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Original Article

Microbiology Section

Epidemiology of Respiratory Syncytial Virus in Hospitalised Adult Patients with Respiratory Tract Infections: A Cross-sectional Study from a Tertiary Care Institute, Amritsar, Punjab, India

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ABSTRACT

Introduction: Respiratory Syncytial Virus (RSV) is increasingly recognised as an important cause of severe Respiratory Tract Infections (RTIs) in adults, especially those with underlying co-morbidities, all over the world. However, the magnitude and nature of the effects of severe RSV infections are underestimated in our geographical area.

Aim: To determine the prevalence of RSV among hospitalised adult patients with RTIs using Real-Time Polymerase Chain Reaction (RT-PCR), along with an assessment of the clinical profile and underlying co-morbidities associated with it.

Materials and Methods: This cross-sectional study was conducted at the Virus Research and Diagnostic Laboratory (VRDL) in the Department of Virology at Government Medical College, Amritsar, Punjab, India. Nasopharyngeal swabs were taken from 158 adult patients with RTIs, belonging to different age groups, genders and localities. The study was conducted from December 2022 to April 2024. Nucleic acid was extracted using the magnetic bead extraction technique, and RT-PCR was performed to detect and differentiate between RSV-A and RSV-B. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 23.0, with a p-value<0.05 considered significant.

Results: Out of 158 nasopharyngeal swabs, 15 (9.5%) tested positive for RSV. Cough, dyspnoea and wheezing, along with normal haematological parameters, were significant clinical features in RSV-positive patients. Most of the RSV-positive patients required intensive care. Diabetes mellitus in 5 (14.3%), Coronary Artery Disease (CAD) in 2 (10.5%), and Chronic Obstructive Pulmonary Disease (COPD) in 10 (10.2%) were significant underlying co-morbidities among RSV patients. The disease exhibited a typical seasonal pattern, with a clear spike in all cases during the winter months. Present study reported that the dominant circulating subtype in the geographical area was RSV-B in all cases.

Conclusion: The burden of RSV infections in adults is underestimated, but it is significant. Accurate estimation of the prevalence of RSV is important to understand its clinical and economic burden, which can guide policymakers in the development of RSV prophylaxis, including RSV vaccines.

Keywords: Co-morbidities, Hospitalised adult patients, Real-time reverse trancriptase polymerase chain reaction

INTRODUCTION

RSV, previously well understood primarily in infants, is now acknowledged as a significant contributor to severe respiratory illness among older adults. It particularly affects individuals with pre-existing pulmonary or cardiovascular conditions, as well as those with severe immunosuppression [1]. Throughout life, RSV infection and reinfection are common occurrences. In healthy younger adults, these infections typically result in mild symptoms. However, in the older adult population, they can lead to serious complications [2]. RSV was estimated to account for approximately 214,000 acute lower respiratory infection hospitalisations per year in adults aged 65 years and above in industrialised countries, with a confidence interval of 95% ranging from 100,000 to 459,000 [3]. According to the Global Burden of Disease Study 2016, there were 25 million episodes of RSV infections worldwide, resulting in 77,000 associated deaths [4].

The virus is primarily transmitted through respiratory secretions and can remain viable for over 24 hours on non porous surfaces. Following an incubation period of approximately 3 to 5 days, patients typically develop symptoms of upper RTIs, including rhinorrhoea, congestion and a non productive cough often accompanied by fever. As the disease progresses to the lower respiratory tract, the cough becomes more pronounced and productive. Wheezing may be detected upon auscultation of the patient [5]. Confirming RSV infection in adults requires laboratory testing of respiratory secretions due to the virus's non specific clinical manifestations. However, detecting RSV in adults can be challenging due to low viral titers and shorter durations of viral shedding compared to young children, which can limit the effectiveness of current diagnostic methods [6].

The four main methods for diagnosing RSV infection in adults include culture, antigen detection via Immunofluorescence Assay (IFA) or Enzyme Immunoassay (EIA), Ribonucleic Acid (RNA) detection through RT-PCR and serological testing, which involves demonstrating RSV-specific IgM acutely or observing a significant increase in RSV-specific IgG antibodies between acute and convalescent-phase sera. However, it's important to note that the serological method only offers a retrospective diagnosis [7].

