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Original Article

Microbiology Section

Surveillance of Swine Flu Influenza H1N1 by Chip Based Real Time PCR Technology from the Clinical Specimens in a Tertiary Care Hospital

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ABSTRACT

Introduction: Influenza viruses are one of the most important viruses which have the ability to cause epidemics and pandemics. Swine flu influenza H1N1 pandemic in 2009 has seen an alarming response from all over the world. Since then continuous surveillance is ongoing to find any new swine flu case. Molecular techniques using real time Polymerase Chain Reaction (PCR) technology gained momentum for the identification of these infections.

Aim: The study aims to identify the prevalence of swine flu influenza H1N1 cases by using Truenat H1N1 micro PCR system and to understand the clinical and demographical distribution of cases attending the tertiary care hospital.

Materials and Methods: This was a prospective study done during the period of Jan 2017 to Dec 2017. All the suspected cases for influenza like illness attending our hospital were included in the study. Only samples from category C patients were subjected to laboratory testing for H1N1. Clinical specimens like nasal or throat or nasopharyngeal swabs were collected by nylon swab and transported in the viral lysis medium. Viral nucleic acid detection was done by Truenat H1N1 micro PCR system (Molbio diagnostics, Bangalore).

Results: A total of 205 samples were obtained during the study period. Out of which 15 samples (7.3%) were tested positive for swine flu influenza H1N1 by Truenat H1N1 micro PCR system. Out of these 15 cases five cases died with the case fatality rate of 33.3%. Majority of the samples were from males accounting for 65.3% followed by females 34.7%. Majority of the cases were in the age group of 30-39 year (24.8%) followed by 40-49 year age group (22.9%). Majority of the patients presented with fever (96.5%) followed by cough (75%) and cold (63.9%). Associated comorbid conditions identified were diabetes (18%), chronic kidney disease (5.3%), pre existing lung diseases (6.8%) and pregnancy (2.4%).

Conclusion: Early and accurate detection of swine flu cases is the best way to undertake any interventions in the management of any epidemic or pandemic. In order to do so, molecular techniques like Truenat H1N1 chip based real time PCR technology systems will be extremely helpful in countries like India where testing is necessary especially in the peripheral settings.

Keywords: Chip based assay, Outbreaks, Rapid diagnostic methods, Truenat

INTRODUCTION

Respiratory infections are among the most common infections which affect the human beings. Influenza virus is one of the most important aetiological agents in the causation of these infections [1]. Influenza virus has the potential to cause regular epidemics as well as occasional pandemics with high mortality and morbidity with the greatest number of people being affected during the Spanish flu over a century ago. During the recent times, 2009 swine flu H1N1 pandemic has caused a great concern because of the rapid dissemination of the virus throughout the world [2]. Outbreaks of swine flu H1N1 continues to be a menace in developing countries like India where it is often difficult to diagnose and treat early because of socioeconomic constraints.

Early detection of the these viral infections will help in the prevention of the spread of infections by taking appropriate treatment and following proper infection control practices which in turn decreases the mortality and morbidity and also decreases the economical burden on the health care system. Nucleic acid detection tests by reverse transcriptase Polymerase Chain Reaction (PCR) have been the tools in the diagnosis of these viral infections. These tests need expertise and also is time consuming. In this scenario it has become absolutely necessary that the suspected cases are diagnosed at the earliest for taking further actions. Truenat H1N1 micro PCR assay (Molbio diagnostics) is a chip based real time reverse transcription PCR test for the diagnosis of H1N1 virus from the clinical samples. This test has been developed indigenous in our country and was evaluated by the Premier institutes for its sensitivity and specificity and has been recommended in the healthcare settings [3]. Having supplied by the government to our institute, we have aimed for the early detection of H1N1 and to know the prevalence of these infections in our geographical area by using Truenat H1N1 system.

The aim of the study was:

1. To know the prevalence of swine flu influenza H1N1 cases by using Truenat H1N1 which is a chip based real time PCR technology.
2. To understand the demographics, signs and symptoms, risk factors, comorbid conditions and their association with the clinical illness.

