Section: Microbiology

Original Research Article

STUDY OF VIRUSES AFFECTING THE UPPER RESPIRATORY TRACT IN PAEDIATRIC PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN THE NORTH EASTERN PART OF INDIA

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ABSTRACT

Background:
Upper Respiratory Tract Illness is mostly caused by viruses in all age groups especially children. It is implicated as a leading cause of mortality amongst the children of the 3rd world countries. Among the viral etiologies common are Parainfluenza virus, Rhino virus, Adeno virus, Respiratory syncytial virus, Human metapneumo virus, Infleunza virus. Laboratory diagnosis of URTI is based on PCR and historical modalities like tissue culture are not done routinely and find use only in reference laboratories and academic institutes.

Material and Methods:
Throat swab, nasopharyngeal swabs and nasopharyngeal aspirates were collected from patients (≤ 3years) and placed inside a viral transport media and transported at 4° C. RNA was extracted and Duplex RtPCR was run.

Results:
In the paediatric patients the most common age group affected was between 1 -2 years,with a slight female predominace. Majority of the patients presented with features of upper respiratory tract infection. High birth order, Low birth weight, undernutrition, poor socio-economic status appear to be significant risk factors.

Conclusion: URTI of viral origin appear to be the most important presentation of ARI in children below 3years of age in the population under study. Demographic factors such as high bith order, low socio-economic background and malnutrition appear to have significant impact on the morbidity due to ARI.

Keywords: URTI, ARI, socio-economic status

INTRODUCTION

Upper Respiratory Tract Infection (URTI) is a disease entity affecting all age groups. Etiology of URTI indicates at the presence of bacteria in only about 10% of the cases, in 90% of the cases the cause appears to be viral in origin.[1] More than two hundred different viruses are implicated in the etiology of URTI.[2] The viral pathogens associated with URTI include picornaviruses (rhinovirus, enterovirus), coronaviruses, adenoviruses, parainfluenza viruses(PIV), influenza viruses, respiratory syncytial viruses (RSV), human metapneumovirus (hMPV), human bocavirus (hBoV) to name a few. Patients usually present with features of involvement of upper respiratory tract which includes fever, running nose, sneezing, cough, throat pain and earache.

URTI in its true nature cannot be thought to be a separate entity without any kind of lower respiratory tract involvement. Thus, URTI is a misnomer because rarely do we see it without any association

with Lower Respiratory Tract Infection (LRTI), and the, symptom complex is termed as Acute Respiratory Infection (ARI).[1] These features seem to be more marked in children, making ARI an important cause of childhood morbidity and mortality. But even after the danger ARI poses to children it usually is considered trivial and is not dealt properly in the medical curricula and most of the time there is an underestimation of its burden.[1] All these factors are compounded by the lack of proper laboratory support to aid in the diagnosis, especially in resource poor countries. In these countries, the medical fraternity and the government are overburdened with managing many other illnesses and donot have the necessary economic resources while diagnosing ARI.

The viruses implicated in the etio-pathogenesis of ARI are basically known as the Respiratory viruses (RV), and the disease is sometimes known as Respiratory Viral Infection (RVI).[2] Among the viruses which affect children notable are the Rhino Virus, RSV, hMPV, PIV, Influenza Virus, alongwith Adenovirus, Coxsackie Virus , hBoV, though they are more common in adults. These viruses have a tropism for the respiratory epithelium and on exposure can lead to various degrees of the manifestations depending on the host factors.

RVIs are implicated in approximately 50% of community-acquired pneumonia (CAP) in young children, over 90% of bronchiolitis cases in infants and young children seeking medical attention and over 90% of asthma exacerbations in children.[3] In addition RVIs predispose those infected to a range of secondary bacterial infections including otitis media, sinusitis and CAP. ARIs are estimated to cause 3.9 million deaths per year and pneumonia alone is the leading single cause of mortality in children under 5 years of age with approximately 1.2 million children dying each year. Estimates indicate that about 99% of these deaths occur in developing countries and 80% occur out of hospitals.[3] Respiratory viruses are the primary agents involved in multiple common respiratory syndromes [e.g. rhinosinusitis, influenza like illness(ILI), bronchiolitis, laryngotracheobronchitis] and that they either directly cause other illnesses affecting the upper respiratory tract (e.g. otitis media and sinusitis) and lower respiratory tract (e.g. pneumonia), or foster secondary bacterial infections at these sites. In addition, some RVIs – particularly influenza and adenovirus – have been associated with severe illnesses involving other organ systems (e.g. encephalopathy–encephalitis and myocarditis). RVIs may sometimes also be associated with chronic sequelae, such as the development of asthma.

WHO has put a huge figure of 6.3 million childhood mortality prior to their 5th birthday in the year 2008, with more than half of these being caused by conditions that could be prevented or treated by access to simple and affordable interventions.[5] About half of the under five deaths occur in 5 countries of China, Democratic Republic of Congo, India, Nigeria, Pakistan.[3] In the Indian context , National Health and Family Surveillance 1 (NHFS) in 1994-95 had reported at an incidence rate of 6.5% which by NHFS 2 in 1998-99 had increased to 19% , again in 2005-6 during the NHFS 3 a declining incidence at 5.8% was seen.[11] A WHO report in 2006-8 had reported ARI rate at 0.38% in the entire SEAR.[5] Reports of incidence rate at 86% incase, of URTI with 6.42 episodes / child/ year have been noted in parts of south India in 2006.[12] By 2013, the ARI incidence dropped slightly down at 60.2%, with 98% contribution by URTI.[13] Thus ARI is a significant problem in the Indian scenario.

The host factors which guide the prognosis of this disease can be divided into two categories – non-modifiable and modifiable. Amongst the non-modifiable factors included are extremes of age, co-morbid conditions, immune-suppression which govern the severity of illness. Patients with decreased respiratory reserves such as those suffering from bronchial asthma, cystic fibrosis, tuberculosis etc are more likely to develop the severe form of the disease. But occurrence of ARI is simultaneously governed by a few modifiable factors foremost being nutritional status of the child. In the developing countries where more than 70% of the population live below the poverty line the children inevitably suffer from both acute and chronic forms of malnutrition, compounding the problem of many childhood illnesses such as ARI, diarrhoea etc. Literacy rate of the mother is another factor which governs the wellbeing of the family in general and children in particular. In many of the developing countries where poverty, disease and day to day difficulties are so overwhelming that education of the girl child takes a back seat leading to their ignorance. Such an illiterate mother does not know how to take care of herself when she is pregnant leading to low birth-weight of the child she carries. On delivery the child is not only malnourished but also susceptible to many illnesses ARI being foremost.

World Bank estimates show that around 12.7% of the total population of the world live at or below the poverty line with income of less than $1.9/day, with most of this population in the developing countries of South Asia, and sub-Saharan Africa.[4] This problem of poverty is compounded by the problem of high birth order which is quite prevalent in these countries. This manifests in children as malnutrition increasing their vulnerability to childhood illnesses like ARI, diarrhoea.

Communities can be both affected directly and indirectly, through the need for outpatient care and hospital services. One recent systematic analysis estimated that, in 2010, 14.[9] million episodes of severe or very severe ARI resulted in hospital admissions in young children worldwide, although among these only 62% of children with severe ARI were hospitalized, indirectly ARIs are responsible each year for major losses in productivity in part due to absenteeism. ARIs are the leading cause of burden

of disease worldwide accounting for 94.5million disability adjusted life years (DALYs), equivalent to 6.2% of total DALYs.[3]

Current management of RVIs is suboptimal in most countries and often results in both use of ineffective treatments and failure to use treatments of proven benefit because it is commonly thought that ARIs are caused by bacteria most such infections are treated with antibiotics. Even when a viral etiology is diagnosed, the illness is unlikely to be treated with specific antivirals because these are generally unavailable except possibly for influenza treatments in some settings.