RSV isolates are categorised into two primary antigenic groups, A and B, each of which can be subdivided further [5]. RSV infections typically follow a seasonal pattern with minor variations depending on the geography of the area. Both groups may co-circulate simultaneously during a seasonal outbreak, but their proportions may vary [8].

At present, the management of RSV infection in adults primarily revolves around supportive therapy, which may involve the administration of bronchodilators, supplemental oxygen, mechanical ventilation, intravenous fluids and antipyretics. Several antivirals and vaccines for the treatment and prevention of RSV infection are currently undergoing various clinical stages of development [9]. A comprehensive understanding of the disease's epidemiology and its clinical outcomes in older adults is necessary to assess their cost-effectiveness and make further recommendations.

In May 2023, the Food and Drug Administration (FDA) approved the first vaccines for the prevention of RSV-associated Lower Respiratory Tract Disease (LRTD) in adults aged 60 years and above. RSVPreF3 (Arexvy, GSK) is a single-dose (0.5 mL) adjuvanted (AS01E) recombinant stabilised prefusion F protein (preF) vaccine. Another approved vaccine, RSVpreF (Abrysvo, Pfizer), is also a single-dose (0.5 mL) recombinant stabilised preF vaccine [10].

In India, there are currently no established guidelines for the prevention or treatment of the disease, particularly within the adult age group. The necessity for an RSV vaccine in India is both evident and critical. However, the epidemiology of RSV in the country is not extensively documented. A collective effort involving microbiologists, physicians and public health experts is necessary to comprehend the disease burden and the seasonality of RSV infections in a country as vast as India [11].

Present study aimed to determine the prevalence of RSV in the study population using multiplex real-time PCR. This study marks a significant step towards addressing the imperative need to understand the epidemiology and disease burden of RSV infections among the vulnerable yet understudied patient group, particularly in our geographical area (Punjab, India).

Therefore, aim was to estimate the prevalence of RSV in adult patients admitted with RTIs in a tertiary care hospital in Amritsar, and the objective of the study was to assess the clinical profile and associated co-morbidities of RSV infections.

MATERIALS AND METHODS

This cross-sectional study was conducted in VRDL, Department of Virology, and the Department of Microbiology at Government Medical College, Amritsar, Punjab, India. Nasopharyngeal and oropharyngeal samples were taken from patients with RTIs admitted to the Guru Nanak Dev Hospital complex during the period from December 2022 to April 2024. The Institutional Ethical Committee (IEC) approved the study with approval number 10749/D-26/2021. Written informed consent was obtained from all the patients.

Inclusion criteria: Patients with RTIs of all genders and age groups: 18 to above 80 years, patients who provided written informed consent were included in the study.

Exclusion criteria: Patients younger than 18 years of age, patients who refused to provide consent were excluded from the study.

Study Procedure

This being a time-bound study, the subjects available during the study duration were included. Nasopharyngeal and oropharyngeal swabs were taken from 158 patients. The patient's head was tilted back to 70 degrees, and a minitip swab with a flexible shaft was inserted through the nostril parallel to the palate until resistance was encountered. The distance was equivalent to that from the nostril to the patient's ear. After gently rubbing and rolling the swab in place for a few seconds, it was removed while rotating. The same swab was inserted in a similar manner into the second nostril. The swab was immediately transported to the laboratory in viral transport medium. The samples were stored at -80°C until further processing.

