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Short communication

Genotype circulation pattern of human respiratory syncytial virus in Iran

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ABSTRACT

In order to have information on the molecular epidemiology and genetic circulation pattern of human respiratory syncytial virus (HRSV) in Iran, we studied the genetic variability of both group A and B HRSV strains during seven consecutive years by sequencing the hypervariable C-terminal domain of G protein. A total of 485 children <2 years of age who were negative for influenza viruses, screened for the presence of HRSV in this research. HRSV was detected in 94 (19.38%) of the samples using nested RT-PCR. Group A viruses were isolated during each year, while group B viruses were isolated during 2009 and 2013. Phylogenetic analysis showed that all HRSV group A viruses belonged to three genotypes: GA1, GA2, GA5 and the group B viruses were in BA genotype.

1. Introduction

Human respiratory syncytial virus (HRSV) is the most important cause of lower respiratory tract infection (LRTI) in infants (Rebuffo-Scheer et al., 2011; Papenburg et al., 2012). Primary HRSV infection usually affects children between 6 weeks and 2 years of age and reinfection could occur throughout the life (Cane, 2011; Tran et al., 2013). Approximately 1–2% of all RSV-infected children require hospitalization which increased in children with underlying disorders (Simon et al., 2007).

HRSV has a ssRNA genome with 10 genes encoding 11 proteins. It has three surface glycoproteins, namely the small hydrophobic (SH), the fusion (F) and the attachment (G). The G protein is a type II glycoprotein consisted of 289–299 aminoacids and responsible for virus attachment. It is the most variable protein between HRSV genotypes. This protein has two hypervariable regions. The region in C-terminal domain which named second variable region shows overall G protein variability and is suitable for molecular epidemiology (Peret et al., 1998; Peter, 2007). HRSV isolates, based on the variability of the G protein, were divided into two antigenic groups, A and B which consisted of related strains. Nucleotide sequencing of the G gene have shown up to 20% amino acid variability within group A and 9% in group B strains (Cane et al., 1991; La Montagne, 1997). HRSV group A consisted of 11 genotypes: ON1, GA1-GA7, SAA1, NA1 and NA2 and HRSV group B has been clustered into following genotypes: BA1-BA10, GB1-GB4 and SAB1-SAB3 (Houspie et al., 2013; Aamir et al., 2013).

Different studies showed that group A and B isolates co-circulate during the most of the epidemics, but usually group A predominates to group B (Tsutsumi et al., 1988).

A better understanding of the strains prevalence shift and antigenic distribution patterns of HRSV infections in the community could be helpful for the prediction of future outbreak subgroups and for the development of useful antiviral therapy or vaccines. The preliminary reports (Faghihloo et al., 2011a,b) described the molecular epidemiology of HRSV from 2007 to 2009 in Iran. In this study we completed and continued the molecular epidemiology and circulation pattern of HRSV during seven successive years from 2007 to 2013 in Iran.

2. Materials and methods

2.1. Patients and definitions

This study was performed in National Influenza Center (NIC) located at virology department, School of Public Health, Tehran University of Medical Sciences from late 2007 through early 2013. The total of 985 children was enrolled in this research. Inclusion criteria were the following: 2 years old age or younger, the children which either received medical attention at an outpatient pediatrics ward or were hospitalized at a pediatrics center because of influenza like illness (ILI) disease and the parents written informed consent.

2.2. Sample collection

Throat swab specimens obtained from the patients with ILI were maintained in a viral transport medium. These swabs with

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patient's information sheets were placed into a Styrofoam container with ice packs and transported to the NIC for influenza surveillance.

2.3. Sample testing

Nucleic acids were isolated from all throat swab samples with the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. Initially all samples were tested for RNase P (an endogenous control) and influenza viruses by SuperScript III Platinum One-Step Quantitative RT-PCR system (Invitrogen, USA). Samples which were negative for influenza viruses underwent for HRSV testing. Nested RT-PCR was performed with primers for HRSV G gene described by Sato et al. (2005). HRSV groups A and B were detected by a strain-specific RT-PCR test (Peret et al., 1998). Thermocycling conditions are available upon request. To check the amplification process to eliminate the presence of carryover contamination, negative and positive controls were run in each PCR.

Sequencing was performed on all original HRSV positive samples using an ABI automatic DNA analyzer (Life Technologies) and the ABI BigDye Terminator V3.1 cycle sequencing kit (Life Technologies) according to the manufacture's instructions.

We performed multiple sequence alignment with the Clustal W program using bioedit version 7.2.5 software. Phylogenetic trees were constructed by using of the neighbor-joining method with MEGA5.2 software.

All sequences were submitted to GeneBank under accession numbers KF171889-KF171947, HM063447-HM063470, GQ259155, GU339396-GU339408.

2.4. Statistical methods

The results were analyzed by using PASW statistics 18 software.

