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Viral etiology in patients hospitalized with Acute Exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD): A case control study

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ABSTRACT: Chronic obstructive pulmonary disease is the leading cause of fatality. The course of COPD is followed by episodes of acute deterioration in respiratory health, referred as 'exacerbations'. Acute exacerbations of COPD contribute substantially to the morbidity and mortality due to number of infectious agents including bacteria, viruses, or both. Therefore, we planned a case control study to know the association of respiratory viruses especially HRSV genotype with acute exacerbation of COPD, if any.

This is a prospective case-control study with two groups of patients (AECOPD and stable COPD). Nasopharyngeal aspirate were tested for the detection of Human Respiratory Syncytial Virus; Influenza Viruses; Human Metapneumovirus; Adeno Virus; Human Boca Virus and Parainfluenza Virus 1,2,3,4 by real time PCR.

Respiratory viruses are more often found in case group (AECOPD patients) 45/ 234 patients (19.23%) than in control group (stable COPD), 8/100 patients (8%; P=0.0330). In case group HRSV was detected in 7.6% (18/234) and was most commonly detected virus followed by INFV-A (11/234; 4.7%), INFV-B (10/234; 4.2%), HMPV (2/234; 0.8%), and ADV (4/234; 1.7%). In control group INFV-A was most commonly detected (4/100, 4%), followed by ADV (2/100, 2%) and HRSV (1/100, 1%). No patient tested positive for more than one virus.

Among respiratory viruses, HRSV-A is the most prominent group associated with AECOPD patients. Present study concluded that respiratory viruses play an important role in exacerbation.

KEYWORDS: COPD, AECOPD, Viral etiology, Real-time PCR, Respiratory Viruses and HRSV.

1 INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the leading cause of fatality worldwide [1], [2]. It is a significant cause of global burden of disease and deaths [3], [4]. COPD is a group of disorders defined by Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria and is characterized by airflow obstruction that can be associated with breathing related symptoms such as chronic cough, dyspnea, and wheezing [5].

The course of COPD includes episodes of acute deterioration in respiratory health, referred as 'exacerbations' [6], [7], [8]. There is presently no specific definition of an exacerbation of COPD. However, some symptoms like sputum volume, purulence and dyspnoea, are important in the description of an exacerbation [9]. There occurs worsening of lung function due to increased airway inflammation and quality of life suffers [10].

Acute exacerbation of COPD (AE-COPD) causes major impairment of health status of COPD patients requiring hospital admission. Recurrent acute exacerbations of COPD contribute substantially to the morbidity and mortality [11], due to number of agents like common pollutant, withdrawal of medication, change in temperature and most importantly infectious

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agents including bacteria, viruses, or both [11], [12]. Bacteria contribute to 50% of exacerbation [13] and viruses are responsible for 30-40% cases among the infectious etiological agents. It is also postulated that bacteria may be secondary invaders following a primary viral infection [14].

Of all respiratory viruses, Human respiratory syncytial virus (HRSV) is the major cause of lower respiratory disease among infants and adults [15], [16] of the lungs and breathing passages causing bronchiolitis, pneumonia, and chronic obstructive pulmonary infections along with other viral infections leading to high mortality and morbidity [17], [18], [19]. However, there had been sparse studies reporting viral etiology in acute exacerbation of chronic obstructive pulmonary disease (AECOPD) patients worldwide. HRSV has been identified as an important pathogen causing exacerbation in COPD patients [20]. Its frequency of infection in AECOPD patients varies and ranges between 0.8 to 22%, depending on the diagnostic methods used [20], [21]. A review from India discusses about 5% prevalence of HRSV in AECOPD patients [22].

It is important to elucidate the etiology of AECOPD so that appropriate therapy can be instituted to reduce exacerbation. Since, the presence of the virus has also been documented in stable COPD patients [23]. It can be hypothesized that viruses may be bystanders in the actual AECOPD events. To evaluate this hypothesis present case control study was planned to know the association of respiratory viruses, especially HRSV group with acute exacerbation of COPD, if any.

