Frequent detection of enterovirus D68 and rhinovirus type C in children with acute respiratory infections

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BRIEF REPORT

Frequent detection of enterovirus D68 and rhinovirus type C in children with acute respiratory infections

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Abstract

This study aimed to evaluate the prevalence of human rhinoviruses (HRVs) and the emergence of enterovirus D68 (EV-D68) in children. A total of 322 nasopharyngeal swab samples were provided from children with an initial diagnosis of upper and lower respiratory tract infections. A total of 34 and 70 cases were positive for EV-D68 and HRV, respectively. The phylogenetic analysis revealed that the clades A and B are the prevalent genotypes for EV-D68 and the HRV-positive samples belong to three types including HRV-A, HRV-B, and HRV-C. The results showed that EV-D68 and HRV-C are circulating in Iran especially in the winter.

Keywords Human rhinoviruses · HRV · Human enterovirus D68 · EV-D68 · Emerging disease · Genotyping

Enterovirus D68 (EV-D68) is a subtype of enterovirus D comprising a blend combination of enterovirus (EV) and rhinovirus (RV) features. It was first isolated in the USA from respiratory specimens obtained from 4 pediatric patients with pneumonia and bronchiolitis [1]. The phylogenetic analysis revealed the predominant prevalence of clade A strains from August 2006 to August 2011 and emerging clade B strains in October 2011 in China [2]. EV-D68 can cause severe respiratory symptoms, which mainly affects the upper and lower respiratory tracts of children [3, 4]. After first detection of EV-D68 in 1962 [1], it was neglected because of negligible outbreak. However, its recent worldwide outbreaks led to attract a new attention [5–15]. Earlier reports show the severity of this infection in children in a way that can even cause death. Human rhinoviruses (HRV) are picornaviruses classified within the same genus (*Enteroviruses*) due to their high sequence homology. Until now, three HRV groups including HRV-A, HRV-B, and HRV-C have been known that can cause respiratory infections, especially in vulnerable groups such as infant, aged, and immunocompromised patients [16]. Considering the common biological characteristics between EV-D68 and HRV, the epidemiological study, frequency of HRV- and EV-D68-infected patients, and

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Table 1 Clinical characteristics associated with EV-D68 and HRV infections in children

Clinical Features	All	EV-D68	HRV
Male	170 (53%)	19 (56%)	32 (46%)
Female	152 (47%)	15 (44%)	38 (54%)
Age	< 24 months Male: 100 (31%), Female: 110 (34%) 24–60 months Male: 52 (16%) Female: 60 (19%)	< 24 months Male: 13 (38%) Female: 11 (32%) 24–60 months Male: 6 (18%), Female: 4 (12%)	< 24 months Male: 20 (29%) Female: 24 (34%) 24–60 months Male: 12 (17%) Female: 14 (20%)
Mild symptoms (fever, cough, sneezing, runny nose, and sputum)	Male: 90 (28%) Female: 99 (31%)	Male: 12 (35%) Female: 7 (21%)	Male: 20 (29%) Female: 21 (30%)
Severe symptoms (shortness of breath, fever, cough, sneezing, runny nose, and sputum)	Male: 80 (25%) Female: 53 (16%)	Male: 7 (21%) Female: 8 (23%)	Male: 12 (17%) Female: 17 (24%)

phylogenetic analysis have not been performed in Iran. In this study, for the first time, we explored the epidemiology of EV-D68 among pediatric patients with acute respiratory. In addition, the prevalence of HRV and its different types were studied.

