ORIGINAL ARTICLE

Clinical Profile and Detection of Novel H1N1 Influenza Virus in Children by Reverse Transcription Polymerase Chain Reaction at a Tertiary Care Center

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ABSTRACT

Background: Influenza is among the most important reasons of hospitalization and mortality in children. Compared to other age groups, children are excessively affected by influenza A (H1N1) pandemic. This study was planned to detect and study the clinical profile of H1N1 virus using real-time reverse transcriptase polymerase chain reaction (rRT-PCR) in pediatric patients. **Materials and Methods:** The H1N1 virus was detected in nasopharyngeal or throat swabs in 510 children suspected with influenza such as illness, using rRT-PCR assays as recommended by the World Health Organization and Centers for Disease Control and Prevention protocol. Patients' biodata and clinical history were recorded. **Results:** Out of 510 suspected influenza cases, majority (61.9%) were males and of 1–5 years' age (44.5%). H1N1 was positive in 100 (19.6%) cases. 6–10 years' age group was most affected (24.1% H1N1 positive). Clinically, all children had flu-like illness. **Conclusions:** The present study revealed that the 6–10 years' age group children were highly susceptible to H1N1 infection. Therefore, early intervention results in good outcome in terms of reduced requirement of invasive ventilation and decreased mortality rate.

KEY WORDS: Child, H1N1 subtype, humans, influenza A virus, reverse transcriptase polymerase chain reaction, virus diseases.

Introduction

Influenza is a very common, highly transmissible viral respiratory tract infection that usually causes yearly epidemics in most parts of the world with its peak prevalence being in winter.[1] The infection can present with a wide-ranging clinical features, from mild symptoms such as pyrexia, cough, and headache, to severe complications such as pneumonia, acute respiratory distress syndrome, or even death. Young children, elderly persons, immunocompromised patients, and pregnant females are more likely to develop such complications.[2] The novel influenza A (H1N1) Influenza virus caused a huge disease outbreak of swine flu in 2009 which was declared pandemic by the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC).[3] In India, Hyderabad was the first to report a laboratory-confirmed case of H1N1 on May 16, 2009, while Rajasthan, the largest state of India, reported its very first case on July 23, 2009.[4]

Children are the most susceptible population for Influenza and are most likely to transmit this infection. Especially the school going children aged 5–17 years are mainly affected during annual flu outbreaks.[5] Furthermore, highest deaths from Influenza are reported among children and elderly patients.[6] Influenza is commonly a vaccine-preventable viral infection. However, currently available vaccines induce only a short lived narrow immunological response.[7]

Influenza virus is an enveloped virus which contains a single-stranded ribonucleic acid (RNA) genome and

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belongs to the Orthomyxoviridae family of viruses. It displays not only genetic variations as a result of its segmented genome but also surface antigenic variation in both hemagglutinin and neuraminidase proteins. Hence, due to antigenic drifts and shifts, it can infect a wide range of hosts including humans, birds, domestic and wild animals and cause potential pandemics. Hence, this study was planned to detect and study the clinical profile of H1N1 virus using real-time reverse transcriptase polymerase chain reaction (rRT-PCR) in pediatric patients.

Materials and Methods
This study was done at virology research laboratory, Dr. Sampurnanand Medical College, Jodhpur, India, after ethical clearance from Institutional Ethics Committee. Children (1 month to 16 years) with suspected influenza-like illness were included in the study. Informed consent was obtained from parents/ legal guardians. Swabs were collected from throat and nasopharynx and sent to virology laboratory in a viral transport medium as per standard prevalent guidelines. Patients' biodata and clinical history were also recorded.

Sample processing and virus detection
The RT-PCR protocol was as per the WHO guidelines.[8] Sample processing was done in a negative pressure chamber Biosafety Cabinet as per the CDC standards.[9] RNA extraction was done from samples by column based RNA kit (QAIamp®, Germany). Primers and probes (Thermo Fisher Scientific, USA) were CDC approved. rRT-PCR machine (Applied Biosystems) was used with a PCR kit (2× master mix; Superscripts™ III enzyme mix; and primer-probe mix for H1N1). Template setup was done according to the PCR kit manufacturer's (Applied Biosystems') instructions.

Statistical analysis was performed using Microsoft Excel 2016 and SPSS® (Statistical Package for the Social Sciences) version 21.

Results
Out of 510 suspected cases of influenza reported during the study period, 100 (19.6%) were positive for H1N1. Among total cases majority ($n = 316$, 61.9%) were males whereas other were ($n = 194$, 38.1%) females.

Table 1 shows the age- and gender-wise distribution of H1N1 cases. Most of the suspected children were

in the 1–5 years' age group ($n = 227$, 44.5%) while the mean age was 5.94 years. Maximum percentage ($n = 19$, 24.1%) of H1N1 positive cases was observed in the 6–10 years of age group.

Geographically, the maximum number of cases was reported from Jodhpur, Western Rajasthan (57.25%). Details of geographical distribution are shown in Figure 1.