2 MATERIALS AND METHODS

2.1 SUBJECT AND STUDY DESIGN

This is a prospective case-control study with two groups of patients. One group with an acute exacerbation of COPD and other control group with stable COPD patients were included in 2:1 ratio (case: control). This study was conducted in Grade 1 Virus Diagnostic Laboratory (VDL) of King George Medical University (KGMU), Lucknow, on patients who had been admitted or referred to department of Pulmonary Medicine, KGMU, Lucknow, during June 2011 to December 2013. The study was approved by the institutional ethical committee. Written informed consent was obtained from each patients enrolled in this study. Nasopharyngeal aspirate (NPA) were obtained from patients for detection of Respiratory viruses such as Human respiratory Syncytial virus (HRSV) and its subtypes (A & B); Influenza viruses (INFV) and its groups (A & B); Human Metapneumovirus (HMPV) and its subtypes (A & B); Adeno virus (ADV); Human Boca virus (HBoV) and Parainfluenza virus 1,2,3,4 (PIV) were being detected routinely in patients presenting as respiratory signs and symptoms.

Inclusion criteria for both group were adult patients (age 18–90 years) with AECOPD (defined below) and stable COPD (defined below). Cases were COPD patients, who consulted or were admitted in a participating hospital for an acute exacerbation of <5 days of onset their illness. Patients with bronchiectasis, lung cancer, cystic fibrosis, hypertension, diabetes mellitus, Tuberculosis, and those presenting only with an exacerbation of asthma were excluded from both the groups. Controls were recruited simultaneously during the study period in order to prevent seasonal selection bias.

Acute Exacerbation of COPD was defined as acute episode in stable COPD subjects with sudden increase in dyspnea, sputum volume and purulence. Cases were categorized into three types according to Winnipeg criteria [24]: Type 1 if they have all the three symptoms of increased dyspnea, sputum volume, and sputum purulence; Type 2 if only two of type 1 and type 3 if only one of the type 1 symptoms were present plus one of the following symptoms; increase in cough, increase in wheeze, increase in heart rate > 20% or symptoms of respiratory tract infection lasting 5 days, fever. A control group of stable COPD patients was defined by GOLD criteria visiting for their regular follow-up.

2.2 DATA COLLECTION

At the time of admission, the clinical and demographic parameters were recorded. Clinical features like fever, chills and rigors, nasal discharge, seizure, headache, bodyache, sore throat, hoarseness, cough expectoration, crepitations; wheezing, breathlessness, increased sputum volume and purulence etc. were noted. Age, sex, Smoking habits, hospital stay, medication, COPD duration were also included.

2.3 SAMPLES COLLECTION AND ITS PROCESSING

Nasophyrengial aspirate (NPA) was collected by inserting a suction catheter into the posterior nasal pharyngeal space via the nostril of the recumbent patient. Approximately 0.5 ml fluid was collected by using a low suction force. The fluid was instantly transferred into 3ml of VTM (Viral transport medium) with penicillin (100unit/ml), streptomycin (100µg/ml) and amphotericin B (2.5µg/ml). NPA was immediately transported to the Virology Laboratory maintaining cold chain. On the

same day the samples were processed by vigorous vortexing for 15 seconds and centrifuged at 500 rpm for 5 minutes. Supernatant was immediately stored at -80°C till further use.

2.4 TOTAL NUCLEIC ACID (TNA) EXTRACTION AND REAL TIME PCR

Total nucleic acid was extracted directly from 115ul supernatant of NPA samples using MagMAX™ total nucleic acid isolation kit (AM1840; Ambion® by life technologies TM, Carlsbad, California, USA), according to the manufacturer's recommendations. Extracted Nucleic acid was eluted in 60µl of elution buffer. A negative extraction control was also included simultaneously with each batch.

