

Molecular Detection of Upper Respiratory Tract Viral Infection with RSV, HPIV & Adenovirus among SARS-CoV2 Negative patients with respiratory illness admitted in tertiary care hospital in Eastern India

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ABSTRACT

The most prevalent disease-causing agents in humans are respiratory viruses, which have a global influence on morbidity and death. The Adenovirus, respiratory syncytial virus (RSV), and parainfluenza viruses (HPIV) are the dominant respiratory viruses that circulate most often throughout all continents. Vaccines and potent antivirals are not yet available, but progress has been made in understanding their biology and the core problems of host-parasite interaction. The current study aims to study respiratory viral infections among clinical manifestations among SARS-CoV-2 negative patients with pseudo symptoms. This cross-sectional hospital-based study was conducted at ICMR-DHR Viral Research & Diagnostic Laboratory (VRDL), Burdwan Medical College where we used real-time PCR for SARS-CoV-2 screening and viral DNA extraction for Adenovirus screening. Real-time PCR through SyBr green was used to detect RSV, HPIV, and Adenovirus in 120 clinical samples. Beta Actin was used as a test control for DNA compatibility for PCR amplification. Among 120 patients studied, 13 were Adenovirus positive, three RSV positive, and two HPIV positive. 35% were infected with Adenovirus. RSV and HPIV also caused infection, with prevalence rates of 5.83% and 14.17%, respectively. Triple infection was observed in 1 neonatal patient among 120. In conclusion, triple infections in RSV, HPIV, Adeno, and neonates were found, but none of the 120 SARS-CoV2 infections were mono-infections. This suggests that respiratory viral infections among pseudo-SARS-CoV-2 signs and symptoms may lead to future pandemics, and further research is needed to confirm this.

Keywords: Adenovirus; RSV; HPIV; RT-PCR; Clinical Manifestations.

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1. Introduction

The coronavirus illness 2019 (COVID-19) was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that first appeared in Wuhan, China, in December 2019 [1]. According to a few studies, the percentage of

SARS-CoV-2 co-infections with other respiratory viruses ranged from 0 to 20%. [2-5]. Also, it has been discovered that for respiratory illnesses other than SARS-CoV-2, lower respiratory tract (LRT) samples considerably increase the efficiency of diagnosis in comparison to upper respiratory tract (URT) samples [6-7]. The most

common cause of death and morbidity among children globally is acute respiratory infection (ARI) [8-9]. According to research, *Haemophilus influenzae* type b and *Streptococcus pneumoniae* are the main bacterial causes of pediatric pneumonia in both developed and developing nations [10-11]. Moreover, viruses are crucial in the development of ARI or pneumonia in young infants. Understanding the functions of viral pathogens, however, can be challenging given the wide range of viruses that have been linked to severe childhood ARI, including pneumonia and bronchiolitis, including respiratory syncytial virus (RSV), human rhinovirus (HRV), influenza viruses (FLU) A and B, parainfluenza virus (PIV), and human metapneumovirus (hMPV). According to systematic research, 22% of children's severe ARI episodes had a relationship. According to a comprehensive analysis, RSV infections account for 22% of children's severe ARI episodes [12]. There is a dearth of concrete data or population-based information about the impact of respiratory viruses on pediatric ARIs in poor nations, particularly in Southeast Asia. However, seasonal variations in viral outbreaks may have consequences on the prevalence of ARI or pneumonia patients admitted to hospitals. Data from healthy children are also important, even while respiratory viruses such as HRV have been identified in 5- 18% of asymptomatic children and their involvement in pediatrics ARI is still disputed [13-14]. Recent advances in molecular microbiology techniques like multiplex PCR [15], which have made it possible to concurrently detect numerous viruses with remarkable sensitivity and specificity, suggest that multiple viral infections in children with ARIs are not atypical. However, more information is required to fully understand how respiratory viruses, either as a single infection or many infections, contribute to severe ARI. Particularly, the confluence of respiratory viruses that raises the risk of lower respiratory tract infections in

children.

