

Prevalence and Molecular Characterization of Circulating Respiratory Syncytial Virus (RSV) in Chennai, South India during 2011-2014

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The objective of the study was to determine the prevalence and genotyping of RSV A and B in Chennai, Tamilnadu, south India. Human Respiratory Syncytial Virus (RSV) is one of the most important respiratory viruses causing acute respiratory tract infections amongst children. Based on genotyping of the attachment glycoprotein (G) gene, it is divided into two groups, RSV-A and RSV-B. We tested 850 samples from patients with influenza like illness (ILI) and severe respiratory illness (SARI) during the period 2011-2014 for RSV using a conventional RT-PCR and found 124 (14.5%) samples to be positive for RSV. Further sub typing by nested PCR in which 84(10%) were RSV A, 40 (4.5%) were RSV B, suggesting that RSV-A was the predominant group circulating in south India during the study period. Among patients with RSV infection, is 47.5% were in less than 1 year age group, 29.8 % were between 1 to 5 year age group, 14.5% were between 6 to 14 years age group and 8 % were above 14 years age. Phylogenetic analysis all RSV-A sequences belonged to ON1 within NA1 genotype and is ON1 with in NA1 genotype and RSV B sequences belonged to the genotype BA9 and BA12.

Key words: Respiratory Syncytial Virus, RSV infections in Chennai, RT-PCR for RSV.RSV Genotyping in India.

Acute respiratory tract infection (ARI) is the leading killer among children in the world with 1.9 million deaths per year occurring in developing countries¹. One fourth (2.5 millions) of the total deaths among children less than 5 years of age occur in India and approximately 20% of these are due to ARTIs²⁻⁴. Other than that bacterial infection, Influenza virus and Human respiratory syncytial virus (HRSV) have been identified as the predominant etiological agents for lower

respiratory tract infections⁵⁻⁷. About 20-40 % of the children are hospitalized due to RSV with ARI in India⁸⁻¹⁰. Human respiratory syncytial virus (RSV) is the second most frequent cause of respiratory infections, and the main cause of severe cases of bronchitis and pneumonia in infants and young children¹¹. RSV has been considered as an important viral infection, there is no antiviral treatment and effective vaccine prophylaxis is currently available against to RSV infections^{12,13}.

The RSV has been classified in the Pneumovirus genus of the family Paramyxoviridae, it is an enveloped virus with negative sense single

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stranded RNA genome that encodes for ten proteins²²⁻²⁴. Glycoprotein (G) and Fusion protein (F) are most immunogenic proteins that are expressed on the virion surface, responsible for inducing production of neutralizing antibodies^{14, 18, 21-23}. Both within and between the major RSV subgroups, the G protein is the most changeable viral protein with minimal conserved ectodomain that contains 2 hypervariable regions (HVRs). The second HVR (HVR2) brings the C terminus of the protein and is commonly sequenced to examine the genetic variability of Human respiratory syncytial virus strains within a given population²⁵⁻²⁷.

The RSV has been classified into two antigenic subgroups RSV-A and RSV-B respectively, initially on the basis of the reactivity of the virus with monoclonal antibodies directed against the attachment G protein^{14-16,22,24,28-30} and currently through genetic analyses^{17,19,23,26,31-33}. The subtypes have been further subdivided into various genotypes. To date, 11 RSV-A (ON1, GA1-GA7, SAA1, NA1&NA2) and 17 RSV-B (GB1-GB4, SAB1-SAB3& BA1-BA10) genotypes have been identified²⁰.

HRSV has been recognized as an important respiratory virus, there is a dearth of information on genetic diversity of HRSV strains among children in India, with few reports from North India^{34-39,52}. In south India reports on RSV molecular epidemiology is limited. Therefore, this is the first study to determine the prevalence and molecular epidemiology of RSV A and B in Chennai, Tamilnadu, south India.