Real time PCR (Polymerase Chain Reaction) was done for all respiratory viruses using published primer and probe sequences by TaqMan chemistry in ABI 31300 real time machine (Applied Biosystem, Carlsbad, California, USA) as detailed in Singh and Jain et al. 2014 [25], [26]. Thermal condition of real time RT-PCR (Reverse Transcriptase- Polymerase Chain Reaction) for RNA viruses were as follows: 50°C for 30 min.; 95°C for 15 min.; 45 cycles of 94°C for 20 sec. and 60°C for 1min. Thermal condition of real time PCR for DNA viruses were as follows: 95°C for 15 min; 45 cycles of 94°C for 20 sec. and 60°C for 1min. RNase P gene was used as internal control to check sample quality [25].

2.5 STATISTICAL ANALYSIS

Appropriate statistical tests were used on the bases of data distribution for analysis using the GraphPad Prism 5.0 version. For comparison of groups, Chi square test was used for categorical data where as Mann–Whitney U test and Kruskal–Wallis test for continuous data. A two sided P value of < 0.05 was considered to be statistically significant. Odds Ratio (95% CI) was also calculated.

3 RESULTS

During the study period, 234 patients with AECOPD (cases) and 100 patients of stable COPD (controls) were enrolled. Respiratory viruses are more often found in case group (AECOPD patients) 45/ 234 patients (18.82%) than in control group (stable COPD), 8/100 patients (8%); P=0.0101.

In case group HRSV was detected in 7.6% (18/234) and was most commonly detected virus followed by Influenza A (11/234; 4.7%), Influenza B (10/234; 4.2%), HMPV (2/234; 0.8%), and ADV (4/234; 1.7%). No patient tested positive for more than one virus. Among HRSV infected subjects, HRSV-A (14/234; 5.9%) is more dominating virus compared to HRSV-B (4/234; 1.7%). In control group (stable COPD). INFV-A was most commonly detected (4/100, 4%), followed by ADV (2/100, 2%) and HRSV (1/100, 1%). The viruses detected in both groups are summarised in table 1. Difference in virus positivity in both cases and controls, was found statistically significant (19.2% vs 8%, p =0.0101, OR=2.7, 95%CI=1.2-6.0). Presence of HRSV infection was also statistically significant (7.6% vs 1%, p=0.0156, OR=8.2, 95%CI=1.0-62.7). HRSV-A was more prevalent at exacerbation than in stable COPD. INFV-A, and ADV were detected in similar rate in both groups.

Table 1: Virus positivity in both groups

Viruses	AECOPD (Case group) N= 234 (%)	Stable COPD, (Control group) N=100 (%)	P value	Odds Ratio (95% CI)
HRSV	**18 (7.6)**	**1 (1)**	***0.0156****	**8.2 (1.0 - 62.7)**
INFV-A	11 (4.7)	4 (4)	0.7770	1.1 (0.3 - 3.8)
INFV-B	10 (4.2)	1(1)	0.1247	4.4 (0.5 -35.0)
HMPV	2 (0.8)	0	--	--
ADV	4 (1.7)	2 (2)	0.8547	0.8 (0.1 - 4.7)
Total	**45 (19.2)**	**8 (8)**	***0.0101****	**2.7 (1.2 - 6.0)**

**P value: test of significance (P values of 0.05 or less were taken as significant).*

Statistical significance is indicated in bold and P values are indicated in italics.

Abbreviation: HRSV – Human Respiratory Syncytial Virus, INFV – Influenza Virus, HMPV – Human Metapneumovirus, ADV – Adenovirus, AECOPD – Acute Exacerbation of chronic obstructive pulmonary disease, COPD - Chronic obstructive pulmonary disease, CI- Confidence Interval

The demographic and clinical characteristics of patients in both the groups were compared. There was no significant difference in demographic characteristic like sex, age, smoking year, and duration of COPD disease. Cases showed significantly higher percentage of nasal discharge (55.5% vs 27%, p= 0.0001), headache (29.9% vs 15%, p= 0.0042), Bodyache (43.1 vs 26, p= 0.0031) sore throat (34.6 % vs 3%, p= 0.0333), hoarseness (20% vs 7%, p= 0.0029, wheezing (41.4% vs 27%, p= 0.0093), breathlessness (49.5% vs 34%, p= 0.0088) purulent sputum (46.1% vs 29%, p= 0.0035). Past history of smoking was significantly present in cases than in controls (51.7% vs 39%, p=0.0142, OR=1.6, CI=1.0-2.6). Details were given in table 2.