The availability of sophisticated diagnostic tests in recent times, multiple respiratory viruses are now often detected in ARIs, especially in children. Such observations raise questions about disease causation, pathogenesis and the dynamics of infection with multiple agents; they also suggest that it would be beneficial to consider innovative therapeutic approaches that do not focus on a single virus. Mixed infections (both viral–viral and viral–bacterial) increase the complexity of pathogen–host interactions. Improved therapeutic strategies for RVIs will depend on a better understanding of the mechanisms of disease in different syndromes. Except possibly during widespread outbreaks due to a specific virus (e.g. epidemic influenza) RVIs cannot be addressed effectively or efficiently with a vertical approach that focuses on one agent at a time from public health and clinical perspectives. A syndromic approach that addresses the pathogenesis prevention and optimal management of clinical problems associated with ARI; it also allows for the introduction of technological advances in diagnostics and therapeutics to those in greatest need.

In the year 2008, WHO reported, 6.3 million childhood mortality prior to their fifth birthday with more than half of these being caused by conditions that could be prevented or treated by access to simple and affordable interventions.[5] More than half of these were due to ARI and maximum were in the developing countries of south Asia and sub Saharan Africa. In context of India a wide variation is seen in the prevalence of ARI among different regions. The southern part of India has reported a prevalence rate at 58.2% of childhood morbidities,[6,7] Jain et al., have reported a prevalence of 11.6% in the state of Rajasthan.8 Deb S K in his study in the north-eastern part of India noted a prevalence of 20%(3% ARI in rural; 7% ARI in urban areas).[9]

The present study has been undertaken in the Dept. of Microbiology in a tertiary care hospital in the north- eastern part of India and has been titled:-

"Study of viruses affecting the upper respiratory tract in paediatric patients attending a tertiary care hospital in the north eastern part of India" with the main objective of :-

Understanding the prevalence of Upper Respiratory Tract Infections of viral origin among paediatric patients attending a tertiary care hospital in the north eastern part of India

MATERIAL AND METHODS

The present study was conducted in the Department of Microbiology, NEIGRIHMS, Shillong, over a period of one year, from January 2014 – December 2014.

Ethical clearance was duly obtained from Institute Ethics Committee, NEIGRIHMS, for conducting the study.

Sample

The samples were collected from children attending Paediatrics Out-Patient Department and also from those who were hospitalised. Samples in the form of nasopharyngeal swab, nasopharyngeal aspirate and throat swab were collected from the patients after obtaining the due Informed consent from the parents/guardians.

Inclusion Criteria

Sample collection was done carefully from those children who fulfilled the following inclusion criteria

1. **Age**: Neonate – 3 years old.
2. **Symptoms:** Sneezing, Running nose, Cough, Throat pain, Earache, Fever.
3. **Signs:** Congested pharynx, bulging tympanic membrane, Raised respiratory rate, Chest in drawing, Stridor.
4. Auscultation- Crepitations, Rhonchi.
5. Chest X-Ray: Hyperlucency

Exclusion Criteria

Children with the underlying features were excluded from the study population

1. Anatomical defect of the Upper or Lower respiratory tract.
2. Co-morbid conditions- Bronchial Asthma, Cystic fibrosis, Tuberculosis, Immunosuppression.
3. Lack of consent from from parents/guardian.

Specimen Collection & Transport

Samples were collected using nylon flocked swabs categorically provided alongwith Himedia Viral Transport Media (VTM).

The samples were then inoculated into VTM transported to the laboratory after maintaining strict cold chain. The samples were then aliquoted stored at -80^{o}C for downstream molecular analysis.

EXTRACTION & PURIFICATION OF VIRAL RNA

Viral RNA was extracted and purified from 140µl of sample collected using QIAamp Viral RNA Mini Kit (spin column- QIAGEN, GmbH, Hilden, Germany) as per manufacturers protocol. Briefly, 560µl of AVL Buffer containing 5.6 µl of carrier RNA was dispensed into 1.5ml micro centrifuge tubes. 140µl of aliquoted specimen was added to AVL buffer and Carrier RNA mixture and vortexed for 15 seconds. The suspension was incubated at room temperature (15-25^{o}C) for 10 minutes followed by brief centrifugation to remove droplets from the inner side of the lid. 560µl of absolute ethanol was added to the sample and mixed by vortexing for 15 seconds followed by brief

centrifugation of the tube to remove droplets from the inner side of the lid. Carefully 630µl of the solution was applied to the QIAamp Mini column with 2ml of collection tube without wetting the rim. The cap was closed and centrifuged at 8000 rpm for 1 min. The QIAamp Mini column was placed into a fresh 2ml collection tube and the tube containing filtrate was discarded. The QIAamp Mini column was opened carefully and 500µl of AW1 Buffer was added to the QIAamp Mini column and centrifuged at 8000 rpm for 1 minute. This was followed by replacement with fresh 2ml collection tube and the tube containing filtrate was discarded. 500µl of the AW2 Buffer was added to the column and centrifuged at 14000 rpm for 3 minutes. This was followed by the replacement with fresh 2ml collection tube and the tube containing filtrate was discarded and centrifuged at full speed for 1 minute. Finally, the QIAamp mini column was placed in a sterile, DNAase, RNAase, free 1.5ml micro-centrifuge tube. The old collecting tube containing the filtrate was discarded and replaced with a fresh 1.5ml MCT. 50µl of AVE Buffer was added to the QIAamp Mini column and was equilibrated to room temperature for 1 minute followed by centrifugation at 8,000g for 1 minute. The eluate which was collected into the MCT consisted of RNA and was stored at -80°C until further use.

PCR ASSAY

The purified and extracted Viral RNA products were amplified using the RSV/hMPV r-gene & Rhino+EV/Cc r-gene (bioMérieux, France) on the ABI 7500 Step One (Applied Biosystems).

Principle

The amplification premix is optimized for amplification and detection of-

Rhinovirus and Enterovirus (at 530 nm)
Cellular control (at 560 nm)
Respiratory Syncytial Virus (at 530 nm)
Human Metapneumo Virus (at 560 nm)

Kit Contents

Amplification premix: 2x 450µl

(dNTP/ MgCl2/ Amplification probe/ Primers and probes/ Taq polymerase/
Passive reference ROX)

Negative amplification & extraction control: 2x1.8ml

Positive control: 1x 300µl

Reverse transcriptase Superscript 3 (concentrated): 1x 15µl

Amplified Sequences

1. Rhinovirus & Enterovirus
   - EV genome: region 5' non coding (146 bp)
   - HRV genome: Region 5' non coding (157 bp
2. Cellular Control: HPRT 1 gene (108 bp)
3. Respiratory Syncytial Virus
   - N gene coding for nucleoprotein (184/ 152 bp)
4. Human Metapneumovirus-M gene coding matrix protein (160bp)

Amplification Preparation

1. Plan n wells (n = number of samples+ positive controls+ negative control).
2. 1µl of Reverse Transcriptase is pipette and diluted with 9µl of nuclease free water in a mct (0.5 ml) this constitutes the Diluted RT.
3. In another mct (2ml), 15µl × (n+ 1) of Amplification Premix is pipetted out, to this is added, 0.15 µl ×(n+1) of the Diluted RT.
4. 15µl of Amplification Mix in added to the n number of wells alongwith 10µl of the extracted RNA onto a microtitre plate (48 well ×0.1 µl).
5. The plates are then placed in the real time PCR instrument and program run asper manufacturers guidelines.

Thermal Profile (Taqman Step one real time PCR- Applied Biosystems)

STEPS		TIME	TEMP(ºC)	CYCLES	FLOURESCENCE AQUISITION
Reverse Transcription		5min	50	1	----------
Taq Polymerase Activation		15min	95	1	----------
Amplification	Denaturation	10 sec	95	45	----------
	Annealing	40 sec	60		530 & 560 nm
	Elongation	25 sec	72		----------

The Cut- Off Threshold (CT) value of the various samples was noted, correlation with the amplification plot obtained during RT-PCR was also observed. Cut off threshold of less than 40 was considered positive any CT value higher than 40 was considered negative.

RESULTS

The present study, "Study of viruses affecting the upper respiratory tract in paediatric patients attending a tertiary care hospital in the north eastern part of India", has been carried out in the Department of Microbiology, NEIGRIHMS, Shillong, during the period January 2014-December 2014.

A total of fifty symptomatic children fulfilling the inclusion and exclusion criteria were enrolled in the study. Respiratory Viruses (RV) were detected in 31(62%) of the patients included in the study.

SEX-WISE DISTRIBUTION OF STUDY POPULATION

RVs were detected in a higher proportion of female patients 19(61.29%) than males 12(38.7%) of the 31 positive patients.

