypertension
Smoking implies current smoking

Table 2: Logistic regression analysis to identify the association between having H1N1 positive and patient characteristics

Variables	Univariate analysis			Multivariate analysis		
	OR	95% C.I	P value	OR	95% C.I	P value
Age	0.98	0.81 – 1.20	0.166	0.96	0.79 – 1.19	0.216
Gender	0.99	0.79 – 1.25	0.371	1.03	0.84 – 1.28	0.170
DM diagnosis	1.03	0.84 – 1.19	0.182	1.01	0.71 – 1.29	0.099
BMI	0.97	0.80 – 1.23	0.296	1.04	0.82 – 1.18	0.167
HbA1C%	1.05	0.88 – 1.29	0.105	1.01	0.78 – 1.22	0.104

OR: Odds Ratio
C.I: Confidence interval
DM: Diabetes Mellitus
BMI: Body Mass Index
HbA1C: Hemoglobin A1C