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Short Communication

Phylogenetic Analysis of Human Astrovirus Infection among Children Suffering from Gastroenteritis Referred to Aboozar Hospital, Ahvaz, Iran

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One of the most common illnesses that have its great impact on children and the elderly is acute viral gastroenteritis (1). After rotavirus human astrovirus may be the second most common cause of viral gastroenteritis in children (2, 3). Human astrovirus (HAstV) is a member of Astroviridae family which contains a single genus, Astrovirus. These viruses are non-enveloped with single stranded positive sense RNA genome (4). The genome of astrovirus is about 7 kb in size and consists of three ORF: ORF1a and ORF1b which encode non structural proteins and ORF2 which encode the precursor of capsid protein (5). All eight known human astrovirus serotypes belonging to the first identified human astrovirus species (HAstV) (6). A second species of astrovirus was found in a child with diarrhea and named AstV-MLB (7). In our best knowledge, genotypes and the strains of HAstV circulating in Iran have not been reported. In our previous study, the relative frequency of astrovirus, seasonal distribution and the patients age range in children suffering from gastroenteritis was reported (8), the aim of the study was to understand the phylogenetic distribution of human astrovirus strains in children suffering from gastroenteritis.

Fecal specimens were taken from children less than five years old with gastroenteritis, referred to Ahvaz, Aboozar hospital. Diagnosis tests to determination of parasitic and microbial infection were done and negative specimens were kept at -80°C. Hundred eighty four specimens were tested for the presence of astroviral infection. Extraction of viral RNA from fecal suspensions and RT-PCR procedure was performed (8), and for clean-up and Nucleotide sequencing, ten out of 29 detected HAstV were selected to analyze the circulating strains pattern. The positive samples were sequenced using the PRISM 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA). The nucleotide sequences obtained from ORF1a were aligned with HAstV sequences from GenBank database by using the CLUSTAL X program (version 1.83). Phylogenetic analysis was done by the Maximum Likelihood method in MEGA software (version 5.2.2), with Kimura-two parameter model. All HAstV sequences were submitted to the GenBank database under the following accession numbers: JX171281 to JX171290.

The phylogenetic analysis revealed that most of Ahvaz human astrovirus strains reported in this study had little divergence amongst them in selected region of ORF1a. Nine of them were grouped in to HAst8 taxon in a clade

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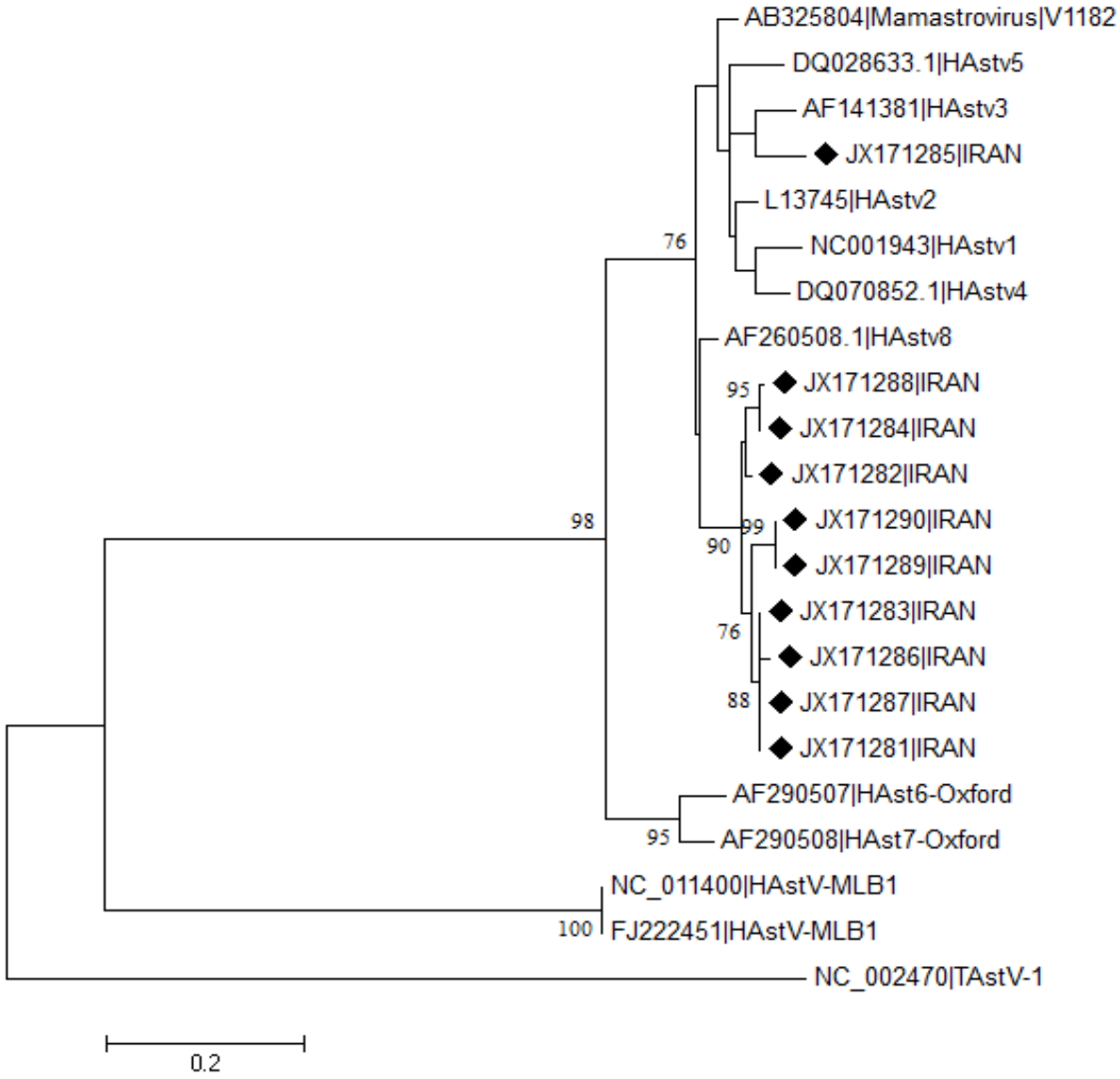