Following symptoms were observed in suspected patients in the decreasing order of frequency: Fever (94%), cough (88%), breathlessness (75%), nasal discharge (70%), sore throat (68%), coryza (60%), vomiting (22%) body ache (17%), headache (12%), and diarrhea (10%), and ear discharge (5%). In contrast, nasal discharge and cough were prominent in positive influenza cases. Following symptoms were observed in positive H1N1 patients in decreasing order of frequency: Fever (96%), breathlessness (80%), cough (78%), nasal discharge (77%), coryza (66%), sore throat (48%), body ache (20%), vomiting (19%), headache (15%), diarrhea (15%), and ear discharge (6%).

Comparison of clinical features of suspected and H1N1 positive patients is shown in Figure 2.

Discussion
Influenza still remains a common cause of respiratory tract infection which seriously threatens pediatric health. While most children show mild illness with flu-like symptoms, some may develop severe pneumonia and/or neurological problems that can be fatal. So early identification of influenza virus is of paramount importance to minimize child mortality. In our study 19.6% os suspected
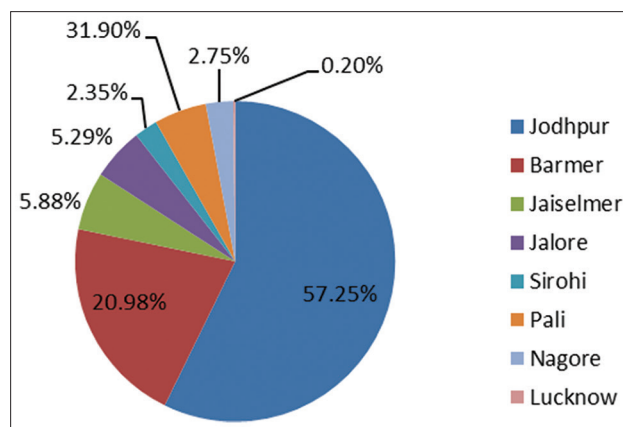
Figure 1: District wise H1N1 positive cases in percentage

Table 1: Age- and gender-wise distribution of H1N1 cases

Age group (in years)	No. of suspected cases	No. of H1N1 positive cases (%)	Male		Female	
			Positive	Negative	Positive	Negative
0–<1	77	17 (22.1)	14	36	03	24
1–5	227	41 (18.06)	28	114	13	72
6–10	79	19 (24.1)	09	35	10	25
11–16	127	23 (18.1)	15	65	08	39
Total	510	100 (19.6)	66	250	34	160
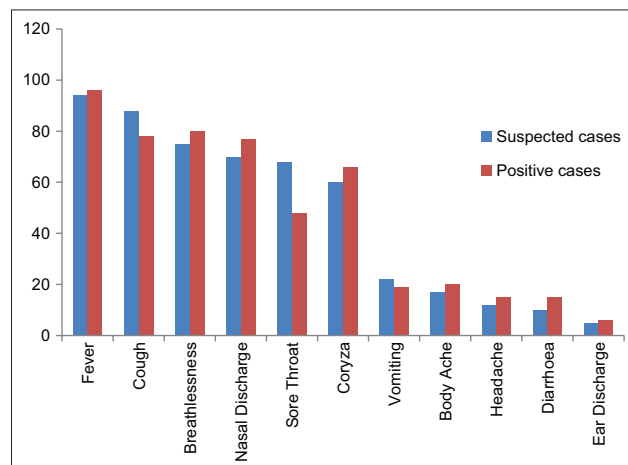
Figure 2: Clinical features of suspected and positive H1N1 cases in %

children were found positive for H1N1 which was in accordance to Li *et al.*[10] and Tresoldi *et al.*,[11] while Mandal *et al.*[12] observed higher positivity rate (47.5%). We found that majority of patients (44.5%) were of 1–5 years' age and were males; similar findings were reported by Pushpalatha *et al.*[13] and Mishra *et al.*[14] We observed that maximum H1N1 positivity (24.1%) was found in the 6–10 years of age group, presumably because they go to schools and hence have a transition in environmental exposure. Chaitanya *et al.*[15] showed highest H1N1 positivity (66.6%) in 1–6 years' age groups of children.

Infantile infection by Influenza virus is usually more frequent in 6–12 months' age group compared to 0–6 months of life, mainly because of circulating maternal influenza antibodies acquired either through placenta or breastfeeding.[16]

We observed that the foremost common clinical features of H1N1 Influenza were fever, cough, breathlessness, rhinorrhea, and coryza. Similar findings were suggested by Mehta *et al.*[17] and Prakash *et al.*[18] However, various studies have

reported that amount of rainfall, relative humidity temperatures differences influence the Influenza outbreaks.[19]

Limitations of the study

We did not include neonates in our study as the sampling is difficult in this age group. As we used convenient sampling method, all the geographical areas could not be represented in our study population. We included only symptomatic cases; hence, asymptomatic carriers could not be detected.

Conclusions

The present study revealed that children belonging to 6–10 years' age group were most vulnerable to H1N1 infection, and common symptoms were fever, cough, breathlessness, rhinorrhea, and coryza. Therefore, early interventions may result in better outcomes in terms of reduced requirement of invasive ventilation and decreased mortality rate. It is of great significance to develop strategies for the prevention and control of Influenza by measures such as social distancing, and educating school children about health and hand hygiene.

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