MATERIALS AND METHODS

This was a prospective study done during the period of January 2017 to December 2017 in the Department of Microbiology, Kurnool Medical College, Kurnool, Andhra Pradesh, India. The study was approved and ethical clearance was obtained by the institutional ethical committee. All the suspected cases for influenza

like illness attending our hospital were categorised in to A, B and C categories based on the revised guidelines of Ministry of Health and Family Welfare, Government of India on categorization of seasonal influenza A H1N1 cases [4]. Briefly, category A included patients presenting with mild fever with cough/sore throat with or without bodyache, headache, vomiting and diarrhea. Category B included category A+ high grade fever and severe sore throat and/or high risk groups. Category C included category A or B with one or more of the signs and symptoms of breathlessness, chest pain, drowsiness, hypotension, cyanosis, irritability or worsening of the existing chronic condition.

All the category C patients of all the age groups were isolated in isolation ward and only samples from category C patients (n=205) were subjected to laboratory testing for H1N1 as per the guidelines. All the category A and B patients were excluded from the study. Viral nucleic acid detection was done by Truenat H1N1 micro PCR assay. Clinical specimens like nasal or throat or nasopharyngeal swabs were collected by nylon swab and transported in the viral lysis medium (both supplied by the manufacturer). As soon as the samples were received in the Microbiology department, viral RNA was isolated by Trueprep MAG sample preparation device (supplied by manufacturer). Following the extraction of viral nucleic acid it was then dispensed into a microtube containing freeze dried PCR reagents including reverse transcriptase. After allowing for 20 seconds, the entire contents were pipetted and dispensed in to the Truenat H1N1 chip and the chip was inserted into Truelab Realtime micro PCR analyzer where the RNA was first converted into complimentary DNA and then further thermal cycling takes place. Conserved sequences of H1N1 swine influenza A virus (swH1) haemagglutinin gene and swine influenza A virus (swInfA) nucleocapsid genes were used as targeted sequences (Developed by Bigtec Labs, Bangalore in collaboration with Molbio, Goa) and human RNase P as full process internal positive control. At the end of test run, results were displayed as H1N1 detected or not detected. This whole procedure takes around an hour time. All the confirmed cases were notified to the concerned authorities through proper channel.

STATISTICAL ANALYSIS

Descriptive analysis was used wherever necessary. The positive rate of influenza was compared with regard to age, gender by using chi-square test and p-value [Table/Fig-1,2].

Gender	Total number	Positives	Negatives
Male	134	9	125
Female	71	6	65
Total	205	15	190

[Table/Fig-1]: Statistical analysis of influenza cases-gender wise.
Chi-square with 1 degree of freedom χ^2 -0.20, p-value 0.65 (not significant)

Age (years)	No of cases	No of positives	No of negatives
Less than 20	16	-	16
20-29	37	3	34
30-39	51	7	44
40-49	47	2	45
50-59	38	2	36
60 and above	16	1	15
Total	205	15	190

[Table/Fig-2]: Statistical analysis of influenza cases-age wise.
Chi-square with 5 degree of freedom χ^2 -5.29, p-value 0.38 (not significant)

RESULTS

A total of 205 samples were obtained during the study period. Out of which 15 samples (7.3%) were tested positive for swine flu influenza H1N1 by Truenat H1N1 micro PCR system.

Majority of the samples were obtained during the period of February to April (73 out of 205) and August to October (86 out of 205) indicating bimodal distribution of the disease pattern [Table/Fig-3].

Month	No. of cases	No. of positives
Jan	4	1
Feb	22	1
Mar	34	4
April	17	2
May	9	-
June	3	-
July	5	-
Aug	18	1
Sept	33	3
Oct	35	3
Nov	13	-
Dec	12	-
Total	205	15

[Table/Fig-3]: Month wise distribution.

Majority of the samples were from males accounting for 134 out of 205 (65.3%) followed by females (34.7%) [Table/Fig-4]. But the positive percentage was higher in females (8.4%) when compared to males (6.7%) [Table/Fig-5].