The nasopharyngeal swab samples were collected from 322 children (0.5–60 months) with the initial diagnosis of lower respiratory tract infections (LRTIs) including bronchitis, bronchiolitis, pneumonia, and upper respiratory tract infections (URTIs) upon admission to the Children's Hospital (Bahrami Hospital, Tehran, Iran) from January to December 2018. The nasopharyngeal swab samples were stored at − 80 °C until use. The informed consent was obtained from parents or legal guardians. This study was approved by ethical committee of Tehran University of Medical Sciences. QIAamp Viral RNA Mini Kit (Qiagen, Germany) was used for RNA extraction according to the manufacturer's instructions. To detect EV-D68 and HRV from the other respiratory infections, the PANEV primers (PANEV-F: 5′-AGCCTGCGTGGCKGC-3′ and PANEV-R: 5′-GAAACACGGACACCCAAAG-3′) and probe (5′-FAM-CTCCGG CCCCTGAATGYGG CTAA-BHQ1-3′) were used. Briefly, 6 μl of RNA was added to a real-time RT-PCR mixture (Qiagen, Germany) in a total volume of 25 μl. Two differently nested RT-PCRs were performed on positive cases by real-time RT-PCR. The VP1 region of the genome of EV-D68 was amplified with specific primers and one-step RT-PCR mixture (Qiagen, Germany) as follows: 1st PCR: F1 (2325): 5′-GGRTTCATAGCAGCAAAAGATGA3-′, R1 (3121): 5′-TAGGYTTCATGTAAACCCTRACRGT-3′ and 2nd PCR: F2 (2547): 5 ′-AAGCCATACAAACT CGCACRGT-3′, R2 (2772): 5′-AGTTGTGAGTATGG TRAYTTCAGCA-3′. The PCR product length of first and second rounds of PCR were 831 and 250 base pairs, respectively. The 5′-UTR region of the HRV genome was amplified with the one-step RT-PCR reaction mixture (Qiagen, Germany) and the following primers: 1st PCR: F1 (OL 26): 5′-GCACTTCTGTTTCCCC-3′, R1 (OL 27): 5′-CGGACACCCAAAGTAG-3′ and 2nd PCR: F2 (OL 26): 5′-GCACTTCTGTTTCCCC-3′, R2 (OL 200): 5′-GGCAGCCACGCAGGCT-3′. The amplicon length for the first and second rounds of PCR were 390 and 175 base pairs, respectively [17, 18]. All positive PCR products were sequenced by 3130 Genetic Analyzer (Applied Biosystems). All sequences obtained in this study have been submitted to

Table 2 The infection chance of EV-D68 and three HRV subtypes were determined for cases with mild and severe respiratory symptoms. The p value < 0.05 was considered significant.

Viruses	Odds ratio (p value)
HRV-A vs. HRV-B	1.25 (n.s)
HRV-C vs. HRV-B	3.05 (0.02)
HRV-C vs. HRV-A	3.81 (0.03)
HRV-C vs. EV-D68	0.9 (n.s)
EV-D68 vs. HRV-A	3.49 (0.01)
EV-D68 vs. HRV-B	4.36 (0.02)

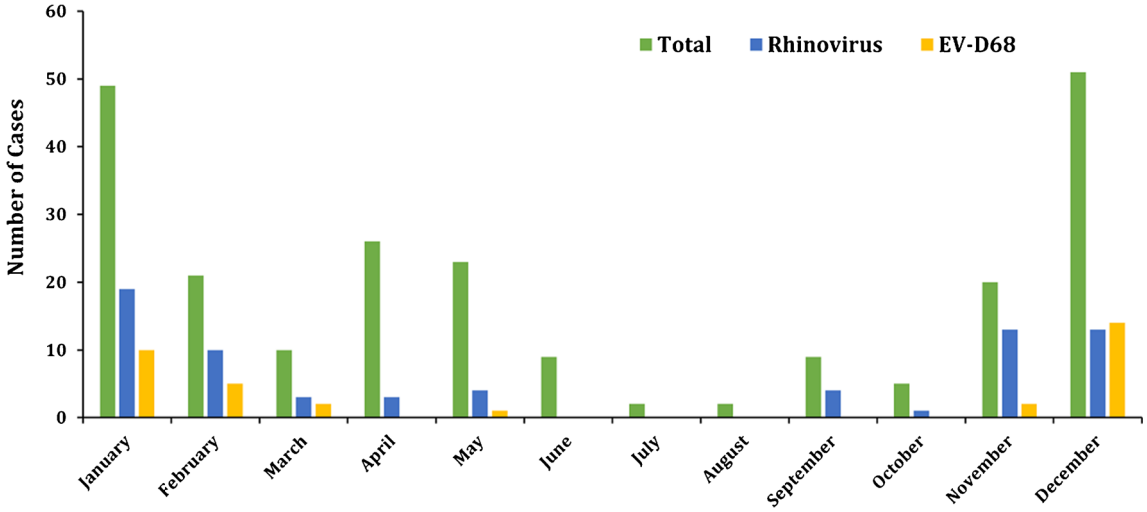

Fig. 1 The distribution of EV-D68 and HRV in different months from January to December 2018