AGE-WISE DISTRIBUTION OF PATIENTS

Majority of the patients were seen in the age group of 12 -24 months (54.8%), followed by children in the age group of 24-36 (32.2%)months and then came the infants (12.9%).

DISTRIBUTION OF PATIENTS BASED ON DIAGNOSIS

Majority of the patients presented with features of URTI 44 (88%), 5 (10%) children presented with features of LRTI and 1(2%) child presented with pneumonia.

DISTRIBUTION OF PATIENTS BASED ON CLINICAL FEATURES

Most of the patients in the study group (90%) presented with fever followed by running nose (64%) and by cough and difficulty in breathing (12% each). Earache found to be one of the symptoms complained by 4% of the patients as depicted in the Table 4.4.

MONTHLY VARIATIONS OF ARI

Table 4.5 depicts URTI (61.16%) to be prevalent throughout the year with slight increase in incidence in the months of November, December, January, February. The number of patients presenting with features suggestive of LRTI (80%) were seen to peak in the cold months of November, December, January.

MONTH- WISE DISTRIBUTION OF VIRUSES

Table 4.6 illustrates month-wise distribution of different viruses. The table showed Rhinovirus to be present as the most prevalent virus with 16 patients (51.61%). RSV was observed to peak in the months of November, December, January with 4 (12.9%) children detected to be suffering from infection, 3 patients were affected by hMPV (9.6%). Co-infection with two viruses were also observed in 8 patients (25.8%). Majority of the cases were clustered in the months of November, December, January, February with 1 patient suffering from dual infection in the month of May.

ASSOCIATION OF DIFFERENT VIRUSES WITH CLINICAL DIAGNOSIS

Table 4.7 shows the association of viruses with pneumonia, LRTI, and URTI. Rhinovirus was most commonly associated with URTI with 16 patients (51.61%). LRTI was associated with RSV and was detected in 4 patients (12.9%) while hMPV was an etiological agent of both URTI (6.4%) and LRTI (3.22%). All patients detected with Co-infection were suffering from LRTI (25.8%). Dual infection of Rhinovirus and hMPV was detected in 3 patients (9.6%), RSV and Rhinovirus in 4 patients (12.9%), while co-infection of RSV and hMPV was observed in 1 (3.22%) child suffering from pneumonia.

AGE-WISE DISTRIBUTION OF VIRAL AGENTS

Table 4.8 shows the age-wise distribution of viral agents. It is seen that maximum number of viruses were detected in the age group of 12-24 months (52.1%).

DISTRIBUTION OF VIRUSES BASED ON SAMPLE

The table 4.9 shows that majority of the sample collected were throat swabs (88%), followed by NPS (10%) and then NPA (2%). Among all the samples NPS and NPA comprise only 12% of the sample collected but viral detection from them were 100%, making them better samples in comparison to throat swab which had a poor yield of 56.81%.

Table 4.10 shows that NPS and NPA constitute only 35.4% and 3.2% respectively of the total positive sample collected but the detection rate of RV from them is 100%. These are especially good samples for detection of RSV (100%). However, throat swab which constituted almost 88% of the total sample collected was efficient at detecting only 61.2% of the viruses.

RT-PCR was done using Argene RMWS Duplex PCR (biomerieux, France). It has documented to have a sensitivity of 91.3% (RSV), 85.7%(hMPV), 80.77%(RhinoV). The specificity of the kit is 99.5% (RSV), 100% (hMPV),98.39% (RhinoV).

A positive sample displays a CT value. If the CT value is less than 40 cycles (40), the sample is considered to be positive. If the CT value cannot be calculated or is more than 40 cycles the sample is considered negative for the virus under study.

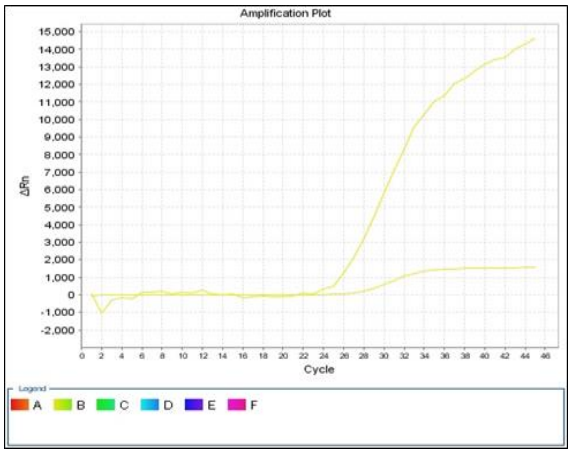

Figure 4.1: Amplification plot of RSV

[Positive Control, CT value = 22-24/ MPT11 (positive Sample), CT value=28]

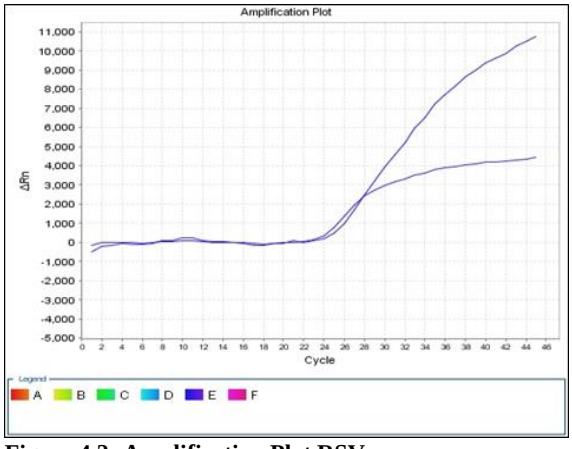

Figure 4.2: Amplification Plot RSV

[Positive Control, CT value= 22-24/ MPT5 (positive sample), CT value=24]

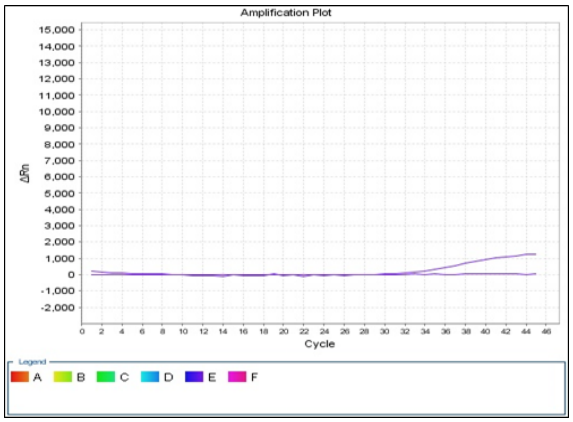

Figure 4.3: Amplification Plot RSV

[MPT42 (positive sample), CT value=32]

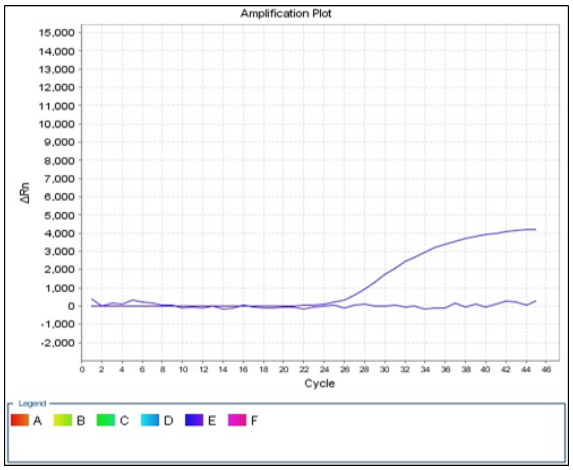

Figure 4.4: Amplification Plot of hMPV

[MPT 2 (positive sample), CT value=24]

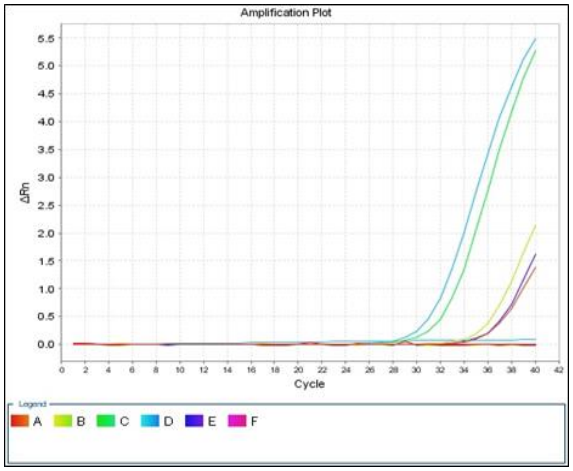

Figure 4.5: Amplification plot of RSV & hMPV co-infection

[Positive Control, CT value = 28 / MPT12 (positive sample) CT value= 34]