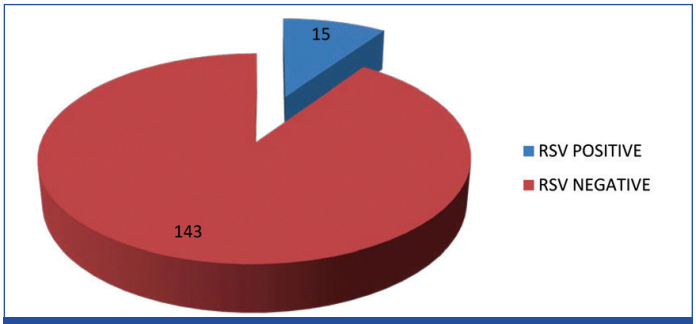
RNA was extracted using the magnetic bead extraction method to obtain total RNA from the sample. The extracted viral RNA was reverse transcribed using the Real Star RSV RT-PCR Kit-3 [12], which is based on RT-PCR technology for the detection and differentiation of RSV A and RSV B specific RNA. The assay was designed for two purposes: firstly, to detect RSV in the specimen and secondly, to differentiate between RSV subgroups A and B. The N gene, being the most conserved gene in the RSV genome, served as the target gene. In the Real Star RSV RT-PCR Kit-3, probes specific for RSV A RNA were labelled with a fluorophore exhibiting characteristics similar to Cy®5, whereas probes specific for RSV B RNA were labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) was labelled with the fluorophore JOE™. The cut-off Ct value was set at 40.

STATISTICAL ANALYSIS

Data were entered into Microsoft Excel sheets, and statistical analysis was conducted using the "Statistical Package for the Social Sciences (SPSS) software version 23.0" for Windows. Categorical data were presented as percentages. The Mann-Whitney U test was used as the test of significance. A p-value <0.05 was considered statistically significant.

RESULTS

The study population included 158 RTI patients, of whom 15 (9.5%) were found positive for RSV on RT-PCR [Table/Fig-1]. Among the RSV-positive cases, the highest prevalence was reported in the age group of 61 to 80 years (12.9%), and the male-to-female ratio was 8:7 [Table/Fig-2].



[Table/Fig-1]: Prevalence of RSV.

Parameters	Total	RSV positive n=15 (%)	RSV negative n=143 (%)
Age group (years)			
18-40	43	5 (11.6)	38 (88.4)
41-60	74	6 (8.1)	68 (91.9)
61-80	31	4 (12.9)	27 (87.1)
>80	10	0	10 (100)
Gender			
Male	93	8 (53.4)	85 (59.5)
Female	65	7 (46.6)	58 (40.5)
Locality			
Rural	77	6 (40%)	71 (49.6%)
Urban	81	9 (60%)	72 (50.4%)

[Table/Fig-2]: Demographic profile of the study population.

The predominant clinical signs and symptoms identified in RSV-positive patients included cough, wheeze, dyspnoea, sore throat and fever. Cough, dyspnoea, wheeze and fever were the most common clinical presentations among the RSV-positive patients [Table/Fig-3]. It was noted that the mean haematological parameters, total leukocyte counts, and differential leukocyte counts were within the normal range among the RSV-positive patients. Among co-morbidities, diabetes mellitus, COPD, and CAD were significantly associated with RSV.

The prevalence of RSV peaked during the winter months, particularly in December and January. The study spanned two winter seasons and one intervening summer season. Out of the total 158 samples, 48 samples were collected during the first winter season from December 2022 to February 2023, while 77 samples were collected during the second winter season from December 2023 to February 2024. Additionally, 33 samples were obtained during the intervening summer season. Notably, all positive samples for RSV were identified during the winter seasons, with no RSV-positive samples recorded during the summer months [Table/Fig-4].

Out of the 158 samples analysed, all 15 positive samples were identified as belonging to the RSV-B subgroup, with none attributed to the RSV-A subgroup. This finding indicates that the RSV-B subgroup was the predominant strain circulating within this population during the period from 2022 to 2024.