Table 2: Clinical comparison between case and control group

Symptoms	Exacerbated COPD N=234 (%)	Stable COPD n= 100 (%)	P value	Odds Ratio (95% CI)
Demographic Characteristics				
Male : Female	221:13	98:2	--	--
Median age (IQR)	60Yr (25-90yr)	55Y (28-85yr)	0.3308	--
Smoking Year	20yr (10-35yrs)	19yr (10-30yrs)	0.3461	--
Duration of COPD disease (years)	6 yrs (2-22yrs)	5yrs (2-20yrs)	0.6230	--
Nasal discharge	130 (55.5)	27 (27)	***0.0001***	3.3 (2.0-5.6)
Headache	70 (29.9)	15 (15)	***0.0042***	1.2 (1.3-4.4)
Bodyache	101 (43.1)	26 (26)	***0.0031***	2.1 (1.2-3.6)
Sore throat	81 (34.6)	03 (3)	***0.0333***	17.12 (5.2-55.7)
Hoarseness	47 (20)	07 (7)	***0.0029***	3.3 (1.4-7.6)
Diarrhea	16 (6.8)	03 (3)	0.1655	2.3 (0.6-8.3)
Crepitations	109 (46.5)	37 (37)	0.1059	1.4 (0.9-2.4)
Wheezing	97 (41.4)	27 (27)	***0.0093***	1.9 (1.1-3.3)
Breathlessness	116 (49.5)	34 (34)	***0.0088***	1.9 (1.1-3.1)
Purulent sputum	108 (46.1)	29 (29)	***0.0035***	2.0 (1.2-3.4)
H/O Smoking				
Past/ Ex- smoker	121 (51.7)	39 (39)	**0.0142**	1.6 (1.0-2.6)
Current smoker	69 (29.4)	37 (37)	0.1767	0.7 (0.4-1.1)
Never/ Non –smoker	44 (18.8)	24 (24)	0.2801	0.7 (0.4-1.2)

P value: test of significance (P values of 0.05 or less were taken as significant).

IQR- Inter Quartile Range, H/O Smoking – History of Smoking, CI- Confidence Interval.

The demographic and clinical characteristic of AECOPD patients having viral etiology and non viral etiology were also compared (table 3). Clinical characteristic like cough (94.4%, p= 0.0148), breathlessness (83.3%, p= 0.0002), increased sputum amount (77.7%, p= 0.0042) and wheezing (72.2%, p= 0.0038) were significantly present at higher percentage in HRSV positive patients than other virus positive. Clinical characteristic like fever (70.3%, p= 0.0002), nasal discharge (96.2%, p= 0.0001) and sore throat (62.9%, p= 0.0034) were found significantly higher in respiratory viruses other than HRSV. There was no significant association found among AECOPD virus positive patients in sputum purulence. In AECOPD patients, majority of them 121/234 (51.7%) had history of smoking and 69/234 (29.4%) were currently smoking. A greater proportion of past smokers were infected with HRSV (83.3%) in comparison to other respiratory virus (55.5%) and with other non-viral infections (48.14%, p= <0.05).

Table 3: Clinical comparison of HRSV positives, other viruses positive and virus negative cases in AECOPD patients