Majority of the cases were in the age group of 30-39 (51 out of 205=24.8%) followed by 40-49 year age group (22.9%). Least number of cases were in the age groups of less than 20 years and more than 60 years. The mean age of the patients in the study was 38.6 and the standard deviation was 13.8.

Highest number of positive cases were in the age group of 30-39 (13.7%) followed by 20-29 years (8.1%). None of the cases were positive in the age group of less than 20 years [Table/Fig-6].

Majority of the patients presented with fever (96.5%) followed by cough (75%) and cold (63.9%) [Table/Fig-7].

All the swine flu influenza H1N1 positive patients presented with high grade fever with or without chills and all were having cough with majority being productive cough. Shortness of breath was observed in majority of the positive cases and bilateral crepts were also observed predominantly [Table/Fig-8].

Associated comorbid conditions were in one fourth of the patients which include diabetes (18%), chronic kidney disease (5.3%) and others. Among the H1N1 positive cases 60% of the patients were associated with type 2 diabetes [Table/Fig-9].

A total of 15 (7.3%) cases were confirmed as swine flu influenza H1N1 by Truenat, out of these five patients died with the mortality rate of 33.3% in positive cases [Table/Fig-10]. Out of these five cases, one patient was postpartum female and one with diabetes, one was having both diabetes and chronic kidney disease as comorbid conditions and the other two patients were not associated with any noticeable comorbid condition.

Age (years)	<20			20-29			30-39			40-49			50-59			60 and above		
Sex	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
No.	10	06	16	21	16	37	39	12	51	32	15	47	20	18	38	12	04	16

[Table/Fig-4]: Age and Gender wise distribution of cases (number of cases).

Sex	Number of cases	Number of positives	Positive percentage
Male	134	9	6.7%
Female	71	6	8.4%
Total	205	15	7.3%

[Table/Fig-5]: Sex wise distribution of cases (n=205).

Age (years)	Number of cases	Number of positives	Positive percentage
Less than 20	16	-	0%
20-29	37	3	8.1%
30-39	51	7	13.7%
40-49	47	2	4.2%
50-59	38	2	5.2%
60 and above	16	1	6.2%

[Table/Fig-6]: Age wise distribution: (n=205).

Clinical presentation	Number of cases (Percentage)
Fever	198 (96.5%)
Cough	154 (75.1%)
Cold/rhinitis/running nose	131 (63.9%)
Sore throat	96 (46.8%)
Dyspnea	40 (19.5%)
Myalgia	87 (42.4%)
Headache	38 (18.5%)
Diarrhea	41 (20%)

[Table/Fig-7]: Clinical presentation of the cases (n=205).

Clinical symptoms	Number of cases (percentage)	Clinical signs	Number of cases (percentage)
Fever	15 (100%)	Altered sensorium	2 (13.3%)
Cough	15 (100%)	Feeble pulse	5 (33.3%)
Cold	11 (73.3%)	Bilateral crepts	10 (66.6%)
Sore throat	5 (33.3%)	Hypoxia	5 (33.3%)
Breathlessness	12 (80%)	Reduced urinary output	7 (46.6%)
Diarrhea	3 (20%)	Hypotension	7 (46.6%)

[Table/Fig-8]: Clinical symptoms and signs of H1N1 positive cases (n=15).

Condition	Total cases (n=205)	H1N1 positive cases (n=15)
Diabetes	36 (18%)	9 (60%)
Chronic kidney disease	11 (5.3%)	1 (6.6%)
Preexisting respiratory diseases	14 (6.8%)	-
Pregnancy/peri and postpartum	5 (2.4%)	1 (6.6%)

[Table/Fig-9]: Comorbid conditions (n=205).

Total number of confirmed H1N1 cases	Deaths (%)
15	5 (33.3)

[Table/Fig-10]: Case fatality rate of confirmed H1N1 cases.

