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Research Article

MOLECULAR DETECTION OF HUMAN RHINOVIRUS IN RESPIRATORY SAMPLES OF SWINE FLU NEGATIVE NORTH INDIAN CHILDREN WITH FLU-LIKE ILLNESS

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ABSTRACT

Objectives: Flu-like illness may also be caused by different respiratory viruses other than influenza. Human rhinovirus (HRV) shows almost flu-like symptoms. The purpose of this study is the molecular detection of HRV in throat swab of swine flu negative North Indian children during the years 2012 and 2013. Reverse transcriptase (RT) - polymerase chain reaction (PCR) amplification of 5'non-coding region (NCR) was used for HRV detection followed by cell culture isolation of HRV.

Methods: PCR confirmed swine flu negative throat swab samples were collected from the Department of Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India. The RNA isolation of samples was done using the QIAamp®Viral RNA Mini Kit (Qiagen), followed by single step RT-PCR amplification (AgPath-ID, Life Technologies). All PCR positive HRV samples were cell cultured in HeLa and HEp-2 cell lines for viral isolation.

Results: 135 swine flu negative throat swab samples were examined. Out of which 34 samples (25.2%) were found HRV positive by RT-PCR, while only four samples (11.8%) were culture positive on HeLa cell line. Younger children (0-4 year) were found more susceptible to HRV infection. This study indicated the highest prevalence of HRV (37.0%) during the months (September-October) of the Autumn season in 2012 and 57% in Winter-spring season (February-March) during 2013.

Conclusion: HRV may be a cause of flu-like symptoms in swine flu suspected North Indian children with a higher rate during Autumn and Spring season. Molecular detection of HRV using RT-PCR is more sensitive than cell culture assay.

Keywords: Human rhinovirus, Swine flu, Influenza-like illness, Lower respiratory tract infections.

INTRODUCTION

Human rhinoviruses (HRVs) association has been seen with severe respiratory illness in upper respiratory as well as lower respiratory tract infections (LRTIs) including influenza-like illness [1]. LRTIs have become the main cause of hospitalization and death of children under 5 years in developing countries [2]. These HRVs are also known as a major cause of common cold among children and immunocompromised patients. Pneumonic and asthmatic children are more susceptible to HRV infection with high mortality rates [3-5]. Symptoms may include a sore throat followed by sneezing, rhinorrhea, nasal obstruction, runny nose, headache, in infants. Sometimes HRV infections lead to muscle weakness, muscle aches, fatigue, malaise, and loss of appetite. Fever is usually common. The complication of HRV infection has been seen with otitis media, sinusitis, chronic bronchitis, and exacerbations of reactive airway disease [6,7]. These HRVs belonging to family Picornaviridae are small, non-enveloped virus with single-stranded, positive-sense RNA genome of 7.2-8.5 KB in length [8]. On the basis of cell-culture and antisera detection, two groups HRV-A, HRV-B, and 101 serotypes are identified. However, recently using polymerase chain reaction (PCR) technique, a different group HRV-C is newly identified concern to many new HRV strains [8,9]. These viruses mainly cause upper respiratory tract infection, but involvement is also found in LRTI [8]. Molecular detection of all prototypes of HRVs has not been completely applied with clinical samples [4,10]. Cell culture for HRV has several limitations as they are typical slow growers and the observation of the cytopathic effect (CPE) of HRV identification takes several days, resulting in poor sensitivity of cell culture assay [11,12]. More than 100 serotypes are in existence that's why serological diagnosis is not possible [11]. According to several studies, reverse transcriptase (RT)-PCR is more sensitive than cell-culture [8,13-17]. Here, we used a 5'non-coding region (NCR) based RT-PCR method for rapid detection of HRV in clinical samples [5].

In previous studies, HRV is found a potent pathogen which causes up to 5% LRTI in children [18] and found associated with 42% of children with influenza-like illness [19] but the involvement of HRV in flu-like symptoms among swine flu negative infants and young children has not been studied previously in North India. The goal of this study is to analyze the prevalence of HRV in North Indian children found swine flu negative in 2012-2013.

METHODS

Sample collection

The PCR confirmed swine flu negative throat swab samples of North Indian children during the year 2012-2013 were collected from the Department of Microbiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.