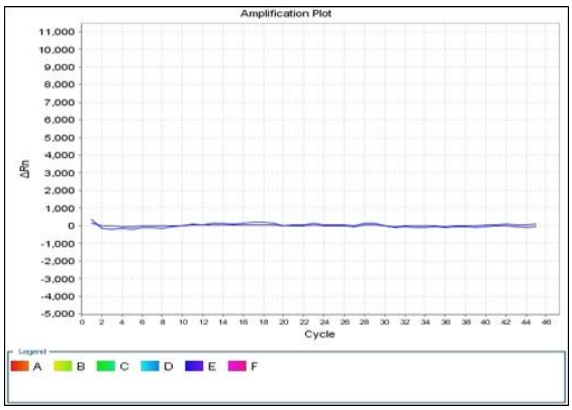

Figure 4.3: Amplification plot RSV

[MPT 1 (negative sample), CT value = Undetermined]

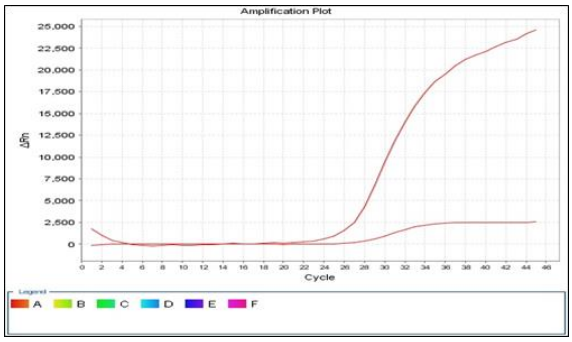

Figure 4.4: Amplification Plot Rhinovirus

[Positive Control, CT value= 22/ MPT29, (positive sample), CT value= 27.494)

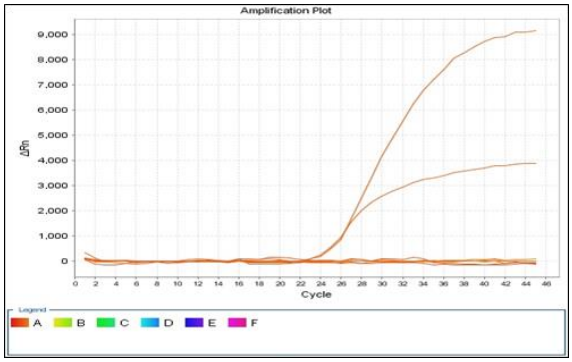

Figure 4.5: Amplification Plot Rhinovirus

[Positive Contol, CT=22/ MPT 20(positive sample), CT value= 23/ MPT 21 (negative sample), CT value= Undetermined, Negative control, CT value = Undetermined]

Assessment of risk factors in Table 4.13 showed a correlation between birth order, birth weight, nutritional status, socio-economic status and literacy rate of the mother and the occurrence of ARI. Among the 31 children who developed RVI 11(35%) children were of birth-order more than 4, while 18(65%) were of birth order 3 or less. Low birth weight was seen in 9 (35.58%)children who

were symptomatic, while 22 (64.51%)had normal birthweight.10(32.25%) number of children were nutritionally under-nourished. On comparison of the socio-economic status, as per the Kuppuswamy scale 10(32.25%) children belonged to the lower class, 14(45.16%) to middle class, and 7922.58%) belonged to the higher class. 9(29.03%) children who were suffering from ARI had mothers who were illiterate, while 22 (70.09%) ARI patients had mothers who had some form of basic literacy.

ASSESSMENT OF RISK FACTORS ASSOCIATED WITH RSV INFECTION

Table 4.14 demonstrates that 75% of the children diagnosed with RSV infection had exposure to risk factors such as low birth weight, high birth order, poor nutritional status, and belonged to lower economic status. Literacy rate of the mother didnot appear to have appear to guide the occurrence of RSV associated ARI.

Table 4.1: Sex- wise distribution of study population presenting with ARI

SEX	TOTAL CASES (n=50)	PERCENTAGE	NO. OF POSITIVE CASES	PERCENTAGE(%)
MALE	18	36	12	38.70
FEMALE	32	64	19	61.29
TOTAL	50	100	31	100

Table 4.2: Age-wise distribution of patients

Age(months)	Total cases (n=50)	Percentage (%)	Total positive cases	Percentage (%)
0-12	7	14	4	12.9
12-24	26	52	17	54.8
24-36	17	34	10	32.2
Total	50	100	31	100

Table 4.3: Distribution of patients based on diagnosis

DIAGNOSIS	TOTAL (n=50)	PERCENTAGE (%)	TOTAL POSITIVE	PERCENTAGE (%)
URTI	44	88	25	80.64
LRTI	5	10	5	16.12
Pneumonia	1	2	1	3.24
Total	50	100	31	100

Table 4.4: Distribution of patients with different clinical features of URTI among the study population

CLINICAL FEATURES	TOTAL CASES (n=50)	PERCENTAGE (%)	TOTAL POSITIVE	PERCENTAGE OF POSITIVE (%)
Fever	45	90	29	64.44
Running nose	32	64	24	75
Cough	6	12	6	100
Earache	2	4	0	0
Difficulty in Breathing	6	12	5	83.3

Table 4.5: Month-wise distribution of ARI patients in the study population

MONTHS	PNEUMONIA	LRTI	URTI	Total
Jan	0	2	7	9
Feb	1	0	6	7
Mar	0	0	1	1
Apr	0	0	1	1
May	0	0	4	4
Jun	0	0	3	3
Jul	0	0	2	2
Aug	0	0	2	2
Sept	0	0	3	3
Oct	0	0	1	1
Nov	0	2	6	8
Dec	0	1	8	9
Total	1	5	44	50

Table 4.6: Month-wise distribution of viruses

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total
RhinoV	1	0	0	0	2	3	0	0	2	1	5	2	16
RSV	1	0	0	0	0	0	0	0	0	0	2	1	4
hMPV	1	0	0	0	1	0	0	0	0	1	0	0	3
>2 V	1	2	0	0	1	0	0	0	0	0	2	2	8
Total	4	2	0	0	4	3	0	0	2	2	9	5	31

Table 4.7: Association of different viral etiologies with clinical diagnosis

VIRUS	URTI		LRTI		PNEUMONIA	
	N	%	n	%	n	%
Rhinovirus	16	51.61	0	0	0	0
RSV	0	0	4	12.9	0	0
hMPV	2	6.4	1	3.22	0	0
RSV+ hMPV	0	0	0	0	1	3.22
Rhinovirus+ hMPV	0	0	3	9.6	0	0
RSV+ Rhinovirus	0	0	4	12.9	0	0

Table 4.8 Age-wise distribution of viral agents

AGE(months)	0-12		12-24		24-36	
VIRUS	n=4	%	n= 17	%	n =10	%
RhinoVirus	3	18.75	8	50	5	31.25
RSV	1	25	3	75	0	0
hMPV	0	0	1	33	2	66
RSV+hMPV	0	0	1	100	0	0
RhinoV+hMPV	0	0	1	33	2	66
RhinoV+RSV	0	0	3	66	1	33

Table 4.9: Sample-wise distribution of viral agents

SAMPLE TYPE	TOTAL SAMPLE	%	POSITIVE SAMPLE	%
THROAT SWAB	44	88	25	56.81
NPS	5	10	5	100
NPA	1	2	1	100
TOTAL	50	100	31	

Table 4.10: Distribution of various viruses depending on sample

Sample	RhinoVirus	RSV	hMPV	RSV+hMPV	RhinoV+hMPV	RSV+RhinoV	Total
ThroatS	16	0	3	0	0	0	19
NPS	0	4	0	0	3	4	11
NPA	0	0	0	1	0	0	1
Total	16	4	3	1	3	4	31