3. Results and discussion

3.1. Patient characteristics and epidemiology

The preliminary reports of this research consisted of 179 specimens with 38 HRSV positive samples under accession numbers HM063447-HM063470, GQ259155, GU339396-GU339408 were described the molecular epidemiology of HRSV in Iran from 2007 until 2009 (Faghihloo et al., 2011a,b). The current study was the complete report of the genotype circulation and molecular epidemiology of HRSV detected from 94 (19.38%) children less than 2 years old in seven consecutive years from November 2007 until April 2013.

HRSV can spread via close contact with respiratory secretions, is highly transmissible and detectable during the cold months of the year. In temperate climates, outbreaks usually occur in the late fall, winter or early spring but not in the summer (Reiche and Schweiger, 2009). During the summer months the less indoor crowding might be one reason for the limited spread of the virus. In this research a continuous activity of HRSV was detected from November to April with a big peak in March and December.

A total of 85 out of 94 patients with HRSV infection (90.43%) were hospitalized (76 HRSV-A, 9 HRSV-B) and all 9 (9.57%) outpatient children were HRSV-A.

About the gender distribution some studies found that boys are more susceptible than girls (Sangaré et al., 2006; Oliveira et al., 2008). In our research, the majority of HRSV positive children were boys (57/94). The mean age was 10 months (Table 1).

3.2. Circulation and distribution of HRSV genotypes

By using nested RT-PCR and partial sequencing of G protein, HRSV strains with different genotypes were detected. Among HRSV positive specimens, 85 (90.43%) were group A and 9 (9.57%) were group B. Group A viruses were isolated during each year, while group B viruses were isolated during 2009 and 2013. The phylogenetic dandrograms based on the nucleotide sequences of the G gene are shown in Fig. 1. Analysis with neighbor-joining tree showed that all HRSV group A viruses belonged to three genotypes: 52 (61%) viruses to genotype GA2, 32 (37.60%) to GA1 and one isolate to GA5.

GA2 was predominant from 2007 until 2008. In 2009 GA1 was detected and GA2 reappeared in 2010 until 2012. During these years only one GA5 was detected in 2008. Circulation of GA1 was started in 2011 with GA2 until 2013 which GA1 was the predominant subgroup. All 9 isolates of group B clustered in genotype BA with the 60 nucleotide duplication in the C terminal region of the G protein which 8 of them was detected in 2009 with GA1 and the last one was detected in 2013 again with GA1 circulation.

Many studies performed the analysis of the G gene for detection of different genotypes and the data showed that very similar virus genotypes have been circulating worldwide. Antigenic analysis of HRSVs during 11 epidemics in UK reported that each epidemic had different genotypes which could be due to the combination of separate epidemics (Cane et al., 1994) and the findings were similar to the research from Seoul, Korea (Choi and Lee, 2000). In one study by Waris an alternating predominance of A and B group viruses were detected in Finland during 2 years (Waris, 1991). The similar study in USA reported different prevalence of group A and B viruses during 15 years from ambulatory and hospitalized children (Hall et al., 1990). During our study both A and B antigenic groups co-circulated with predominance of group A which is consistent with some published studies by Zhang et al. that showed the predominance of group A virus strains circulation but there are some studies which showed the HRSV-B as the dominant group (Zhang et al., 2007). This suggests that HRSV has varied circulation pattern in different seasons and geographic regions. These variations in G protein may result in antigenic changes, the host immune system evading, reinfections and effective spread of new strains (Etemadi et al., 2013). This hypervariabilty and being target for protective and neutralizing antibodies are the reasons for sequence study of

Table 1
Demographic details of suspected and confirmed HRSV cases from 2007 to 2013 in Iran.

Epidemic season	No. samples	Median age (months)	No. of HRSV infections	Median age of HRSV infections	November total/RSV	December total/RSV	January total/RSV	February total/RSV	March total/RSV	April total/RSV
2007	29	13	3	11	2/–	3/–	5/–	7/1(GA2)	10/2(GA2)	2/–
2008	36	14	10	11	6/2(GA2)	4/1(GA2)	10/4(GA2)	5/1(GA5)	11/2(GA2)	–/–
2009	46	12	24	10	3/–	8/6(GA1)	12/5(GA1&BA)	10/5(GA1&BA)	10/7(GA1)	3/1(GA1)
2010	53	13	14	11	5/–	22/6(GA2)	10/–	6/1(GA2)	10/7(GA2)	–/–
2011	75	10	15	9	5/1(GA2)	11/5(GA2)	12/2(GA2)	25/4(GA2)	13/2(GA2)	9/1(GA1)
2012	108	14	19	13	18/4(GA1)	22/5(GA2)	23/2(GA2)	27/2(GA2)	10/3(GA2)	8/3(GA1)
2013	138	15	9	12	10/–	34/5(GA1)	28/2(GA1)	33/1(GA1)	31/1(BA)	2/–