Clinical feature	Total	RSV (+) with C/F	%RSV + with C/F (n=158)	RSV (-) with C/F	%RSV - with C/F (n=158)	p-value
Cough	152	15	9.5	137	86.7	**0.001**
Dyspnoea	140	14	8.9	126	79.7	**0.001**
Nasal congestion	43	11	6.9	32	20.3	0.142
Sore throat	46	9	5.7	37	23.4	0.112
Fever	68	6	3.8	62	39.2	**0.004**
Wheeze	102	14	8.9	87	55.0	**0.001**
Co-morbidity	**Total**	**RSV +**	**%RSV +**	**RSV -**	**%RSV -**	**p-value**
Diabetes mellitus	35	5	14.3	30	85.7	**0.001**
COPD	98	10	10.2	88	89.8	**0.001**
CAD	19	2	10.5	17	89.5	**0.001**

[Table/Fig-3]: Distribution of clinical features and comorbidities.
(Statistical test- Mann-Whitney U test was applied with p-value <0.05 considered significant)
(C/F: Clinical feature); COPD: Chronic obstructive pulmonary disease; CAD:Coronary artery disease

Seasonal distribution	Total samples	RSV positive samples
Dec 2022 to Feb 2023 (First winter season)	48	6
Mar 2023 to Nov 2023 (Summer season)	33	0
Dec 2023 to Feb 2024 (Second winter season)	77	9

[Table/Fig-4]: Seasonal distribution of the study population and RSV positive case.

DISCUSSION

Despite previous efforts to estimate the prevalence of RSV, there is little to no data and very low awareness regarding RSV as a cause of disease among adults in low- and middle-income countries like India [11]. The present study holds particular importance as it focuses solely on RSV in adults within a specific geographical area, utilising the most sensitive diagnostic technique, RT-PCR.

The observed prevalence rate of 9.5% in present study aligns with previous global reports. Falsey AR et al., and Tin Tin Htar M et al., reported rates ranging from 4 to 10% and 1 to 10%, respectively [13,14]. It is worth noting that a study conducted by Hindupur MT et al., in Chennai reported a prevalence rate of 22% [15]. However, it is important to mention that more than 90% of the study population in that study comprised paediatric patients, although adults were also included. In India, various hospital-based studies are primarily conducted in children, and the rates of RSV detection have varied widely, ranging from 5 to 54%. A few studies conducted in India also reported rates of RSV detection that ranged from 8 to 15% [11]. These variations could be attributed to differences in methodologies used, as well as demographic profiles, climatic conditions, environmental factors and the severity of epidemics.

It was surprising to see that the prevalence of RSV in individual age groups was notable, being highest in the age group of 61 to 80 years. Additionally, present study finding that RSV-positive patients in this vulnerable age group required ICU care reflects the serious nature of the disease in this demographic. This makes members of this age group ideal candidates for vaccine prophylaxis. A similar higher incidence among older patients was reported in a study by Branche AR et al., [16]. A study by Savic M et al., also highlighted the need for RSV prophylaxis in individuals over 60 years of age [17].

The clinical manifestation of RSV infection is quite variable, with symptoms ranging from a mild cold to severe respiratory distress. It is important to mention that almost all patients presented with complaints consistent with the expected clinical manifestations of RSV infection, such as upper respiratory symptoms-including cough, nasal congestion and sore throat-and, in severe cases, dyspnoea, wheezing and rhonchi. These findings are in accordance with studies by Bandla SS et al., and Dowell SF et al., [8,18].

At the same time, it is noteworthy that the mean haematological parameters, such as total leukocyte count and differential leukocyte count, were within the normal range among the RSV-infected patients, as reported by Bandla SS et al., and Dowell SF et al., Therefore, keeping all these findings in mind, clinicians in our geographic area should have a strong suspicion of RSV infection in adult patients hospitalised with lower respiratory tract symptoms during the winter season, especially those who present with wheezing, rhonchi and a normal WBC count [8,18].

While the prevalence of RSV in adults is notably lower compared to paediatric populations, the severity of RSV infections in adults necessitates aggressive medical intervention. This is particularly evident in ICU settings where higher positivity rates are observed, indicating a progression to more severe illness. Out of the 15 patients found positive in present study, 12 were from ICU settings rather than general wards. A similar finding was reported in the 'Morbidity and Mortality Weekly Report' by the CDC in its October 2023 edition [19].

