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Molecular epidemiology and genetic variability of respiratory syncytial virus (RSV) in northern India (2013-15) with emphasis on novel RSV A ON1 genotype

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Background: Respiratory syncytial virus (RSV) in infants and young children is considered as a major pathogen of lower respiratory tract causing significant mortality and morbidity. Due to genomic diversity RSV possesses several genotypes and because of highly variable 'G' glycoprotein, in alteration in the epitope site might alter the pathogenecity. The novel RSV A ON1 genotype with 72nt duplication in G gene, replacing the circulating NA1, has been documented recently in several countries including India. The aim was to carry out phylogenetic analysis of circulating RSV strains isolated from hospitalized children with ALRTI in a tertiary care hospital, North India.

Material/methods: Nasopharyngeal samples from 194 hospitalized children suffering from ALRTI collected in between 2013 December to March 2015 were screened for the presence of RSV by reverse-transcriptase PCR. Second hypervariable 'G' gene region of RSV (n=20) was amplified and sequenced for phylogenetic analysis. Sequences were aligned with Clustal X2.1 and divergence at the level of nucleotide and amino acid was evaluated using MEGA6.0 software. Construction of phylogenetic tree for RSV A, RSV B and sub-classification of RSV A ON1 type and RSV BA type was done using Maximum likelihood model with 1000 bootstrap replicates by MEGA 6.0 software. Potential N-glycosylation and O-glycosylation sites were predicted using NetNGlyc 1.0 and NetOGlyc 3.1.

Results: Seventy-six (39.18%) children were classified as ALRTI, 82 (42.3%) severe ALRTI and 36 (18.6%) very severe ALRTI. Mechanical ventilation as a part of IPPR was required for 18 patients. RSV RNA was detected in 75 (38.7%) children. Of the 20 RSV strains sequenced, 11 belonged to the recently discovered RSV A ON1 genotype with 72 nucleotide G gene duplication resulting in an increase of 24 amino acids in antigenic site and 2 belonged to RSV A NA1 ancestor genotype. Among 11 patients infected with RSV ON1 genotype, patients belonged to ALRTI, severe ALRTI and very ALRTI are 3, 7 and 1 respectively. Phylogenetic analysis shows that ON1 genotype has largely replaced RSV A GA2 and NA1 genotypes from northern India. Upon further classification of highly divergent RSV ON1 genotype, 6 strains belonged to lineage 1 and 5 strains under lineage 3. RSV genotype BA subtype 9 with 60bp duplication was found to be also circulating in 7 study strains.

Conclusions: Acquisition of the 72 nucleotide duplication appeared to have improved the fitness of this virus, thereby novel RSV A ON1 strains may need to be evaluated for their efficacy, so that their inclusion in the future vaccines may be warranted