Table 4.13: Assessment of risk factors associated with ARI in Study Population

RISK PARAMETER	TOTAL CHILDREN POSITIVE	%
BIRTH ORDER		
>4	11	35
<3	20	64.51
BIRTH WEIGHT		
LOW BIRTH- WEIGHT	11	35.48
NORMAL BIRTH-WEIGHT	20	64.51
NUTRITIONAL STATUS		
UNDER NOURISHED	10	32.25
NORMAL	21	67.74
SOCIO-ECONOMIC STATUS		
LOWER	10	32.25
MIDDLE	14	45.16
HIGHER	7	22.58
LITERACY RATE OF MOTHER		
ILLITERATE	9	29.03
LITERATE	22	70.09

Table 4.14: Assessment of risk factors associated with RSV infection

RISK FACTORS	PARAMETERS	TOTAL CHILDREN POSITIVE	%
BIRTH ORDER	>4	3	75
	<3	1	25
BIRTH WEIGHT	LOW BIRTH- WEIGHT	3	75
	NORMAL BIRTH-WEIGHT	1	25
NUTRITIONAL STATUS	UNDER NOURISHED	3	75
	ORMAL	1	25
SOCIOECONOMIC STATUS	LOWER	3	75
	MIDDLE	1	25
	HIGHER	0	0
LITERACY OF MOTHER	ILLITERATE	2	50
	LITERATE	2	50

DISCUSSION

The present study, "Study of viruses affecting the upper respiratory tract in paediatric patients attending a tertiary care hospital in the north eastern part of India" was carried out with the main objective of determining the prevalence of RVs in the paediatric population presenting with features of URTIs with special attention to the cases caused by RSV and correlate the association of such infection with various host profiles.

A total of 50 numbers of children were incorporated into the study. Throat swab, NPS, NPA were collected from the patients after taking due Informed Consent from the parents/guardian and a brief clinical history. The samples were analysed by RT-PCR and viral isolation for RVs especially RSV.

The present study showed that females (61.29%) were more affected by RVs and suffered from ARI in comparison to males (38.7%) which is consistent with the findings of Wadgave et al., 2007,[56] they have reported a higher prevalence of ARI in female child seen (46.49%) in comparison to males (36.32%). However, compared to our study Moura et al.,2006 (57.3% Males,42.7%Females),[57] Pore et al., 2010 (62.5%,37.5%);58 Bonfim et al.,2011(56.4%,43.5%),[59] Ahmed et al., 2012 (54.2%, 45.8%),[60] Singh et al.,2014 (61%,25%),[55] in all these studies a higher prevalence of RVI was noticed in the male children. The study conducted by Broor et al., 2001,[61] and Njouom et al., 2012,[62] have not reported any relationship between the sex of the affected child and occurrence of ARI. Such difference in observation might be due to comparatively limited sample size in our study.

The present study observed that the most commonly affected age group was that between 12-24 months (54.8%) by the RVs like Rhinovirus, RSV, hMPV. This observation was similar to the findings of Tsai et al.,2001 (12-36 months),[51] Williams et al.,2004 (25%, 13-24 months)59, Kwofie et al., 2012 (6 months- 24months),[60] Pretorius et al.,2012 (24-48 months).[63] But there is difference in the observation with a few studies, Bharaj et al., 2006,[53] and Shafik et al.,2012,[66] where they have observed a higher prevalence of RVI in infants. However, the studies of Matu et al., 2014,[67] observed a higher prevalence of RVI in children above 3 years of age as the study population included more number of children in the age group of 36- 60 months.

The month-wise distribution of patients suffering from ARI showed that URTI was prevalent throughout the year with slight predominance in the months of November to February (61.16%), while LRTI mostly presented in the cold months of winter (80%). These observations are consistent with the findings of studies of Monto et al., 1993,[50] Shafik et al.,2012,[66] Singh et al., 2014,[55] in all these studies month-wise variation of prevalence of ARI was observed with more number of cases being reported in the winter months. However in the studies of Tsai H et al., 2000.[51] Moura et al., 2006,[57] no month- wise or seasonal variation was observed.

Majority of the patients presented with chief complaints of fever (90%), with running nose (64%) Difficulty in breathing (12%) and cough (12%) was seen in patients presenting with LRTI and pneumonia. These findings compare favourably with those of Do a H L et al., 2010,[54] where fever was the presenting complaint in majority of the patients (97%) followed by rhinorrhoea and cough 96% and 97% respectively. However, these observations differ from the study of Shafik et al., 201266 and Singh et al.,2014,[55] wherein they observed a higher presentation of cough at 98% and 90% respectively. This difference in observation could be due to the incorporation of higher number of patients with LRTI while our study includes majority (88%) patients with features of URTI.

It is observed in our study that Rhinovirus was significantly associated with URTI (51.61%). These findings were similar to the observations of de Freitas Souza L S, et al.,2003,[52] while it was contrary to the findings of Choi, et al.,2006,[68] and Miller et al.,2007,[69] where an association with LRTI was seen. RSV was associated with more chances of developing LRTI (12.9%) this is in agreement to the studies of Tsai et al.,2001,[51] Bharaj et al., 2009.[53] In our study hMPV was associated with cases of LRTI which is similar to the observations of Williams et al.,2004,[63] and Ebihara et al., 2004,[70] but differs from the study of Choi et al.,2006,[68] where they observed it to be associated with pneumonia. The reason for this variation could be difference in geographic and genetic variability of the study population.

In our study the total prevalence of viral etiology was 62% which was similar to the studies conducted by Jain et al.,1991(22%);8 Arruda et al.,1991(35%),[73] Monto et al.,1993(22.1%),[50] Weigl et al.,2000(34%);72 Nokso- Koivitso et al.,2002(62%),[74] Manzarrez et al.,2003(35%),[75] Louie et al., 2005(38%);76 Miller et al.,2007(61%); 69 Chonmaitree et al.,2008(24.8%),[77] Bharaj et al., 2009(35.2%),[53] Bonfim et al.,2011(37.6%),[59] Gorjipour et al., 2012 (40.7%),[2] Ahmed et al.,2012(49.8%),[60] Matu et al., 2014 (44.9%).[67] In all these studies, viral etiology was implicated as the most common causative agent in children presenting with ARI.

The present study observed that Rhinovirus was detected in 51.61% samples, followed by RSV which affected 12.9% children, and hMPV with 9.6% cases. These findings were similar to the findings of Arruda et al. ,1991(45.6%),[73] Monto et. al.,1993(39%),[50] Fin OM cohort study 2000(40.7%),[78] Miller et al.,2007(26%),[69] Chung et al.,2007(33.3%),[79] Bonfim et al., 2011(37.7%),[59] Costa et al.,2014(41.7%),[80] Moreno-Valencia et al., 2015(26.6%),[81] in all these studies Rhinovirus has been detected to be the most

prevalent virus affecting children especially under the age of 5 years . However, studies of Jain et al., 1991(5%),[8] Lina et al.,1996(31.03%),[82] Weigl et al.,2000(12.1%),[71] Andreoletti et al.,2000 (53.6%),[83] Louie et al.,2005(12%),[76] Bharaj et al.,2009 (47.16%),[53] Fujitsuka et al.,2011(40.9%);84 Ahmed et al., 2012(12.5%),[60] Matu et al., 2014(16.7%),[67] Nasreen et al.,2014(34%),[85] RSV was detected as the most prevalent virus causing ARI. The variation in observation of these studies is because they had included infants with features of LRTI. However, in our study patients presenting with URTI were included.

The detection rate of dual infection in our study was 25.8%, 12.9% was caused due to Rhinovirus and RSV, 9.6% was caused by Rhinovirus and hMPV, 3% of the cases showed co-infection of RSV and hMPV. These observation are in close relation with the observations in a few other studies,such as, Bonzal et al., 2008 (20%),[86] Canducci et al., 2008(23%),[87] Calvo et al., 2010(22%) ;88 Matu et al., 2014 (29.5%),[67] Lopez et al., 2015 (29.9%).[89] However a few studies had shown slightly diminished rate of viral co-infection in comparison to our study Miller et al.,2007(13%),[69] Chung et al., 2007 (16%),[79] Bharaj et al.,2009 (18.86%),[53] Fujitsuka et al., 2011(12.2%).[84] This difference in observation was due to the larger sample size in these study and the variations in the race and ethnicity of the study population.