MATERIALS AND METHODS

Sampling site, collection and transport of clinical specimens

During the study period from January 2011 to December 2014, 850 Influenza virus negative clinical samples (throat/nasal swab) were subjected to detection of other respiratory viruses like, Respiratory Syncytial Virus, Para influenza viruses, Human Metapneumo viruses, samples were collected from out patient of Chennai Government Hospitals (Saidapet Government Hospital, Stanly Government Hospital, Periyar Nagar peripheral Government Hospital, Institute of Child Health). In the present study we are

included only RSV data. Throat and nasal swab were collected following standard protocols (WHO criteria), Patient demographic details, clinical symptoms were recorded. Samples were transported to the laboratory with virus transport medium (Hanks' balanced salt solution with 200 IU penicillin ml⁻¹, 200 mg streptomycin ml⁻¹ and 2% BSA) The present study was approved by the Institutional Ethical Committee of the King Institute of Preventive Medicine and Research, Gundy, Chennai. The present study was carried in the Dept of Virology King Institute of Preventive Medicine and Research, Guindy, Chennai.

Viral RNA Extraction

Viral RNA was isolated from the respiratory specimens using Viral RNA mini kit (Qiagen) followed by manufacture's directions. The RNA was stored in aliquots at 80°C until use.

RT-PCR for detection of HRSV strains

Nucleic acid amplification was performed on GeneAmp PCR system 9600 thermal cycler (ABI-Applied Bio systems) using with Invitrogen Superscript III platinum one step RT-PCR kit for detection and sub typing of the RSV as described earlier⁴⁰. The amplified products were analyzed on a 1.5 % Agarose gel electrophoresis.

RSV Genotyping

RSV-A and RSV-B specific oligonucleotide primers (second hypervariable region of the G-protein) were used for genotyping and sequencing of the G protein. PCR protocols were described earlier⁴².

RT-PCR and Nested PCR for RSVA Genotyping

Genotyping of RSV A, amplification of the second hypervariable region of the Glycoprotein gene by either external or nested PCR. The external PCR was carried out with 10 pmol of forward primer RSVA-G513-F (5' AGTGTTCAACTTTGT ACCCTGC3') and reverse primer RSVA-F131-R (5' CTGCACTGCATGTTGATTGAT3')⁴¹ with Superscript III platinum one step RT-PCR kit (Invitrogen). Thermal cycling conditions are 50°C for 30 min 94°C for 5 min, followed by 40 cycles of PCR, 1 cycle consisting of 30 s at 94°C, 30 s at 58°C, and 1 min at 72°C, and a final extension is 10 min at 72°C. The 583 bp amplified products were analyzed by electrophoresis. In the case of external PCR results negative, 5 µl of the external PCR product was used as a template for nested PCR with 10 pmol of primers RSVA-G606-F

(5' AACCACCACCAAGCCACAA3') and RSV-F22-R (5' CAACTCCATTGTTATTTGCC3')⁴¹, with Phusion DNA PCR kit (New England Bio labs). The cycling conditions were the same as for the external PCR (absence of RT step), except annealing temperature, which was 53°C. The nested amplicons of 391 bp were visualized by agarose gel electrophoresis.

RT-PCR for RSV-B Genotyping

For RSV B, RT-PCR assay was performed by using Superscript III platinum one step RT-PCR kit (Invitrogen) in a 25µl volume containing 20 pmol each of forward primer BGF (5' GCAGCCATAAT ATTCATCATCTCT3') and reverse primer BGR (5' TGCCCCAGRTTTAATTTTCGTTTC3')⁴¹, and 5µl of the extracted RNA used. PCR reactions were conducted on a GeneAmp PCR system 9600 thermal cycler (ABI) thermal Cycling conditions are 50°C for 30 min for reverse transcription and 94°C for 5 min, 40 cycles of 94°C for 30 s, 63°C for 1 min, 72°C for 1 min, and the final extension is, at 72°C for 10 min. Amplified products were subjected to 1.5% agarose gel electrophoresis.