The most frequent cause of morbidity in high-income nations is acute lower respiratory infection, but in low- and middle-income countries, the situation is significantly worse [16-17]. The most frequent causes of acute lower respiratory infections in newborns and young children are viral pathogens [18]. Respiratory syncytial virus (RSV) is the most common viral cause of acute lower respiratory infections globally [19]. According to Langley and Anderson (2011), RSV is also the most significant viral pathogen in infancy. RSV is unquestionably a significant respiratory pathogen in young children, but other viruses, including adenoviruses, influenza, parainfluenza, rhinoviruses, and human metapneumovirus, have also been reported to be significant causes of acute lower respiratory infection in young children [20]. Adenoviruses are the second most common respiratory viral pathogens after RSV, so they have a substantial role in pediatric acute lower respiratory infection [21-22]. According to certain studies, adenoviruses are to blame for 4-10% of pneumonia cases and up to 10% of bronchiolitis cases [23].

Adenoviruses and RSV may both be isolated year-round, with no discernible seasonal trend and in certain circumstances; they can cause more severe illness than RSV. Studies are yet required to comprehend the epidemiology, clinical traits, seasonality, and risk factors that can make a kid more vulnerable to viral infections. To lessen the heavy burden of the disease, these studies are crucial for identifying the prevalence and seasonal pattern of the viruses, forecasting epidemics, and implementing preventative measures, particularly for high-risk pediatric populations [24]. Lower respiratory tract infections (LRTI) in young infants are most frequently caused by the respiratory syncytial virus (RSV), an enveloped RNA virus of the

Paramyxoviridae family and Pneumovirinae subfamily. It spreads by respiratory tract secretions and may persist on non-porous surfaces for more than 24 h. Infants may have upper respiratory tract sickness, including rhinorrhoea and congestion, with or without a fever, following the infection's incubation period of three to five days. Up to 40% of newborns develop LRTI, which ranges in severity from mild to moderate illness to potentially fatal respiratory failure and cyanosis. RSV infects almost all people throughout the early years of life, but the ensuing immunity is neither comprehensive nor maintained [25].

RSV infections occur from late fall through early spring in temperate climates over a season of 4–6 months, with a clear pattern of winter incidence. In climates with high annual precipitation, RSV infections usually peak during wet months, while in warm/hot climates and arid climates, RSV incidence peaks during cooler months. Higher-latitude locations tend to have broader variation, even within individual temperate zones, with peak activity outside of typical winter months [25].

To precisely determine the incidence and impact of single and multiple respiratory viral infections on hospitalization with ARI and the risk of RTIs in central Vietnam, we conducted a population-based prospective surveillance study and a case-control study over a period of 12 months with various co-morbidities across studies to provide a comprehensive representation of the burden of RSV, Adeno, and HPIV.

ICMR-DHR Viral Research & Diagnostic Laboratory (VRDL), Department of Microbiology at Burdwan Medical College in West Bengal undertook an institutionally based cross-sectional study from January to October of 2022. In a viral transport medium (VTM), 120 Nasopharyngeal and oropharyngeal throat swabs

in total were collected. Samples were collected with a properly completed proforma that included information on the patient's demographics, symptom onset date, co-morbidities, travel/contact history, etc. Within 2-3 hours after receiving the samples, these samples were processed at a Bio Safety Level 2 (BSL2) laboratory.

2. Material & Methods

2.1. Ethical Statement

This cross-sectional hospital-based study has been approved by the Institutional Ethics Committee (IEC) of Burdwan Medical College, West Bengal.

2.2. Viral RNA Extraction

According to the manufacturer's instructions, the HiPurA Viral Automatic RNA Purification Kit (HiMedia) was used to separate the patient's RNA from Nasopharyngeal and oropharyngeal swabs found in VTM. The concentration of RNA will be determined using a spectrophotometer to detect OD values in 260nm.