In our present study, we observed that all the RSV and hPMV associated with LRTI were detected from NPS (100%) and NPA (100%) samples although they constituted only 10% and 2 % of the total sample collected. Throat swab which constituted 88% of the total sample collected contributed only 56.8% of the positive samples. In throat swab Rhinovirus detection rate was as high as 100%, while no RSV was detected. This showed that throat swab was not a very good sample for viral detections. This observation is in corroboration with the findings of Do a H L et al., 2011,[54] where they had seen a higher positivity of NPA and Naso-tracheal aspiration with a positivity rate of 90% and 93% respectively.

In our study we observed that nutritional status is associated with 4 times more risk of acquiring ARI but no statistical significance can be associated due to the small sample size in this study. Significant association has been attached to the nutritional status and development of ARI in many studies which include those of, Broor S et al.,2001,[61] Savitha M R., et al.,2007;91 Pore et al.,2010;52 Prajapati et al.,2011,[91] Arun A., et al;2014.[92]

Literacy rate of the mother is negatively associated with affection of ARI in the children, in our study also we see quite a similar association limited sample size statistical significance cannot be associated with this factor. In our study there is 7.3 times more chance of developing ARI in children of mothers who are illiterate. These findings are seconded by the findings of Broor S et al., 2001,[61] Savitha M R., et al.,2007,[91] Pore et al.,2010,[52] Prajapati et al.,2011,[92] Arun A., et al;2014.[93]

Thus, the present study showed a significant prevalence of viral etiology in the causation of ARI, with Rhinovirus being the commonest followed by RSV and then HMPV, in children under the age of 3 years. Patients presenting with severe illness may be suffering from co-infection due to 2 or more viruses. In addition, there are many predisposing factors which make a child susceptible to developing ARI in varying severity. It can range from non-modifiable factors such as birth order and birth weight to modifiable factors such as malnutrition in the child, socio-economic status of the family and also the literacy rate and knowledge of the the mother who is the primary care provider for a child.

The results and observations in the present study show associations and variations, with similar and related studies. A number of factors are responsible for these differences such as duration of the study, selection of study population, limited sample size, variation in genetic, demographic and socio-economic status of the patients under study.

CONCLUSION

Summary

The present study, "Study of viruses affecting the upper respiratory tract in paediatric patients attending a tertiary care hospital in the north eastern part of India" with the objective of understanding the prevalence of Upper Respiratory Tract Infections of viral origin among paediatric patients attending the Paediatrics Department, NEIGRIHMS, Shillong.

The present study was conducted on 50 children in the age group of 12-36 months presenting with features of URTI to the outpatient and in-patient department of the Department of Paediatrics, NEIGRIHMS by selecting patients fulfilling the inclusion and exclusion criteria for a period of one year. Samples in the form of throat swab, NPS, NPA were collected in VTM subjected to RT-PCR.

- were enrolled in the study of which 18 (36%) patients were males and 32 (64%) were females and the highest number of patients 26 (52%) belonged to the age group of 12- 24 months (54.8%).
- Majority of the patients presented with features of URTI (88%), followed by LRTI (10%), pneumonia (2%).
- Among the patients suffering from URTI 80.64% showed positivity for RVs. Rhinovirus was the most prevalent virus causing URTI (88%) followed by hMPV (12%). There was no association between URTI and RSV. However, a positive association was observed between RSV and LRTI. An 80% correlation was observed between occurrence of LRTI and RSV infection.

- The months of winter November, December, January, February were associated with higher prevalence of URTI (60.16%) and LRTI (80%).
- Assessment of the factors which govern RSV associated ARI showed association with low birth weight, high birth order, poor nutritional status and low socioeconomic status (75%).

Conclusion

The present study entitled, "Study of viruses affecting upper respiratory tract with special reference to Respiratory Syncytial Virus in paediatric patients attending, NEIGRIHMS, Shillong" was conducted with a small sample size for a period of one year in consideration of limited resources available.

The study shows the association of Respiratory viruses in 61% of RVI among the paediatric population included in our study where 40.3% were associated with URTI. Rhinovirus was found to be the most common etiological agent of such URTI (88%).

The study has highlighted 83% of association of RV in LRTI, with RSV as the commonest RV infecting the lower respiratory tract (83.3%).

Among the associated factors Rvs were seen mostly to affect the age group of 12-24 months (54.8%) with a female preponderance (61.29%). A positive association was observed between low birth weight, high birth order, overcrowding, lower socioeconomic status, poor nutritional status and negatively with the literacy rate of the mother.

Thus, Acute Respiratory Illness among paediatric age group is a continuing challenge. Proper laboratory diagnosis needs to be undertaken for correct distinction from bacterial disease so that unnecessary antibiotic usage can be halted. Improvement of the demographic features will also go long way in preventing such a killer disease.

REFERENCES

1. Cotton M F, Innes S, Jaspan H, Madide A, Rabie H. Management of upper respiratory tract infection in children. S A Fam Pract.2008; 50(2):6-12.
2. Gorjipour H, Karimi A, Fahimzad A, Shiva F, Fallah F, Shamshiri AR. The seasonal frequency of viruses associated with upper respiratory tract infection in children. Arch Paediatr Infect Disease. 2012; 1(1): 9-13.
3. WHO. Research needs for the Battle against Respiratory Viruses (BRaVE).2013;-:1-6.
4. [Internet] Available from: http://iresearch.worldbank.org/PovcaINet/index.htm/2005-2012 .
5. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull World Health Organisation.2008; 86: 408-416.
6. Selvaraj K, Chinnakali P, Majumdar A, Krishnan I.S. Acute respiratory infections among under-five children in India: A situational analysis. JNSBM. 2014; 5(1):15-20.
7. Gladstone BP, Muliyil JP, Jaff ar S, Wheeler JG, Le Fevre A, Iturriza-Gomara M, *et al*. Infant morbidity in an Indian slum birth cohort. Arch Dis Child .2008; 93:479-84.
8. Jain SK, Khan JA. Epidemiological study of acute diarrhoeal disease and acute respiratory infection amongst under-five children in Alwar district (Rajasthan), India. Indian J Practising Doctor. 2006; 3 (5):11-2.
9. Deb SK. Acute respiratory disease survey in Tripura in case of children below five years of age. J Indian Med Assoc. 1998; 96:111-6.
10. CDC. NHSN Surveillance definitions for specific types of Infections.2015 Jan (modified Apr, 2015):13-14.
11. NFHS 1-3. Causes of neonatal and child mortality in India: A nationally representative mortality-survey-Causes-of-neonatal-and-childmortality-in-India-2010.pdf.Available from:http://cghr.org/wordpress/wp-content/uploads/Causes-of-neonatal-and-child mortality-in-India-2010.pdf
12. Acharya D, Prasanna KS, Nair S, Rao RS. Acute respiratory infections in children: A community based longitudinal study in south India. Indian J Public Health 2003; 47:7-13.
13. Sarkar R, Sivarathinaswamy P, Thangaraj B, Sindhu KN, Ajjampur SS, Muliyil J, *et al*. Burden of childhood diseases and malnutrition in a semi-urban slum in southern India. BMC Public Health 2013; 13:87.
14. Burden of Acute Respiratory tract Infections in India. National Health Profile. CBHI.2015.
15. Turner R B, Cough R. Rhinoviruses. In: Knipe DM, Howley PM(eds).*Field's Virology*. 5th ed. Philadelphia : Lippincott Williams & Wilkins;2007,pp 896-909.
16. Pelon W, Mogabgab WJ, Phillips IA, Pierce WE. A cytopathogenic agent isolated from naval recruits with