The heightened severity of RSV-related disease in adults may be attributed to underlying co-morbidities. Among the positive patients, a significant proportion had pre-existing conditions such as COPD, diabetes mellitus and CAD, which are known risk factors for RSV infections. This was consistent with previous studies by Branche AR et al., and Shi T et al., underscoring the increased morbidity associated with RSV infections in individuals with these co-morbidities [16,20]. Cardiopulmonary conditions and immunocompromised status are positively correlated with the severity of RSV infections necessitating ICU admissions, as noted by Haver FP and Mac S et al., [19,21].

These findings emphasise the importance of routine screening for RSV alongside COVID-19 and influenza in these high-risk patients with respiratory illnesses. Furthermore, they highlight the urgency of considering vaccination strategies targeting this vulnerable patient population.

In this study, the prevalence of RSV infections was higher in patients from urban settings than in those from rural settings. This finding was strongly supported by Tin Tin Htar M et al., and Rowlinson et al., [14,22]. Similar findings were reported by Branche AR et al., [16]. Due to the longer survival of RSV in the environment, indirect contact (via fomites) can be an efficient means of transmission of RSV infection [23]. Authors hypothesise that the higher rates in urban settings may be attributed to a denser population, crowding and higher frequency of public transport use in these areas. Thus, social distancing and hand hygiene can be important non pharmaceutical measures to prevent the spread of RSV infection.

Even after the decline of COVID-19, our society should continue to uphold norms of social distancing and maintain the hygiene habits that were developed during the pandemic. These practices have proven effective in reducing the transmission of various infectious diseases, including respiratory infections like RSV. Continuing these habits can help protect public health by minimising the spread of infections, ultimately contributing to a healthier society.

The description of seasonality patterns of RSV is of utmost importance, as it would help in planning intervention strategies, saving costs and allowing maximum benefits. In present study, there was a clear seasonal pattern with peaks in the winter months of December to January. Similar patterns have been confirmed in studies in India by Baroor S et al., Hindupur A et al., and Grewal GK et al., [11,15,24]. At the same time, a study in India by Bandla SS et al., reported a correlation between RSV and the monsoon season [8]. This information will guide authorities in issuing timely warnings and necessary guidelines regarding non pharmaceutical measures such as masking, social distancing and staying at home during the seasonal epidemic of RSV, especially for vulnerable groups within the population.

The RSV-B subtype prevailed across both consecutive seasons. A study in India by Bandla SS et al., reported co-circulation of RSV-A and RSV-B in 2016, 2017, and 2018, with RSV-A dominance in 2016 and RSV-B dominance in 2017 and 2018 [8]. However, a study in Korea by Kim T et al., reported alternate dominance of the two subtypes in different seasons of RSV [23].

Limitation(s)

The study has some limitations. First, the research was conducted in only one hospital. The epidemiology of the disease could be better understood if more hospitals were included. Second, the study period included only two winters and one summer season. Including more seasons would definitely provide better data. Third, cases of some underlying diseases, such as solid cancers and haematological malignancies, were not available. Nevertheless, this study will be helpful in recognising the magnitude of RSV infection in the adult age group and in taking necessary countermeasures.

CONCLUSION(S)

It was observed that the underestimated burden of RSV in adults stems from a lack of awareness. Present research highlights the crucial necessity of monitoring RSV infections, particularly during annual influenza outbreaks, within the growing elderly and high-risk populations. Learning from the lessons of the tripledemic of Influenza A virus, SARS-CoV-2, and RSV experienced in the United States, the diagnostic panel for every patient with respiratory tract disease during the season should include RSV testing.

Authors unequivocally advocate for the development of RSV prophylaxis, such as an RSV vaccine, using shared clinical decision-making, following the lead of initiatives in the USA. An accurate estimation of the prevalence of RSV in the adult age group is important for fully understanding its clinical and economic burden, so informed policy decisions can be made regarding the implementation of vaccines once approved in our resource-limited settings.

Present study proposes making RSV a notifiable disease. It also emphasises the necessity of a national program for continuous surveillance to monitor the burden, control, prevention and treatment of viral respiratory diseases such as RSV, Influenza and COVID-19 in our country. These diseases significantly contribute to the economic burden on society and improved surveillance and management could help mitigate their impact.

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