Viral RNA isolation

The RNA Isolation of samples was done using the QIAamp®Viral RNA Mini Kit (Qiagen) as per protocol provided with the kit.

Molecular detection by RT-PCR

RT-PCR was done by using 5 µl of extracting viral nucleic acid and one-step RT-PCR Kit (AgPath-ID, Life Technologies), as per the manufacturer's instructions. The forward primer 5'-GGGACCAACTACTTTGGGTGTCCG-3' and reverse primer 5'-CACGGACACCCAAAGTAGT-3' were used to amplify a region within the 5'NCR as previously described [6]. The conditions during the single step PCR were as follows- reverse transcription for cDNA synthesis at 50°C (30 minutes), Initial denaturation 95°C (10 minutes), followed by 40 cycles of denaturation at 95°C (30 seconds), annealing at 55°C (30 seconds), and elongation at 72°C (45 seconds). Resulting in amplified fragment, approximately 400 bp in length confirmed in agarose gel electrophoresis.

Cell culture

Monolayer of HeLa and HEp-2 cells were grown separately in cell culture flasks (Corning), using the minimal essential medium (Sigma-Aldrich) supplemented with Earle's salts, L-glutamine, Penicillin-Streptomycin (Sigma), and 10% Fetal Bovine Serum (Gibco-Life Technologies). Subconfluent HeLa and HEp-2 cell monolayers were washed with Dulbecco's phosphate buffered saline (PBS) (Sigma) three times, and then inoculated with filtered (0.22 µm filter by Millipore), PCR confirmed HRV positive throat swab samples. After 45 minutes at room temperature, the cells were washed with PBS, overlaid with 2% MEM, then moved to an incubator (35°C, 5% CO_2), under observation for 5 days [20]. The passage was done for confirmation of CPE.

Statistical analysis

All statistical analysis was done using GraphPad Prism software, Version 5.01. (GraphPad Software, Inc., USA)

RESULTS

In this study, molecular detection of HRV in swine flu negative samples was done by RT-PCR amplification of 5'NCR region. Among 135 samples, 34 samples (25.2%) were HRV positive (Fig. 1). 55.9% of total HRV positive children were between 0 and 4 year age group. 35.3% children were 5-9 years old while 8.8% HRV positive children were 10-14 years old (Fig. 2). All PCR positive samples were found cell culture negative in HeLa and HEp-2 cell lines in primary inoculation, while only four samples (11.8%) showed CPE in HeLa cell line in the first passage (Table 1 and Fig. 3). The positive rate of HRV was found highest (37%) during Autumn season (September-October) in 2012 and (57%) in Winter-Spring season (February-March) during 2013 (Fig. 4).

DISCUSSION

In clinical samples, detection of HRV has increased with the improvement of molecular techniques [7,16], providing the evidence of HRV association with LRTI including influenza-like illness [21]. The molecular method based on the 5'NCR has been used previously for rapid detection and typing of all serotypes of HRV [6]. HRVs have been successfully cultured on HeLa cell lines [22]. However, in this study, the cell culture rate of HRV was found very low, and only four samples (11.8%) were cultured successfully. A recent study showed 26% prevalence of HRV among children under 5 years of age, hospitalized with flu-like symptoms [14,23].

The HRV infection occurs around the year, but during Autumn (September-November) and Spring (March, April) seasons, the incidence of HRV associated infections increased up to 80% with flu-like symptoms [24]. In a previous study, the prevalence of HRV found 15.4% by RT-PCR method during 2000-2006 in Beijing with highest positive results 32.61% in September 2004 and 35.3% in February 2005. Among these HRV positive patients, 44.8% were under the age of 1 year [25]. However, the association of HRV in flu-like symptoms among swine flu negative infants and young children has not been studied previously in North India. In a flu-like illness, clinical specimens are tested for common respiratory viruses such as Influenza A and B, parainfluenza 1, 2, 3, and RSV, while HRV may also play a role in flu-like illness in influenza suspected children.