NCBI's GenBank sequence database. The accession numbers MH250142-MH250148 and MH356731-MH356744 were assigned for partial VP1 gene sequences of EV-D68 and 5′-UTR sequences of HRV, respectively. The VP1 gene and the 5′-UTR sequences were utilized to identify the EV-D68 and HRV genotypes, respectively. The phylogenetic analysis was conducted using maximum likelihood method by MEGA software (version 7, http://www.megasoftware.net) and a bootstrap value of 1000. Statistical analysis was carried out using the SPSS statistics software package (SPSS 16). The chi-square test was used to compare the variables. P values < 0.05 were considered statistically significant.

Of the 322 samples, 170 (53%) cases were male and 152 (47%) cases were female. The percent of patients with age less than 24 months was 65% (210 cases) and the age between 24 and 60 months was 35% (112). Also, 189 and 133 cases showed mild respiratory symptoms and severe respiratory symptoms, respectively (Table 1). Totally, EV-D68 and HRV were detected in 104 samples, of which 34 samples were recognized as EV-D68 and 70 samples were found as HRV. A significant difference (p value = 0.001) was observed between the age groups with the EV-D68 infection; however, such meaningful difference did not observe in the HRV samples. The number of patients with mild symptoms was higher than patients with severe symptoms. There were no remarkable differences between male and female groups in terms of clinical symptoms in both HRV and EV-D68 infections (Table 1). EV-D68 and HRV were followed throughout all seasons of the study. The sharp peaks were observed in the winter and then autumn. These observations disclosed the preferable prevalence of HRV and EV-D68 in the cold seasons (Fig. 1). The phylogenetic analysis based on viral protein (VP) 1 region of EV-D68 displayed three major clades, A, B, and C (Fig. 2). The clades A and B were found as the prevalent genotypes. Another phylogenetic tree was constructed according to 5′-UTR region of HRV, in which three groups including A, B, and C were specified for Iranian isolates (Fig. 3). The infection chance of EV-D68 and three HRV subtypes were determined for cases with mild and severe respiratory symptoms as mentioned in Table 2. The results showed the higher infection chance of HRV-C and EV-D68 compared with HRV-A and HRV-B in patients with severe respiratory symptoms than those with mild respiratory symptoms. [10, 19–22] In this survey, the incidence of EV-D68 was 10.5% in children with respiratory infection that were similar to the previous reported in Denmark and Norway [19, 23]. The prevalence of HRV varies from 10 to 60% depending on the duration of the study in different populations. In this study, 21% of nasopharyngeal specimens had positive results for HRVs, which is similar to 21% prevalence among infants less than 36 months old in France [24]. Moreover, our results showed that types of HRV-C and HRV-A