2.3. Real-Time PCR for SARS-CoV-2 Screening

By using real-time PCR, the nucleic acid isolated from the samples was examined. We employed the COVIPATH Applied Biosystems Kit (Thermo Fisher, USA) for this procedure, which uses two probes for two target sequences that are unique to SARS-CoV-2 and one target sequence that is unique to RNase P. Positive samples are taken out of the research when the results are interpreted. In addition to the history of ARDS and ILS, we also processed samples that were negative for the SARS-CoV-2 virus.

2.4. Viral DNA Extraction

Among SARS-CoV-2 negative patients, we further extracted DNA from the NP/OP swab for the Adenovirus screening process so according to

the manufacturer's instructions, the HiPurA Viral DNA Purification Kit (HiMedia, India) was used to separate the patient's DNA from NP-OP swabs found in VTM. With the OD value's help, we will determine the DNA concentration in the respective samples.

2.5. C-DNA Synthesis

The Extracted RNA of the patient's sample was then preceded for the C-DNA synthesis process by HiMedia, India C-DNA Synthesis Kit (MBT076). As Per the Manufacturer's Protocol,

we converted all our RNA into C-DNA by using this kit.

2.6. Oligonucleotide Design and Synthesis

In this study, 2 sets of primers were used for Respiratory Syncytial Virus (RSV) A & B. Single set for Adenovirus and 3 sets for Human Parainfluenza virus (HPIV) were used. The primers were designed from NCBI Primer-Blast online. The primer sequences are listed in **Table 1**.

Table 1. Demographic data of viral infection among SARS-CoV-2 negative patients

Age Groups	Total Patients	RSV Positive	HPIV1 Positive	HPIV2 Positive	HPIV3 Positive	Adeno Positive
≤ 15 Yr	48	3	2	3	2	13
16-30 Yr	17	2	2	0	0	2
31-45 Yr	19	0	1	0	0	9
46-60 Yr	21	2	3	1	0	13
61-75 Yr	11	0	1	2	0	3
≥ 76 Yr	4	0	0	0	0	2
SEX						
Male	56	4	3	4	2	20
Female	64	3	6	2	0	22

2.7. Real-Time PCR through SyBr green

The total 120 SARS CoV-2 negative samples go for further viral panel testing. The qualitative SyBr green dye manufactured by Hi-Media, India, was utilized to carry out real-time PCR reactions that focus on the different regions of the RSV, HPIV, and Adenovirus. The components of each reaction were a 25-l reaction, a 1X master mix, and specially crafted primers for a brief sequence of the different genes mentioned in Table 1. Bio-Rad CFX initial denaturation incubation at 95 °C for 2 minutes was followed by 38 cycles of alternate 95 °C incubations for 5 seconds, 56 °C incubations for 30 sec, and 72 °C incubations for 10 sec. With each 72 °C extension incubation, fluorescence was seen. This procedure allowed us to establish the presence of the viruses in 120 clinical samples. The presence

of the housekeeping gene Beta Actin was used as a proxy for the DNA compatibility of each material for PCR amplification.

3. Results

We studied 120 patients and discovered that the majority of patients (n= 48) were under the age of 15, that 13 individuals were Adenovirus positive, three individuals were RSV positive, and two individuals were HPIV 1, two, and three positive, respectively. Another group (n= 17) was discovered that was divided into Adeno 2 positive, RSV 2 individuals positive, and HPIV1 2 positive. In the third category, the age group 31–45 years (n= 19), nine individuals are Adenovirus positive, and one individual is HPIV1 positive. We had a category 4 group from 46–60 years (n = 21) and found 13 individuals were Adenovirus positive, followed by 2 RSV

infected, 3 HPIV1 positive, and 1 HPIV2 positive. In the fifth age group, 61-75 years (n= 11), three people tested positive for Adenovirus, one tested positive for HPIV1, and two tested positive for HPIV2. The final category included patients more than 76 years old (n= 4); among

these individuals, 2 were Adenovirus positive. All demographic data is illustrated in Table 1. All 120 patients tested negative for SARS-CoV2, and 35% were infected with Adenovirus. RSV and HPIV also cause infection, with prevalence rates of 5.83% and 14.17%, respectively (**Table 2**).