Fig. 1. Phylogenetic relationship of human astroviruses detected in Ahvaz and other control genotypes for the 289 bp amplicon within the conserved domain of ORF1a of their genome. Corresponding sequences of HAstV isolate V1182 (AB325804) for the genus Mamastrovirus and AstV-MLB1 (NC_011400 and FJ222451) were included to show a comparison of evolutionary distances and relationships. Turkey astrovirus TAstV-1 (NC_002470) was used as an out-group.

(Fig. 1). One of our samples (JX171285) was grouped in to HAst3 taxon. Geographical analysis showed that nine of our samples clustered together with close proximity to strains from Kolkata (AB126670, AB191789) and Seoul (AY962542). The Seoul (AY962543) is another control strain in this clade. One more of our strain (JX171285) was near to HAst/3/ Oxford (AF290504) and HAst/5/ Oxford (AF290506) taxa. Two strains from Korea (AY027809, KS106203), were close to Iran (JX171285) in this clade. Korea (KS106210, KS106209), HAst/7/Oxford (AF290508), HAst/6/Oxford (AF290507), clustered in to distinct lineage respectively (Fig. 2).

From our previous studies (8, 9), it is clear that astrovirus is an important viral etiological agent having a significant role in infantile gastroenteritis, other than rotavirus in Ahvaz.