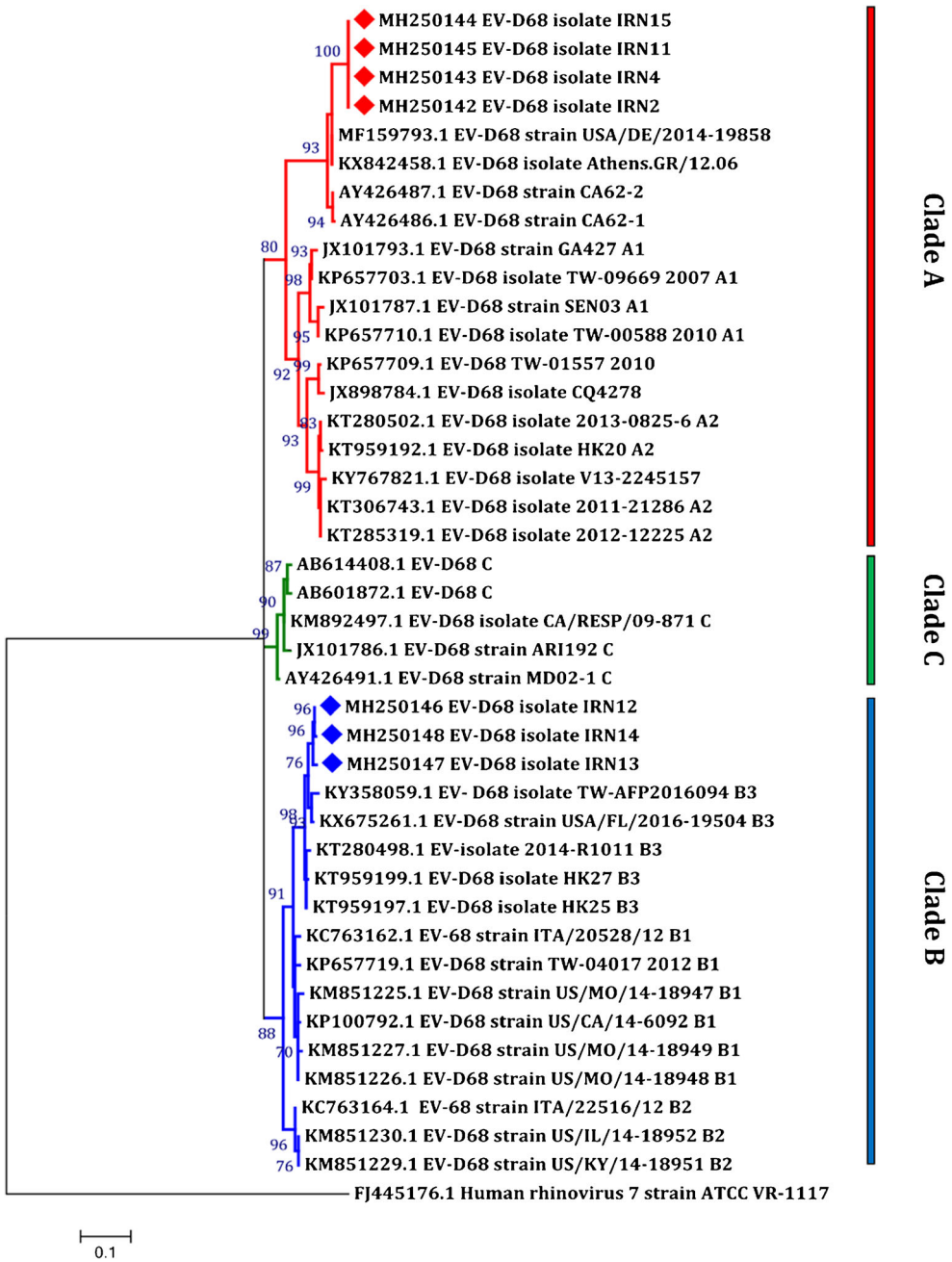

Fig. 2 Phylogenetic tree of seven Iranian EV-D68. The tree was rooted with human rhinovirus 7 strain ATCC VR-1117 (accession number FJ445176.1); the percentage of replicate trees in which the associated taxa cluster together in the bootstrap test (1000 replicates) is presented next to the branches; the red and blue diamonds represent the 2 different genotypes of Iranian isolates

may be more dominant in our population and related to higher hospitalization rates. In addition, the percentage of HRV-C associated with severe clinical symptoms was higher than other HRV types (46%).

In conclusion, this study is the first epidemiological report regarding to the EV-D68 infection of the Iranian children with the age lower than 5 years. Since, there is no antiviral drug or vaccine for EV-D68 and HRV-C, the epidemiological investigation of these viruses in different seasons will help the healthcare organization to make proper strategies for prevention of further outbreaks in the future.