mild respiratory illness. Proc Soc Exp Biol Med 1957; 94: 262-267.
17. Tyrrell DAJ, Chanock RM. A description of rhinoviruses. Science 1963; 141:152-153.
18. Kapikian AZ, Conant RM, Hamparian VV, Chanock RM, Chapple PJ, Dick EC,*et al*. Rhinoviruses, a numbering system. Nature 1967; 213: 761-763.
19. Kapikian AZ, Conant RM, Hamparian VV, Conant RM, Dick EC, Gwaltney JM, *et al*. Collaborative report: rhinoviruses-extension of the numbering system. Virology 1971; 43:524-526.
20. Louie JK, Yagi S, Nelson FA, Kiang D, Glasor CA, Rosenberg J, *et al*. Rhinovirus outbreak in a long term care facility for elderly persons associated with unusually high mortality. Clinical Infect Dis. 2005; 41(2):262-265.
21. Jawetz E, Melnick J L, Adelberg E. Medical Microbiology.26th ed. Atlanta: McGraw Hill; 2013.
22. Papadopoulos N G, Xatzipsalti M, Johnston SL. Rhinoviruses. In: Zuckerman *et al* (eds). *Principles & Practice of Clinical Virology*.6th ed. West Sussex, UK: John Wiley &Sons, Ltd., publications; 2009,pp 399-510.
23. Igarashi Y, Skoner DP, Doyle WJ, Fireman P, White MV, Kaliner MA. Analysis of nasal secretions during experimental rhinovirus upper respiratory infections. TJACI.1993; 92: 722-731.
24. Johnston SL, Papi A, Bates PJ, Mastronarde JG, Monick MM, Hunninghake GW. Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. J of Immunology.1998; 160: 6172-81.
25. Papadopoulos, N.G. and Johnston, S.L. Rhinoviruses as pathogens of the lower respiratory tract. Canadian Resp J, 2000;7: 409–14.
26. Schroth MK, Grimm E, Frindt P, Galagan DM, Konno SI, Love R, *et al*. Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. American J of Respiratory Cell and Molecular Biology.1999; 20:1220–28.
27. Bossios A, Psarras S, Gourgiotis D, Skevaki CL, Constantopoulos AG, Papadopoulos NG, *et al*. Rhinovirus infection induces cytotoxicity and delays wound healing in bronchial epithelial cells. Respiratory Research.2005.6, 114.
28. Wilson R., Alton E, Rutman A, Higgins D, Al Nakib W, Geddes DM, *et al*. Upper respiratory tract viral infection and mucociliary clearance. European Journal of Respiratory Diseases. 1987; 70: 272–79.
29. Yuta A, Doyle WJ, Gaumond E, Ali M, Tamarkin L, Baranuik JN, *et al*. Rhinovirus infection induces mucus hypersecretion. American Journal of Physiology.1998; 274: L1017–L1023.
30. McNamara P, Smyth R. The pathogenesis of respiratory syncytial virus disease in childhood. British Medical Bulletin 2002; 61: 13–28.
31. Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis associated hospitalizations among US children, 1980–1996. JAMA. 1999; 282: 1440–6.
32. Stang P, Brandenburg N, Carter B. The economic burden of respiratory syncytial virus associated bronchiolitis hospitalizations. Arch Pediatr Adolesc Med. 2001; 155: 95–6.
33. Simoes EA. Respiratory syncytial virus infection. Lancet. 1999; 354: 847–52.
34. Hall CB. Respiratory syncytial virus. In: Feigin RD, Cherry JD. (eds) Textbook of Pediatric Infectious Diseases. Philadelphia, PA: WB Saunders, 1998; pp 2084–111.
35. van den Hoogen BG, de Jong JC, Groen J, Kuiken T, deGroot R, Fouchier RAM, *et al*. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 2001; 7:719-24.
36. Papadopoulos N G, Psarras S, Johnston SL. Rhinoviruses. In: Zuckerman *et al* (eds). *Principles & Practice of Clinical Virology*.6th ed. West Sussex, UK: John Wiley &Sons, Ltd., publications; 2009,pp 409-439.
37. Ralston S, Hill V. Incidence of apnea in infants hospitalized with respiratory syncytial virus bronchiolitis: a systematic review. J Pediatr. 2009; 155:728–733.
38. Freshney R.I. *Culture of Animal cells: A Manual of Basic Techniques.*5th ed. Hoboken, New Jersey. John Wiley & Sons ,Inc. 2005.
39. Arceneaux C J(ed). Proceedings: Twenty-Sixth Annual Meeting,In: Electron Microscopy Society of America, Baton Rouge: Claitor's Publishing Division; 1968.
40. Bradbury S. The Microscope Past and Present. London: Pergamon Press; 1968.
41. Gabor D. The Electron Microscope. NewYork : Chemical Publishing Company, Inc; 1948.
42. Goldstein J I, Yakowitz, H. Practical Scanning Electron Microscopy. NewYork : Plenum Press; 1975.
43. Marton, L.L. Early History of the Electron Microscope.San Francisco: San Francisco Press, Inc; 1968.
44. McDevitt, H O. Albert Hewett Coons: Biographical memoirs of the National Academy of Sciences; 2012.
45. Mohan K H, Pai S, Rao R, Sripathi H, Prabhu S. Techniques of immunofluorescence and their significance. Indian J Dermatol Venereol Leprol. 2008:74(4); 415-419.
46. Mullis, K. and Faloona, F. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol.1987; 155: 335-350.
47. Saiki R., Scharf S, Faloona F, Mullis K, Horn G, Erlich H. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science. 1985; 230: 1350-54.
48. Lawyer F, Stoffer S, Saiki R., Chang S., Landre P, Abramson R, Gelfand D. High-level expression, purification, and enzymatic characterization of full-length Thermus aquaticus DNA polymerase and a truncated form deficient in 5' to 3' exonuclease activity. PCR Methods and Applications.1993; 2: 275-87.
49. Joshi M, Deshpande J D. Polymerase chain reaction: methods, principles and application. IJBR. 2010; 1 (5):81-97.
50. Monto, A S,Sullivan K M. Acute respiratory illness in the community. Frequency of illness and the agents involved. Epidemiol Infect.*1993;* 110(1): 145-60.
51. Tsai H P, Kuo P H, Liu C C, Wang J R. Respiratory Viral Infections among Pediatric Inpatients and Outpatients in Taiwan from 1997 to 1999. J Clinic Microbiol. 2001; 111-118.
52. de Freitas Souza LS, Ramos EAG, Carvalho FM, Guedes VMCR ,Souza LS, Rocha CM, *et al*. Viral respiratory infections in young children attendingday care in urban Northeast Brazil. Pediatr Pulmon. 2003; 35: 184–191
53. Bharaj P, Broor S, Dar L, Sullender WM, Kabra SK, Kaushik S, *et al*. Respiratory viral infections detected by multiplex PCR among paediatric patients with lower respiratory tract infections seen at an urban hospital in Delhi fro 2005 to 2007. Virology J. 2009; 6:89.
54. Do A H L, vanDoorn H R, Ngheim M N, Bryant J E, thi Hoang T H, Do Q H, *etal*. Viral etiologies of Acute Respiratory Infections among Hospitalized Vietnamese Children in Ho Chi Minh City, 2004–2008. PLoS ONE. 2011; 6(3): e18176.
55. Singh AK, Jain A ,Jain B, Singh K P, Dangi T, Mohan M, *et al*. Viral aetiology of acute lower respiratory tract illness in hospitalised paediatric patients of a tertiary hospital: One year prospective study. Indian J Med Microbiol. 2014; 32(1): 13-18.
56. Wadgave H V, Godale L B. Burden of Acute respiratory tract infections in under-Fives in urban areas. NJCM. 2012; 39-42.
57. Moura F E, Nunes IF, Silva GB Jr, Siqueira M M. Respiratory syncytial virus infections in northeastern Brazil: seasonal trends and general aspects. Am J Trop med Hyg. 2006; 74(1):165-7.
58. Pore P, Ghattargi C H, Rayate MV. Study of risk factors of acute respiratory infection (ARI) in underfives in Solapur. NJCM .2010; 1(2): 64-7.