Sequencing and Phylogenetic Analysis

The PCR products were purified with a Purification kit (Qiagen), according to the manufacturer's directions. The purified products were sequenced in both directions on an ABI Prism 3130 genetic analyzer (ABI) by using an ABI Prism BigDye Terminator cycle sequencing kit 3.1 (ABI). Sequences were edited manually in Bioedit software version 7.0.9.0⁴³. The reference global sequences were downloaded from NCBI GenBank. All the Phylogenetic analysis and multiple sequence alignment were conducted with MEGA software version 5⁴⁴.

Statistical analysis

Statistical analysis were carried out with SPSS16.0 software. Significance of differences in

frequencies of age groups were analyzed using a chisquare test. P value was found to be statistically significant (P<0.05).

RESULTS

Yearly Distibution of RSV infections in chennai

Out of 850 samples, 124 (14.5%) samples were positive for RSV in which 84(9.8%) were RSV A, 40 (4.7%) were RSV B, (Table I). There was an increase in the percentage positivity in 2012 when compared to remaining years. During the study period from 2011-2014,

Prevalence of RSV infection in different age groups

Among patients with RSV infection, 47.5% were less than 1 year age group, 29.8 % were between 1-5 year age group, 14.5% were between 6-14 years age group and 8 % were above 14 years age (Figure I).

Relation of meteorological factors with RSV infection

During the study period, January 2011- December 2014 RSV positivity and number of samples tested were compared with meteorological data (Rain fall and Temperature) obtained from the Meteorological Department, Chennai, Government of India. The percentage of RSV positivity was found to be higher during the months of October to December in all the four years (Figure II).

Phylogenetic analysis

During the study period, out of 124 positive samples 27 samples (20 RSV A, 7RSV B) were subjected to sequencing. Phylogenetic tree was constructed with RSV global reference sequences and Indian sequences (Dibrugarh, Delhi and Pune, Kolkata, Lucknow). Phylogenetic analysis detected, detected that all RSV-A glycoprotein sequences obtained in this study were clustered in ON1 group of NA1 subtypes. A

Table 1. Yearly Distribution of RSV infections in Chennai

Year	No of samples	No of positives (%)	Sub typing	
			RSV A (%)	RSV B (%)
2011	230	32 (13.9%)	20 (2.3%)	12 (1.4%)
2012	210	32(16.6%)	25 (2.9%)	10 (1.1%)
2013	190	27 (14.2%)	17(2.0%)	10(1.1%)
2014	220	30 (13.6%)	22 (2.5%)	08 (0.9%)
Total	850	124 (14.5%)	84 (9.8%)	40 (4.7%)

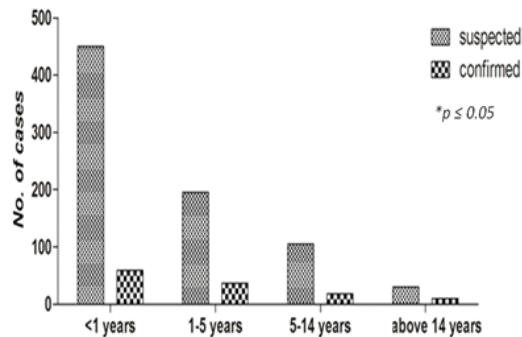


Fig. 1. Proportion of suspected and confirmed cases of RSV infection by age group, during the study period in Chennai

newly designated genotype ON1, within the NA1 genotype was first documented from Ontario, Canada and those samples were collected in the period Dec 2010⁴⁷. Phylogenetic analysis revealed that all RSV-B glycoprotein sequences obtained in this study clustered in genotype BA, in which 4 sequences clustered in genotype BA9, 3 sequences clustered in newly designated BA12 genotype which was first observed in Malaysia.