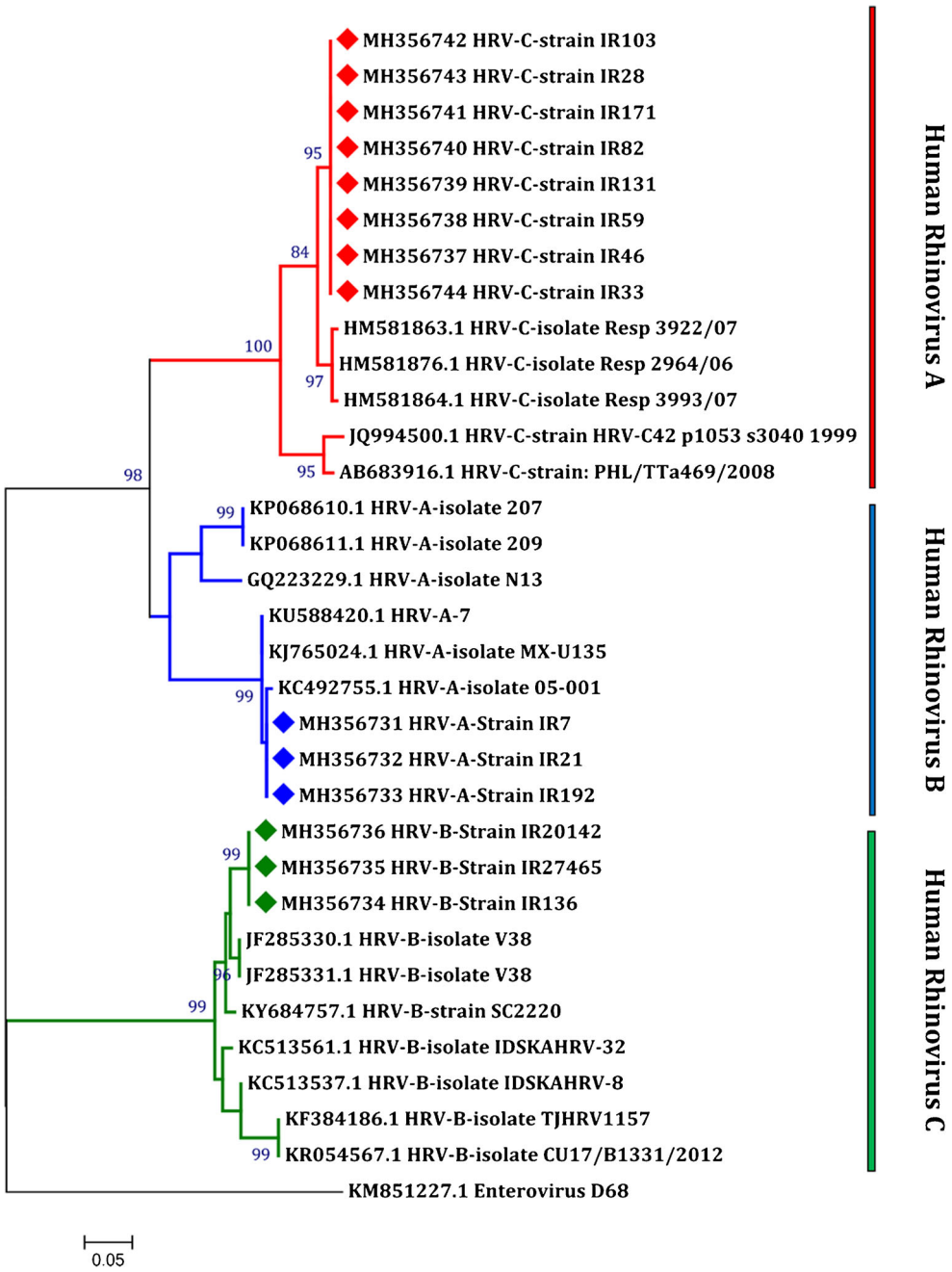

Fig. 3 Phylogenetic tree analyses of Iranian rhinovirus isolates. All the Iranian isolates clustered with types A, B, and C reference genotypes. All of the reference sequences are represented by their accession number. Enterovirus D68 (accession number KM851227.1 enterovirus D68) was used as an out-group

Authors' contributions SHM, MZGh, MK, and FR performed bioinformatics and statistical analysis. SHM, MZGh, and FR interpreted and wrote the manuscript. FR, NM, HN, VS, TMA, SAN, and NGh performed experiments. NM contributed with patient samples. FR obtained study funding. FR and TMA supervised the study. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article. In addition, all sequences obtained in this study have been submitted to NCBI's GenBank sequence database and accession numbers MH250142-MH250148 and MH356731-

MH356744 were assigned for partial VP1 gene sequences of EV-D68 and 5′-UTR sequences of HRV, respectively.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate This study was approved by ethical committee of Tehran University of Medical Sciences.

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