59. Bonfim CM, Nogueira LM, Simas PVM, Gardinassi LGA, Durigon EL, Rahal P, *et al*. Frequent respiratory pathogens of respiratory tract infections in children attending daycare centers. J Pediatr (Rio J). 2011; 87(5):439-44.
60. Ahmed JA, Katz MA, Auko E, Njenga MK, Weinberg M, Kapella BK *et al*. Epidemiology of respiratory viral infections in two long-term refugee camps in Kenya, 2007-2010.BMC Infectious Dis. 2012; 12 :7.
61. Broor S, Pandey RM, Ghosh M, Maitreyi RS, Lodha R, Singhal T,*et al*. Risk factors for severe acute lower respiratory tract infection in under five children. Indian Pediatrics .2001; 38: 1361-1369.
62. Njouom R, Yekwa EL, Cappy P, Vabret A, Boisier P, Rousset D. Viral Etiology of Influenza-Like Illnesses in Cameroon, January–December 2009.The J of Infectious Diseases 2012;206(S1):S29–35.
63. Williams J V, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM,Edwards KM, *et al*. Human Metapneumovirus and Lower Respiratory Tract Disease in otherwise healthy infants and children. NEJM. 2004; 350(5): 443–450.
64. Kwofie T B, Anane YA, Nkrumah B, Annan A, Nguah SB, Owusu M. Respiratory viruses in children hospitalized for acute lower respiratory tract infection in Ghana. Virology J.2012, 9:78.
65. Pretorius M A, Madhi SA, Cohen C, Naidoo D, Groome M, Moyes J, *et al*. Respiratory Viral Coinfections Identified by a 10-Plex Real-Time Reverse-Transcription Polymerase Chain Reaction Assay in Patients Hospitalized With Severe Acute Respiratory Illness—South Africa, 2009–2010. The J of Infectious Diseases.2012;206(S1):S159–65.
66. Shafik CF, Mohareb EW, Yassin AS, Madgy AA, El Kholy A, Youssef FG, *et al*. Viral etiologies of lower respiratory tract infections among Egyptian children under five years of age. BMC Infectious Dis. 2012; 12:350.
67. Matu M, Kikuvi G, Wanzala P, Karama Md, Symekher S .Aetiology of Acute Respiratory Infections in Children under Five Years in Nakuru, Kenya. J Microbiol Exp 2014, 1(4): 00021.
68. Choi E H, Lee H J, Kim SJ, Eun B W, Kim N H, Lee JA, *et al*. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. Clinical Infectious Dis. 2006; 43:585–92.
69. Miller EK, Lu X, Erdman DD, Zhu Y, Poehling KA, Griffin MR, *et al*. Rhinovirus-Associated Hospitalizations in Young Children.J Infectious Dis . 2007; 195 :773-81.
70. Ebihara T, Endo R, Kikuta H, Ishiguro N, Ishiko H, Hara M . Human Metapneumovirus Infection in Japanese Children. J Clinical Microbiol. 2004; 42(1): 126–132
71. Liu C Y, Xiao Y, Xie ZD, Ren LL , Hu YH, Yao Y,*et al*. Viral etiology of acute respiratory tract infection among paediatric inpatients and outpatients from 2010-2012 in Beijing, China. Chinese J paediatr. 2013; 51(4): 255-9.
72. Weigl JA, Puppe B, Grondahl ,Schmitt HJ. Epidemiological investigation of nine respiratory pathogens in hospitalized children in Germany using multiplex reverse- transcriptase polymerase chain reaction. Eur J Clin Microbiol Infect Dis. 2000 ; 19(5): 336-43.
73. deArruda NE, Hayden FG, McAuliffe JF, deSousa MA, Mota SB, McAuliffe MI, *et al*. Acute respiratory viral infections in ambulatory children of urban northeast Brazil. J Infect Dis .1991; 164(2): 252-8.
74. Nokso-Kovitso J, Pitkaranta A, Blomqvist S, Jokinen J, Kleemola M, Takala A, *et al*. Viral etiology of frequently recurring respiratory tract infections in children. Clinical Infectious Dis. 2002; 35:540-6.
75. Manjarrez, ME, Rosete DP, Rincon M, Villalba J, Cravioto A, Cabrera R. Comparative viral frequency in Mexican children under 5 years of age with and without upper respiratory symptoms. J Med Microbiol. 2003; 52(7): 579-83.
76. Louie JK, Hacker J K, Gonzalez R,, mark J, Maselli JH, Yagi S, *et al*. Characterization of Viral Agents Causing Acute Respiratory Infection in a San Francisco University Medical Center Clinic during the Influenza Season. Clin Infectious Dis. 2005; 41: 822-8.
77. Chonmaitree T, Ravei K, Grady JG,Clos A,Patel JA, Nair S, *et al*. Viral Upper Respiratory Tract Infection and Otitis Media Complication in Young Children. Clin Infectious Dis. 2008; 46:815–23.
78. Nokso–Kovitso J. *Viral upper respiratory tract infections in young children*. Academic dissertation. National Public Health Institute Helsinki, Finland. 2004.
79. Chung J Y, Han T H, Kim SW, Kim CK Hwang S. Detection of viruses identified recently in children with acute wheezing. J Med Virol. 2007; 79(8):1238- 43.
80. Costa L F, Yokosawa J, Mantese OC, Oliviera TFM, Silveira HL, Nepomuceno LL, *et al*. Respiratory viruses in children younger than five years old with acute respiratory disease from 2001 to 2004 in Uberlândia, MG, Brazil. Mem Inst Oswaldo Cruz. 2006; 101(3):301-306.
81. Moreno-Valencia Y, Hemandez-Hernandez VA, Romero-Espinoza JAI, tellez RHC, Lopez MC, Hernandez A, Padilla RP, *et al*. Detection and characterization of respiratory viruses causing acute respiratory illness and asthma exacerbation in children during three different seasons (2011–2014) in Mexico City. Influenza and Other Respiratory Viruses. 2015; 9(6): 287–292.
82. Lina B, Valette M, Foray S, Luciani J, Stagnara J, See DM *et al*. Surveillance of Community-Acquired Viral Infections due to Respiratory Viruses in Rhone-Alpes (France) during Winter 1994 to 1995. J Clinical Microbiol. 1996; 34(12):3007-3011.
83. Andreoletti L, Lesay M, Deschildre A, Lambert V, Dewilde A, Wattre P. Differential detection of rhinoviruses and enteroviruses RNA sequences associated with classical immunofluorescence assay detection of respiratory virus antigens in nasopharyngeal swabs from infants with bronchiolitis. J Med Virol.2000; 61(3): 341-6.
84. Fujitsuka A, Tsukagoshi H, Arakawa M, Goto-Sugai K, Ryo A, Yoshimichi O, *et al*. A molecular epidemiological study of respiratory viruses detected in Japanese children with acute wheezing illness.BMC Infectious Dis. 2011, 11:168.
85. Nasreen S, Luby SP, Brooks WA, Homaira N, AlMamun A, Bhuiyan MV, *et al*. Population-Based Incidence of Severe Acute Respiratory Virus Infections among Children Aged ,5 Years in Rural Bangladesh, June–October 2010. PLoS ONE. 2014; 9(2): e89978.
86. Bonzel L, Tenenbaum T, Schroten H, Schilden O, Adams O, Schweitzer-Krantz S, *et al* . Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. Paediatr Infect Dis J. 2008 Jul; 27(7): 589-94.
87. Canducci F, Debiaggi M, Sampaolo M, Marionozzi MC, Berre S, Terulla C, *et al*. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. J Med Virol. 2008 Apr;80(4):716-23.
88. Calvo C, Pozo F, Garcia-Garcia ML, Sanchez M, Lopez-Volero M, Casas I, *et al*. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. Acta Paediatr.2010; 99(6): 883-87.
89. Cebey-López M, Herberg J, Pardo-Seco J, Gómez-Carballa A, Martinón-Torres N, Salas A, *et al*. Viral Co-Infections in Pediatric Patients Hospitalized with Lower Tract Acute Respiratory Infections. 2015. PLoS ONE 10(9): e0136526.
90. Rajala MS, Sullender WM, Prasad AK, Dar L, Broor S. Genetic variability among Group A and B Respiratory Syncytial Virus isolates from a large referral hospital in New Delhi,India. J of Clinical Microbiol. 2003; 41(6): 2311-2316.
91. Savitha MR, Nandeeshwara SB, Kumar PMJ, ul-Haque F, Raju CK. Modifiable risk factors for acute lower respiratory tract infections. Indian J Pediatr. 2007 ;74 (5):477-82.

92. Prajapati B, Talsania N, Lala M K, Sonalia KN. A study of risk factors of acute respiratory tract infection (ARI) of under five age group in urban and rural communities of Ahmedabad district**,** Gujarat. NJCM .2011; 2(2): 255-59.

93. Abhishek A, Gupta P, Sachan B, Srivsatava J P. Study on prevalence of acute respiratory tract infections (ari) in under five children in Lucknow district. NJMR. 2014; 4(4):298-302.