DISCUSSION

RSV is one of the most common viral pathogens causing lower respiratory tract infections among infants, young children and elderly individuals worldwide. Different studies detected RSV positivity in the range from 15% to

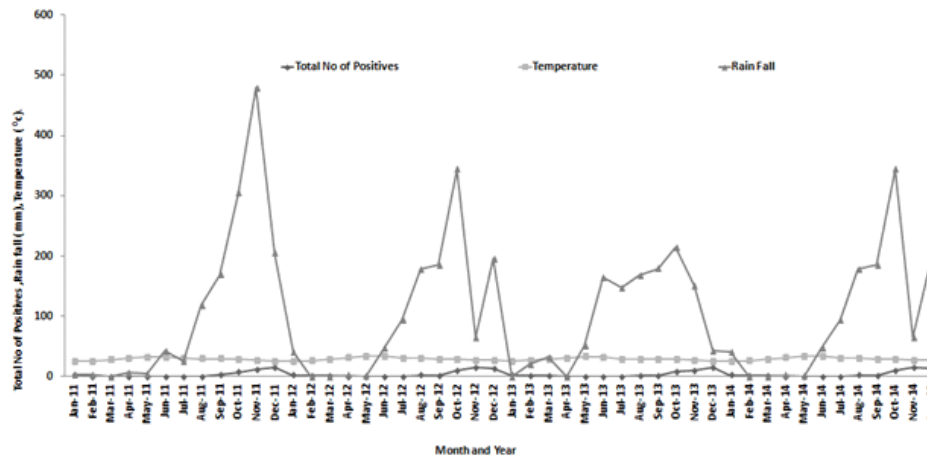


Fig. 2. The correlation between meteorological variables and the number of RSV positive samples obtained during the study

35% in pediatric population^{45,46}. In India, Kolkata (Eastern India) 10.3%³⁹, Assam (North East) 7.9%⁵², Delhi (Northern India) 17%³⁵, Pune (Northern India) 18.61%³⁴ were detected positive, in the present study during the study period from 2011–2014 positivity rate was 14.5%. Previous studies show that RSV group A is more predominant than group B infections in globally^{33,49-51}. RSV-A was the predominantly circulating group in 2011, RSV-B was the predominantly circulating in 2005–2006 while RSV-A in 2007–2008 in Kolkata³⁹ and RSV-A during the 2001–2005 in Delhi³⁵. RSV-A was the predominant circulating strain in Assam during 2009–2012⁵². Our present study shows that, 9.8%

were RSV A, 4.7% were RSV B, suggesting that RSV-A was the predominant subtype circulating in Chennai, south India. Little is known regarding the seasonality of RSV in tropical and subtropical countries like India.

Most infected individuals were children less than 2 years of age. Globally, the rate of RSV infection has been reported to decrease with increasing age^{46,53,54}. We observed an increased prevalence of RSV in the less than one year (47.5%) and 1-5 years (29.8%) age groups compared with greater than 5-14 (14.5%) and above 14 years old children, which is similar to previous studies done in Kolkata, India³⁹. We observed that RSV infection