Symptom & Signs:	HRSV Positive N=18 (%)	Other Viruses Positive N=27 (%)	Virus Negative N=189 (%)	P Value
Demographic Characterstics				
Male : Female	18:0	27:0	176 : 13	--
Median age (IQR)	60Yr (30-83yr)	55Y (28-90yr)	60 (25-90yrs)	0.4669
Smoking Year	21yr (12-30yrs)	18yr (10-35yrs)	15yrs (10-35yrs)	0.8635
Duration of COPD disease (years)	6yrs (4-20yrs)	5yrs (2-22yrs)	5yrs (2-24yrs)	0.7636
Clinical Characterstics				
Fever	07 (38.8)	**18 (70.3)**	52 (27.5)	**0.0002**
Chills & rigors	5 (27.7)	06 (22.2)	46 (24.3)	0.9134
Nasal discharge	13 (72.2)	**26 (96.2)**	91 (48.1)	**0.0001**
Seizures	01 (5.5)	02 (7.4)	12 (6.3)	0.9666
Headache	07 (38.8)	07 (25.9)	56 (42.1)	0.6365
Bodyache	09 (50)	10 (37)	71 (37.5)	0.5770
Sore throat	07 (38.8)	**17 (62.9)**	57 (30.1)	**0.0034**
Hoarseness	03 (16.6)	04 (14.8)	40 (21.1)	0.6622
Cough	**17 (94.4)**	22 (81.4)	104 (55.0)	**0.0148**
Expectoration	11 (61.1)	12 (44.4)	84 (44.4)	0.3946
Crepitations	12 (66.6)	11 (40.7)	86 (45.5)	0.1848
Wheezing	**13 (72.2)**	15 (55.5)	69 (36.5)	**0.0038**
Breathlessness	**15 (83.3)**	18 (66.6)	83 (43.9)	**0.0002**
Increased sputum amount	**14 (77.7)**	16 (59.2)	78 (41.2)	**0.0042**
Increased sputum purulence	12 (66.6)	13 (48.1)	94 (49.7)	0.3726
H/O Smoking				
Past/ Ex- smoker	**15 (83.3)**	15 (55.5)	91 (48.14)	**0.0174**
Current smoker	02(11.1)	07 (25.9)	60 (31.74)	0.1693
Never/ Non –smoker	01(5.5)	05 (18.5)	38 (20.10)	0.3197

P value: test of significance (P values of 0.05 or less were taken as significant).

IQR- Inter Quartile Range, H/O Smoking – History of Smoking, CI- Confidence Interval.

Distribution of viruses was observed in three severity groups of acute exacerbation of COPD, detailed in table 4. In case of AECOPD, type 1 AECOPD was significantly more severe and associated with viral positivity by 20.7%. Among viruses, HRSV was maximally present in type 1, AECOPD patients (14.4% vs 6.3%, p= 0.0459). Viruses other than HRSV were maximally present in type 2 and type 3 AECOPD.

Table 4: Distribution of viruses in severity group of acute exacerbation of chronic obstructive pulmonary disease (AECOPD) patients

Types of AECOPD patients	HRSV Positive N=18 (%)	Other Viruses Positive N=27 (%)	Total Viruses Positive N=45 (%)	P Value
Type 1 (n=111)	**16 (14.4%)**	7 (6.3%)	23 (20.7%)	0.0459
Type 2 (n=71)	1 (1.4%)	***11 (15.4%)***	12 (16.9%)	0.0026
Type 3 (n=52)	1 (1.9%)	***9 (17.3%)***	10 (19.6%)	0.0080

P value: test of significance (P values of 0.05 or less were taken as significant).

P value calculated between HRSV positive and other viruses positive.

HRSV- Human respiratory Syncytial Virus

Control group of patients were categorized according to the stages of COPD defined by GOLD (Global Initiative for Chronic Obstructive Lung Disease), in which virus positive patients were compared with very sever COPD (stage 4; FEV1 < 30%

predicted) to those with mild, moderate and severe COPD (stage 1, FEV1 >80% predicted; stage 2, FEV1 > 50% to 80% predicted; stage 3 and FEV1 > 30 to 50% predicted, respectively). However, there was no such significant result found (table 5).