Table 2. Prevalence rate of infected patients

Virus Name	Positive Sample	Negative Sample	Prevalence (N= 120)
SARS-COV2	0	120	0%
RSV	7	113	5.83%
HPIV	17	103	14.17%
ADENO	42	78	35%

In our study total of 120 patients' signs and symptoms were illustrated concerning RSV, HPIV (1-3) adeno-infected patients. Some

dominant parameters were discussed in **Tables 3 & and 4**. In between 10-35%, all parameters are hiked in adeno virus-positive patients.

Table 3. Signs & Symptoms of infected patients in URTVI (Upper Respiratory Tract Virus Infection), (n=120)

Parameters	RSV Positive	HPIV1 Positive	HPIV2 Positive	HPIV3 Positive	Adeno Positive
Wheezing	7(5.83%)	6(5%)	5(4.17%)	1(0.83%)	40(33.33%)
Fever	6(5%)	8(6.67%)	4(3.33%)	1(0.83%)	41(34.17%)
Diarrhea	5(4.17%)	1(0.83%)	2(1.67%)	1(0.83%)	13(10.83%)
Vomiting	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	8(6.67%)
Cough	3(2.5%)	3(2.5%)	6(5%)	2(1.67%)	38(31.67%)
Pneumonia	5(4.17%)	2(1.67%)	5(4.17%)	1(0.83%)	14(11.67%)
Runny Nose	6(5%)	4(3.33%)	3(2.5%)	2(1.67%)	37(30.83%)
Cyanosis	1(0.83%)	3(2.5%)	4(3.33%)	1(0.83%)	10(8.33%)
Tachypnoea	2(1.67%)	2(1.67%)	5(4.17%)	2(1.67%)	12(10%)
Shortness of breath	4(3.33%)	5(4.17%)	2(1.67%)	1(0.83%)	29(24.17%)
Laryngitis	2(1.67%)	1(0.83%)	3(2.5%)	1(0.83%)	21(17.5%)

Table 4. Age and clinical manifestations of co-infection with viral infections (n=120)

Parameters	RSV + Adeno	Adeno + HPIV	RSV+ HPIV	RSV+ Adeno +HPIV
Age				
Total Paediatric Population	3(2.5%)	2(1.67%)	1(0.83%)	1(0.83%)
• Neonates	3(2.5%)	2(1.67%)	1(0.83%)	1(0.83%)
• Infants	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
• Children	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
• Adolescence	0(0.00%)	5(4.17%)	0(0.00%)	0(0.00%)
Total Adult Population	2(1.67%)	5(4.17%)	0(0.00%)	0(0.00%)
Clinical Manifestations				
• Wheezing	5(4.17%)	3(2.5%)	1(0.83%)	1(0.83%)
• Fever	5(4.17%)	4(2.5%)	1(0.83%)	1(0.83%)
• Diarrhea	2(1.67%)	0(0.00%)	0(0.00%)	1(0.83%)
• Vomiting	3(2.5%)	1(0.83%)	0(0.00%)	0(0.00%)
• Cough	2(1.67%)	3(2.5%)	1(0.83%)	0(0.00%)
• Pneumonia	1(0.83%)	0(0.00%)	0(0.00%)	0(0.00%)
• Runny Nose	2(1.67%)	2(1.67%)	1(0.83%)	1(0.83%)
• Cyanosis	0(0.00%)	0(0.00%)	0(0.00%)	1(0.83%)
• Tachypnea	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)
• Shortness of breath	1(0.83%)	0(0.00%)	0(0.00%)	1(0.83%)
• Laryngitis	0(0.00%)	0(0.00%)	0(0.00%)	1(0.83%)

4. Discussion

In this study, we observed an increase in the positivity rate of the adenovirus concerning RSV and HPIV infections. The Abnormal re-emergence of RSV, HPIV, and Adeno during COVID-19 was also observed during the year 2022.