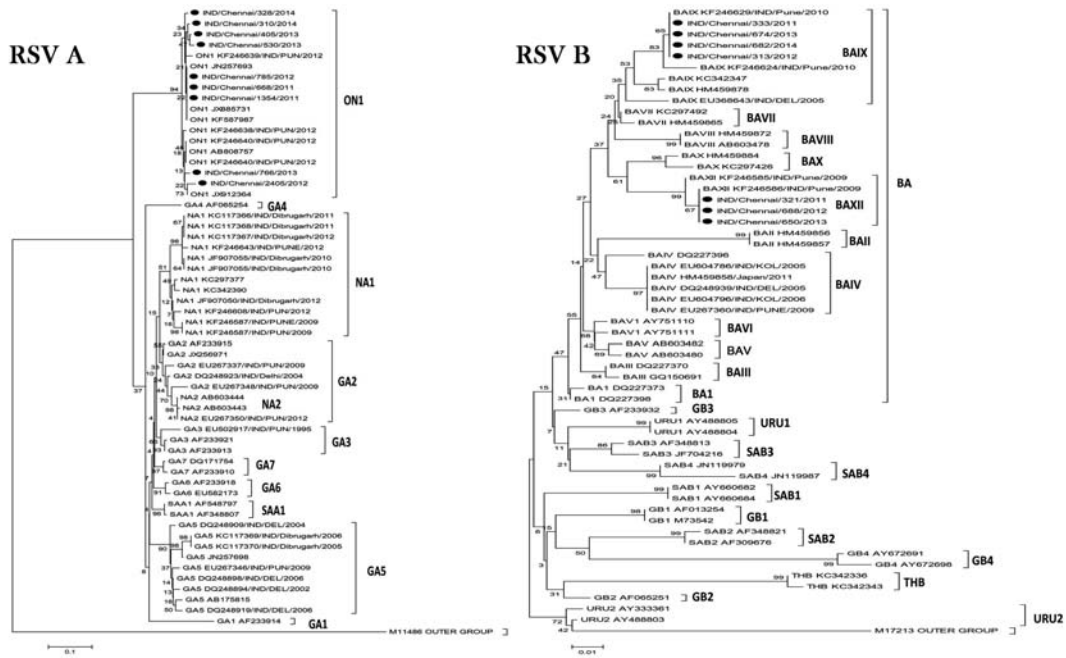


Fig. 3. Phylogenetic Analysis of RSV A and RSV B

revealed seasonal variations which peaked during the monsoon and post monsoon. Similar findings were reported in Pune which will come reported a peak in monsoon and winter³⁴, while Kolkata and Delhi reported peaks in the winter months^{36, 39}. Similar findings were reported from Cambodia⁴⁶, Germany⁵³ and Belgium⁵⁴.

In recent years, GA2 and GA5 were the most familiar genotypes among RSV-A worldwide. GA2 was the most common genotype of RSV-A found around the world and has persisted for many years^{36, 46, 53, 54}. In earlier studies from Delhi, subgroups GA2 and GA5 were prevalent among the circulating RSV-A strains during the 2002 to 2003 season and genotype GA5 was predominant in the 2003 to 2005 seasons. Sequences from Pune area in 2006 clustered in genotype GA2, GA5 and the recently designated group NA2. Sequences previously documented from different parts of India clustered together in the GA2, GA3, GA5 and NA2 genotypes. Sequences from Dibrugarh, eastern India from 2009 to 2012 mainly clustered in NA1 genotype and two sequences in GA5 genotype⁵². In the present study we had not described about amino acid analysis which is the drawback of our

study, further studies will be performed to rectify the hitch. In our study, phylogenetic analysis revealed that all RSV-A strains are clustered in newly designated ON1, within the NA1 genotype.

RSV group B genotype BA, first detected in Argentina in 1999 and has since become the predominant worldwide^{36, 39, 46, 54}. BA genotype is further classified from BA1 to BA12. BA genotype has been the most reported genotype in previous studies from India^{35, 39}. Our BA sequences clustered along with the BA9 and BA12 genotypes. Four sequences from the present study clustered in cluster BA within BA9 genotype, and three sequences in genotype BA12. However, it required long term surveillance throughout the country.

CONCLUSION

This is the first report contributing preliminary data on the RSV molecular characterization in Chennai, South India. In the present study concludes the sentence is In the present study concludes that RSV group A viruses predominated than group B infections during the study period in Chennai. RSV

infection is predominant in the age group between less than one to five years children. In Chennai RSV infection exhibited seasonal variations which peaked during the monsoon and post monsoon. Phylogenetic analysis of RSV-A belonged to the ON1 within NA1 genotype, RSV-B belonged to genotype BA of BA9 and BA12. The present study had not described about amino acid analysis, further studies will be carried out. However further studies will be required from India subcontinent to arrive at a better understanding to circulating genotypes of RSV. This will help in selecting candidate vaccines in future.

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