Table 5: Chronic obstructive pulmonary disease (COPD) patients with virus positivity were categorized in different stages

Viruses	All cases N=100	GOLD Stages Stage 1 & 2 n= 30 (%)	Stage 3 n=34 (%)	Stage 4 n=36 (%)
Negative	92	28 (96.6)	31 (91.1)	33 (91.6)
HRSV	1	--	--	1 (2.7)
INFV-A	4	1 (3.3)	2 (8.8)	1 (2.7)
INFV-B	1	1 (3.3)	--	--
ADV	2	--	1 (2.9)	1 (2.7)
Total Viruses	8	2 (6.6)	3 (8.8)	3 (8.3)

HRSV- Human respiratory syncytial virus; INFV-Influenza virus; ADV- Adenovirus; GOLD- Global Initiative for Chronic Obstructive Lung Disease

Stage 1= FEV1 > 80% predicted (mild)

Stage 2= FEV1 > 50% to 79% predicted (moderate)

Stage 3= FEV1 > 30 to 49% predicted (severe)

Stage 4= FEV1 < 30% predicted (very severe)

FEV1= Volume that has been exhaled at the end of the first second of forced expiration.

4 DISCUSSION

An exacerbation is a critical episode in life of COPD patients that frequently causes rapid decline in lung function. Due to the existence of various triggering factors, it is important to establish exact etiology in each episode. It has been observed that different respiratory viruses are frequently detected in the lungs of COPD patients, but up to what extent they have role in causation of exacerbation, is yet to be determined precisely.

Very few studies have been done to know the viral etiology in AECOPD patients. Up to 40% of COPD exacerbations are associated with viral infections, although this may be an underestimate, due to difficulties in sampling and its processing at the very onset of an exacerbation when there is highest probability of detection of virus and due to difference in detection method used [20], [27]. Most studies have shown high prevalence of HRSV in AECOPD patients among respiratory viruses [28].

In present study viruses were found to be significantly associated with exacerbation (19.2%) in comparison to stable COPD (8%) group, which is a consistent finding with other studies too [20], [29]. Such finding clearly states the association of viruses with AECOPD, particularly HRSV in AECOPD patients. According to Gaston et al. in 2009, 31% of viral etiology had been seen in which HRSV accounts for 7% and was the most commonly triggering viral agent in AECOPD patients [29]. In our setup HRSV accounts for 7.6% in AECOPD patients. According to Chandra S, 9.09% viral etiology was observed in AECOPD patients which was less to the present study, the reason could be that they have used RT-PCR and culture for detection while in present study the more sensitive real time PCR method was used [30]. Influenza virus along with ADV and HMPV, follow HRSV in causing exacerbation similar to our findings [7], [14].

Comparison between the AECOPD patients with and without viruses revealed significant difference in presentation. Virus infected patients were having more fever, nasal discharge sore throat, cough, wheezing and breathlessness as expected, but HRSV was having lesser of these features except cough, wheezing, breathlessness in comparison to those AECOPD patients infected with viruses other than HRSV. HRSV is a known lower respiratory tract pathogen that damages airway epithelium causing loss of ciliated cells, increased plasma exudation and increase mucus production [31], therefore is responsible for more cough and wheezing.

Smoking is significant risk factor in COPD patients. Patients with history of smoking have more damages in lungs and are more prone to acquire viral infection leading to exacerbation. We found cigarette smokers (past smokers) are significantly

more prone towards HRSV which is similar to other studies [32]. Groskreutz et al. in 2009, stated cigarette smoke causes necrosis which results in increased inflammation and enhanced HRSV replication [33].

Depending upon the severity of AECOPD patients, distribution of viruses were studied among their types. The results showed that viruses were equally distributed but HRSV was significantly detected in patients with highest severity (type 1). This clearly denotes the causation of severity of AECOPD due to HRSV infection.

5 CONCLUSION

Etiology of AECOPD is not clear to the clinicians due to association of number of factors. To the end, present study concluded that respiratory viruses play a vital role in exacerbation and especially HRSV-A. To the best of our knowledge, this is the first study from northern part of India in acute exacerbation of COPD patients describing the dominancy of HRSV-A over HRSV-B group. This will help clinicians to impart proper and timely management to patients. Reduction of exacerbation in COPD will definitely have a vital impact on morbidity and mortality of AECOPD. It also paves the way for future detailed research in AECOPD patients.

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