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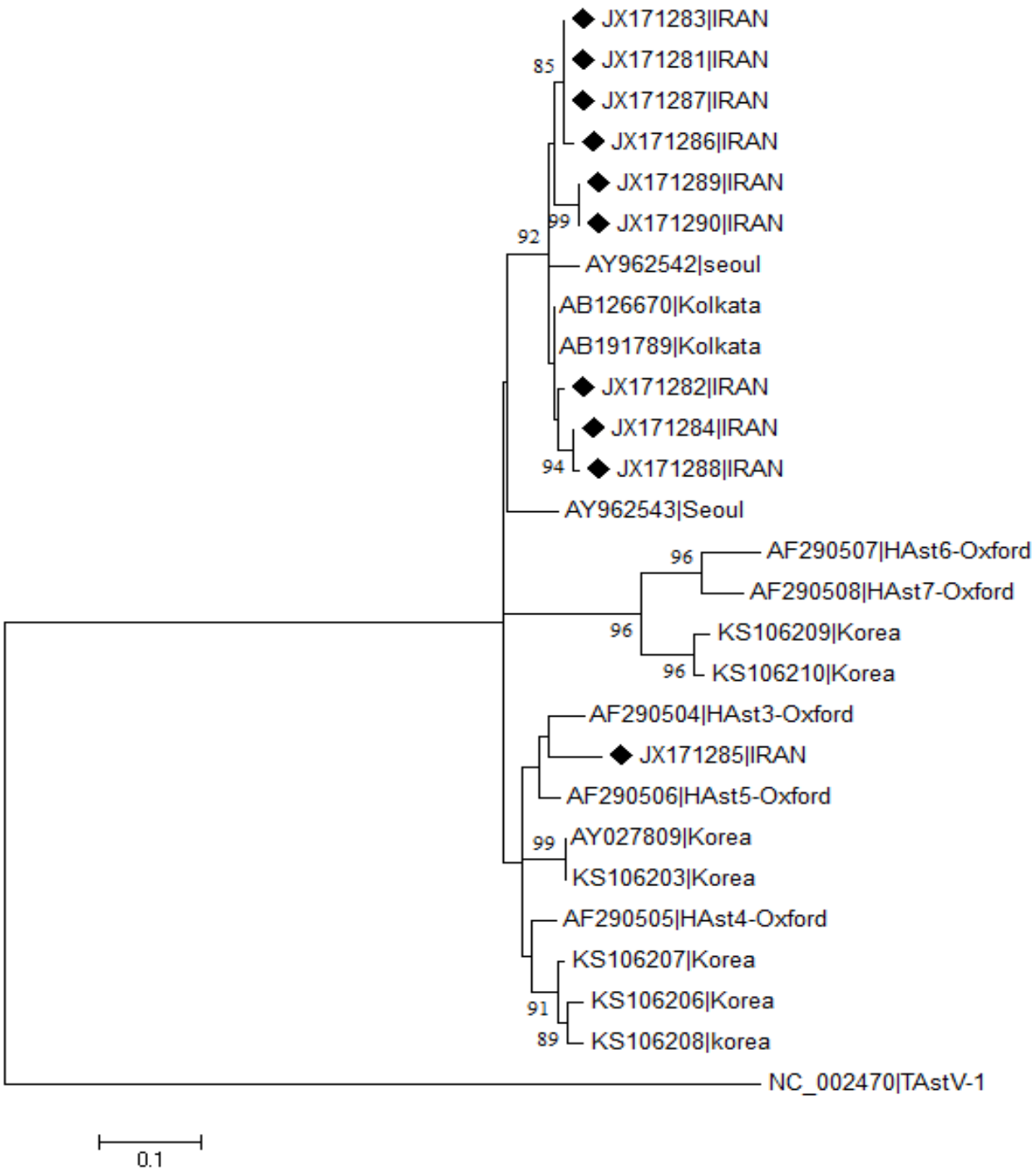


Fig. 2. Geographical distribution of human astrovirus detected in Ahvaz and other control strains for the 289bp amplicon within the conserved domain of ORF1a of astrovirus genome. Turkey astrovirus TAstV-1 (NC_002470) was used as an out-group.

The phylogenetic tree which was constructed based on different astrovirus isolates of different genomic groups and Turkey astrovirus TAstV-1 (NC_002470) as an out-group, showed all but one isolate belong to genotype 8. However, these sequences were grouped in to a separate sub cluster with bootstrap value of 90, indicating that a majority of Iranian sequences might be grouped in to another yet unsigned new

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genomic group. One isolate ((JX171285) was grouped in to genotype 3. In previous study (8) we reported that this isolate belongs to genotype 4, but this study revealed this sample was grouped in to genotype 3. According of a study that was done by Bhattacharya et al to determine molecular epidemiology of human astrovirus infection in Kolkata, with specific primers to the conserved region of ORF1a, maximum homology to the Seoul strain (AY962542) was observed (10). In this study nine of ten samples were in the same lineage to the Kolkata strains and this result may indicate similarity between viruses circulating in these two different zones of area with the Seoul strain. One of our samples: (JX171285) is near to HAst/3/ Oxford (AF290504) and HAst/5/ Oxford (AF290506) in the same taxa, and according of the study that was done by Bhattacharya et al: this two (AF290504 and AF290506) were in the same taxa (10). It can be said that human astrovirus strain (JX171285) has relative identity (%92.2) in sequence identity matrix to Oxford strains (AF290504). Altogether the Iranian sequences showed a homological pattern in the tree that indicating a slow recent evolution compared to the other isolate in the tree. In conclusion this study was partly determines the phylogenetic distribution of HAstV strains, in children suffering from gastroenteritis referred to Aboozar hospital, Ahvaz, and may be useful for epidemiological study, control and prevention of HAstV gastroenteritis.

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