The SARS-CoV-2 pandemic affects RSV just like it does other infectious illnesses. Our results support the shift in seasonality noted in previous studies conducted throughout the globe and highlight the significance of monitoring systems for understanding disease dynamics and foreseeing potential repercussions for medical services. One of the variables that might have contributed to the epidemics' delayed commencement and the low number of cases detected is viral interference. Other potential causes include SARS-CoV-2 control efforts and individual behavioral changes brought on by the pandemic [26].

There aren't many studies from underdeveloped nations on etiological agents in the same patient cohort that take the shape of viruses and atypical organisms. It's also crucial to note that the topics of these investigations are either certain age groups or the upper or lower respiratory systems. With a focus on potential pathogens, viruses, and atypical agents, we looked at the etiology of upper and lower respiratory tract infections in a variety of age groups of children. In 57.9% of cases, specific etiological agents could be identified. The percentage of etiological agent identification in children with ARI ranges from 2% to 94%. Its rate relies on a variety of variables, including laboratory and procedural variations, illness severity, kind of studied materials, number of stages of the diseases, potential infections, etc. [27-29].

According to past research published in several journals, the majority of viral pathogens were found in children under the age of three. B,

10 RSV is the pathogen that is most often found in children with ARI in the vast majority of investigations. ~I, 12, 13 Only children between the ages of 6 and 12 months were included in our study when RSV was the main viral pathogen. Among the entire group of patients, parainfluenza, adenovirus, and influenza A were the most common causes of ARI. There are just a few reports that do not indicate the RSV's dominance, together with the occurrence of parainfluenza, adenovirus 14, and influenza 15. This unexpectedly low prevalence of RSV in our study can be attributed to the age of the patients: although most studies focused on children under the age of one or three when the risk of RSV is highest, our study also looked at older children. Moreover, the geo-regional characteristics of respiratory infections should be considered [30].

Our result showed that the adenovirus was the dominant common viral pathogen, followed by Human Parainfluenza and respiratory syncytial virus. The higher number of adenovirus-positive patients was in the age groups below 15 years and the other age group was 46 to 60 years. A higher number of mixed-infection was established in neonate populations, where triple viral infection was observed. The prevalence rate of triple infection was 0.83% and it also reported that mixed infection occurred below 3 years of age group in a published article [30].

The clinical manifestations of the respiratory tract viral infection are more prominent in adenoviral infections than in others. Co-infection with various combinations of RSV, HPIV, and Adenovirus was observed in our study along with triple infections.

In conclusion, the outcome of our study was the finding of co-infections in RSV, HPIV, and Adeno as well as triple infections also found in neonates in the pediatric population. Surprisingly, we have also found within 120 SARS-CoV2

negative samples no infections were mono-infections. Rather all infections were co-infections; Adenovirus is predominant in all the co-infections. The burden of respiratory viral infections among pseudo-SARS-CoV-2 signs and symptoms patients may lead to future pandemics. There is a scope for clinicians and researchers to do further study with the help of our study.

Declarations

Ethics approval and consent to participate

This cross-sectional hospital-based study has been approved by the institutional Ethics Committee (IEC) of Burdwan Medical College, Memo No: BMC/I.E.C/58 dated 09.02.2023.

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ICMR-DHR Viral Research & Diagnostic Laboratory, Department of Microbiology, Burdwan Medical College, Burdwan.

Authors Contribution

NM, Research Plan Implementation, Draft writing, Data analysis; SN, SM & TB, Review literature and draft editing; AN, Research plan conceptualization, plan designing, draft editing, data interpretation.

Conflict of Interest

No conflict of interest.

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