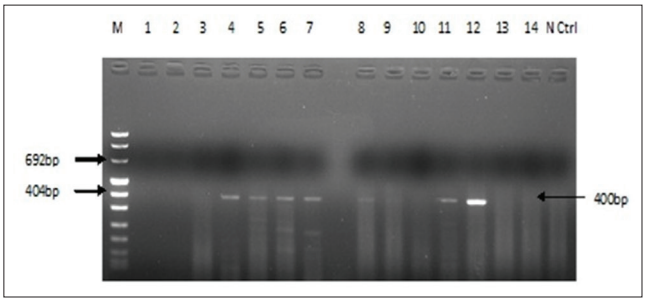

Fig. 1: Agarose gel image showing polymerase chain reaction amplification of the 5' non-coding region (NCR) region of viral RNA. Lane M: Marker 1000 bp. Lane 4 to 7 and Lane 11 to 12: Amplified 5' NCR region of 400 bp

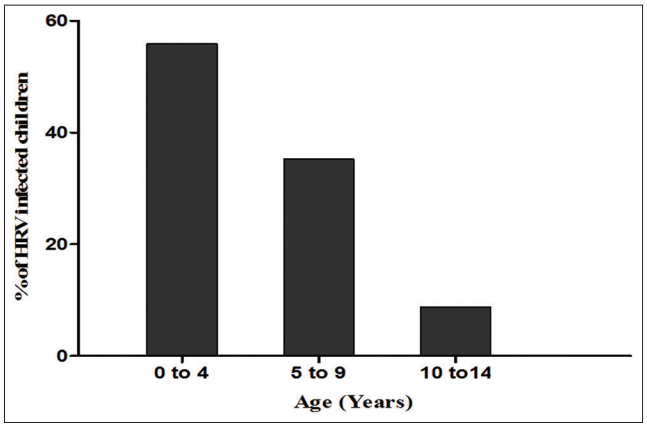

Fig. 2: Percentage of human rhinovirus infected children with respect to their age groups

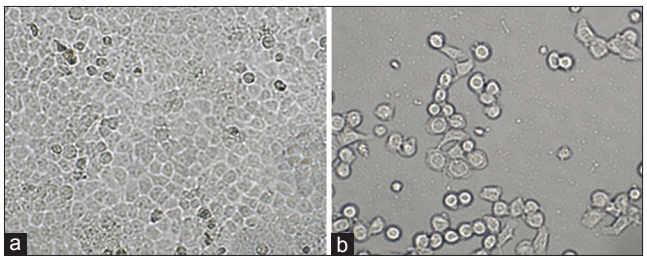

Fig. 3: (a and b) Cell line images (40× magnification). (a): HeLa cell line (Control), (b): Inoculated HeLa cells showing cytopathic effect in the first passage

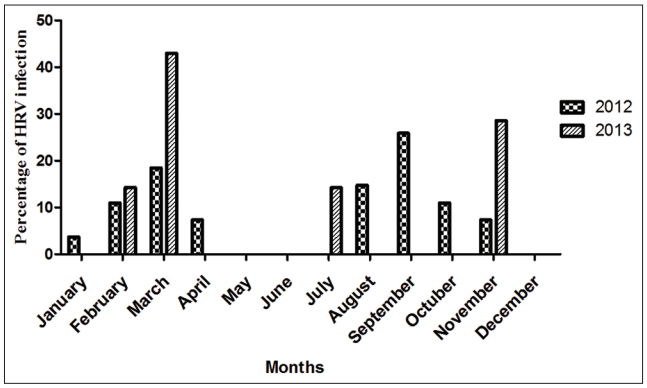

Fig. 4: Percentage of human rhinovirus infection in different months of years 2012-2013

Table 1: Cell culture result of PCR positive HRV samples inoculated in cell lines

Serial number	Cell lines	Total number of PCR positive HRV samples inoculated on cell lines	Cell culture results	
			Primary inoculation	First passage
1	HeLa	34	All negative	Four positive
2	HEp-2	34	All negative	All negative

PCR: Polymerase chain reaction, HRV: Human rhinovirus

CONCLUSIONS

HRV may be a cause of flu-like symptoms in swine flu suspected North Indian children with a higher rate during Autumn and Spring season due to rapid fluctuation in climate conditions. RT-PCR is rapid and sensitive molecular method for diagnosis of HRV in clinical swab samples. Younger children under the age of 5 years are more susceptible to HRV infection. The cell culture of HRV is not very sensitive for isolation of all HRV strains.

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