DISCUSSION

After the 2009 pandemic, frequent outbreaks of swine flu cases have been reported in the Indian subcontinent [5,6]. World Health Organization (WHO) emphasised that pandemic virus may continue as seasonal influenza and local outbreaks can be expected in the post pandemic phase after the 2009 pandemic. Between 2010-2015, many laboratory confirmed influenza A (H1N1) cases have been reported in India [6]. Though rapid antigen diagnostic tests and immunofluorescence tests are widely available, they lack the sensitivity and specificity. Viral isolation and nucleic acid amplification tests such as real time PCR are the most reliable diagnostic tests for H1N1 with greater performance [7]. However, they are limited only to centralised labs and needs skilled manpower and infrastructure and also are time consuming. We need a rapid, reliable, cheap and point of care testing methodology with less turnaround time for the detection of suspected cases even in the peripheral settings. Truenat micro PCR system is one such system developed by Bangalore based Molbio company for the purpose of detecting infectious diseases like tuberculosis, malaria, swine flu influenza H1N1 etc., in the health care settings. This technology has been evaluated extensively and was found satisfactory [8,9]. It is a chip based technology where single sample can also be tested with accurate results within one and a half hour time. This user friendly system enables decentralisation and near patient diagnosis of H1N1 in resource poor settings.

In the present study a total of 205 suspected cases of influenza cases were tested for swine flu influenza H1N1 by truenat micro PCR system where 15 samples came as positive for H1N1. Our results were similar to the results of Nandhini G et al., where 12.7% of the samples were positive for pandemic H1N1 influenza virus though the percentage of positivity varied from year to year from 2009 to 2013 [5]. Other studies from India have shown different positivity rates from 7% to 27% [10-13].

In the present study, maximum number of cases were males and younger population with patients between 20-40 years was affected more. Around half of the cases screened were younger population and among the confirmed H1N1 cases 10 out of 15 (66.6%) were in this age group. Similar findings were observed in other studies. In Siddharth V et al., study 81.4% of the affected population was younger population below the age of 40 years and majority of them (56.48%) were males [14]. In a study from Maharastra conducted by Gurav YK et al., the most affected age group was 20-39 years [15].

Majority of the cases presented with symptoms of fever (96.5%) followed by cough (75.1%) and cold. All the H1N1 positive cases presented with fever and cough and majority of them (80%) were having shortness of breath. On auscultation, crepts were noted in majority of the positive cases. In Nandhini G et al., study also fever was the most common symptom (98%) followed by cough (85%) [5]. Similar findings were also observed in Choudhry A et al., study [16].

Five out of the 15 confirmed H1N1 cases died in the present study with a case fatality ration of 33.3%. In Siddharth V et al., study the case fatality rate was 25.49% which is similar to this study [14] where as in Nandhini G et al., study the mortality rate was 7.6% [5]. This high mortality rate may be attributed to various reasons including study population age, occupation, geographical location, underlying comorbid conditions, socioeconomic status etc.

In the present study, diabetes was the most commonly observed comorbid condition especially in confirmed cases with 60% of the positive cases being diabetic. Other underlying conditions like pregnancy, chronic kidney diseases, pre existing lung diseases like asthma, COPD were also observed in some patients. Similar findings were observed in other studies [5,17,18]. Swine flu when associated with the other comorbid illness can be devastating with great mortality as observed in the present study where three out of five deaths were associated with underlying comorbidity.

LIMITATION

Present study is limited only to the detection of pandemic swine flu H1N1 from the clinical samples. Other circulating/seasonal influenza viruses have not been accounted. This is a small study done in a single centre and the results may not be generalised.

CONCLUSION

Majority of the cases of Swine flu influenza H1N1 in the studied geographical location have been reported between February to April and August to October. Comorbid conditions like diabetes, chronic kidney disease and pregnancy were associated with increased mortality in swine flu cases.

Truenat H1N1, a chip based molecular test for detection of swine flu influenza H1N1 helps in the rapid diagnosis of the swine flu cases. User friendliness of the instrument allows less expertise which can be utilised even in the peripheral settings. This in turn helps in the rapid diagnosis and better management of the patients.

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