Abstract

Non-polio enteroviruses (NPEVs), known to cause a variable spectrum of infections in humans from subclinical to fatal, are implicated in the classic manifestation of acute flaccid paralysis (AFP). These viruses belong to the genus *Enterovirus* of the family *Picornaviridae* and contain a positive-sense, single-stranded RNA of 7.5 kb. Flanked by two highly structured non-coding regions (NCRs) at the 5' and 3' ends, a single large open reading frame (ORF) of the genome encodes four structural (VP1, VP2, VP3 and VP4) and the seven non-structural (2A, 2B, 2C, 3A, 3B, 3C and 3D) viral proteins.

Isolation of NPEVs from AFP cases has been frequently reported in polio surveillance programs (PSPs) conducted worldwide. However, there is lack of limited understanding of the characteristics of the NPEV infections in different geographical/meteorological zones, and the genomic variations among strains of the same NPEV genotype related to the diverse infection sequelae, from no symptoms to minor febrile illness to irreversible paralysis.

This study was conducted to understand the characteristics of NPEV infections among AFP cases, investigated during PSPs in 2009–2010, in different geographical/meteorological zones of south-western India. NPEV cell culture isolates were derived from the stool specimens that were collected from 422 of 2186 AFP cases (<1-14 years age) and 17 of 41 asymptomatic contacts. The isolates along with the details of all AFP cases/contacts were obtained from National Polio Laboratory (NPL), Bangalore. The distribution of NPEV infections among AFP cases and circulation pattern of NPEV strains were determined by statistical analysis of the data. Genotyping of all NPEV isolates was carried out by partial VP1 gene sequencing and phylogenetic analysis.

NPEV positive AFP cases were significantly higher in children aged < 2 years; with residual paralysis; in summer months; and in the regions with relatively hot climate (p-values: <0.0000001–<0.05). Genotyping of NPEVs
identified predominance of enteroviruses (EV)-B species [81.9% - Echoviruses (E): 57.3%; coxsackieviruses (CV) B: 15%; numbered EVs: 8.9%; CV-A9: 0.7%] and lower levels of EV-A [14.5% - CVA: 6%; numbered EVs: 8.5%] and EV-C [3.6% - CVA: 2.6%; numbered EVs: 1%] species, encompassing 63 genotypes. EV-A76 (6.3%) and each of E3, CV-B3 and E9 (~5%) were found frequently during 2009 while E11 (6.7%), CV-B1 (6.1%), E7 (5.1%) and E20 (5.1%) were detected commonly in 2010. Infections with E/numbered EVs were higher than that found with CVA/CVB in AFP cases from children aged < 2 years; presenting with fever; and from north and south interior parts of Karnataka state. All types detected in asymptomatic contacts, except EV-B69, were found in association with AFP.

The full-length genomes of CV-A24 strains, detected in four paralytic cases, were described for the first time in this study.

A phylogenetic tree constructed on the basis of entire genomes displayed topology similar to that of the full-VP1 tree, classifying the study strains in genogroup CV-A24vGIV along with their temporal counterparts in strains from non-paralytic cases. The strains of the study formed a single genetic cluster C4 within CV-A24vGIV indicating 3.5-19.4 % nucleotide sequence divergence, with 2-4 novel nucleotide mutations in the 5'NCR and 3-8 unique amino acid substitutions in the polyprotein, with respect to the CV-A24 strains associated with non-paralytic cases. Only one unique nucleotide mutation, A299U, was identified in the 5'NCRs of all of the study strains. However, these mutations are too few to classify the AFP-associated strains of this study in a genogroup different from that of the strains circulating in non-AFP cases from close or distant geographical regions.

CV-B3 strains of EV-B species, detected in 10 AFP cases and 5 asymptomatic contacts, were considered for complete genome sequencing and characterization to examine the genomic variations, phylogeny, recombination and secondary structure of the 5'NCR of the viral strains.
Phylogenetic analyses of complete VP1 gene sequences of global CV-B3 strains classified Indian CV-B3 strains into genogroup GVI along with south-central Asian strains and a new genogroup GVII. Genomic divergence between the genogroups of study strains was 14.4%, with significantly lower divergence (1.8%) within GVI (n=12) as compared to that (8.5%) within GVII (n=3). The strains, from both AFP cases and asymptomatic contacts, identified mainly in coastal Karnataka and Kerala, belonged to the dominant genogroup GVI while the GVII strains were recovered from AFP cases in north interior Karnataka. All study strains carried inter-genotypic recombination, with the structural region similar to reference CV-B3 strains and 5'NCR and non-structural regions closer to other Enterovirus-B types. The domain-II structures of 5NCRs, described to modulate the virus replication, were predicted to have varied structural folds in the two genogroups, and attributed to differing recombination patterns.

In summary, this study highlights the extensive genetic diversity and diverse circulation patterns of NPEV strains in AFP cases from different populations and climatic conditions. This study has revealed a notable genomic diversity (3.5–19.4%) including a unique nucleotide mutation (A299U) in the 5'NCR of all of the AFP-associated CV-A24v study strains differentiating them from other known CV-A24 strains from non-AFP cases. The findings of the study reveal distinct genomic composition of CV-B3 strains circulating in different geographical regions of India. This study suggests concurrent analysis of viral and host factors, to further understand the varied manifestations of these infections.