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Effect of Roflumilast As Add On Therapy in Airway Inflammation and Serum Inflammatory Markers in COPD Patients

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Abstract: Chronic Obstructive Pulmonary Disease is associated with increased inflammatory cells. Combination of long acting beta agonist and inhaled corticosteroids are standard treatment. Roflumilast targets inflammatory cells and decreases the frequency of exacerbation. This study looks at the effect of Roflumilast on inflammatory cells in sputum and inflammatory markers in the blood when used as add-on therapy in COPD patients. This observational comparative study conducted in Department of Pulmonary Medicine for 1.5 years duration. 70 COPD patients were divided into two Group A (Roflumilast + Standard treatment) and Group B (Standard treatment). Pulmonary Function Test, Sputum Eosinophil & Sputum Neutrophil, CRP were measured at baseline and after 4 months of treatment in both groups. Intergroup analysis between Group A and Group B was done at baseline and after 4 months. Mean age of patients in Group A was 64.3 years and in Group B was 61.2 years. Maximum patients were in age groups 51-60 and 61-70 years. Of total 70 patients, 60% were males and 40% females. Majority of patients in both groups had low BMI. Sputum inflammatory cells were significantly lower in Roflumilast group. Pulmonary function parameters showed significant improvement in Roflumilast treated group. There was no significant decrease in CRP when compared between two groups. Roflumilast, add-on therapy to standard treatment in COPD patients have improved lung function and decreased level of inflammatory cells in airway significantly.

Keywords: Chronic Obstructive Pulmonary Disease, Roflumilast, PDE -4 inhibitors, inflammatory mediators, Pulmonary Function Test, C-reactive protein.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD), is a major problem and global burden on public health services[1]. It causes tremendous difficulties for individuals and societies, being a cause of severe lifelong disability and even premature death. COPD is one of the leading cause of morbidity and mortality worldwide.[1] COPD has been defined as "disorder", "disease state", or "pathologic condition" resulting from chronic bronchitis, pulmonary emphysema, and small airway disease[2].

Globally, in 2010, COPD affected 329 million people (4.8% of the population)[3]. The disease affects men and women almost equally, as there has been increased tobacco use among women in developed world[4]. In 2015, it resulted in 3.2million deaths, up from 2.4 million deaths in 1990[5]. More than 90% of these deaths occur in developing world[6].

Most cases of COPD is prevented by reducing exposure to risk factors[7]. This includes decreasing rates of smoking, improving indoor and outdoor air quality. While treatment can slow worsening, there is no cure[1]. COPD treatments include smoking cessation, vaccination, respiratory rehabilitation and inhaled bronchodilator and steroids[8]. Some people benefit from long term oxygen therapy/lung transplantation. In those who have periods of acute worsening, increased use of medications and hospitalizations are needed[9].

Diagnosis of COPD is confirmed by spirometry, which measures post-bronchodialator force expiratory volume in the first second (FEV1). COPD is referred to as severe when FEV1 is 50-30% and very severe when it is less than 30% of predicted value[10]. Recent global guidelines for the management of COPD recommend grouping patients into four labeled A, B, C or D according to symptoms, airflow limitations, risk of exacerbations and presence of co-morbidities[10,11].

The predominant inflammatory cells in COPD are CD68 macrophages and CD8 T lymphocytes with polymorphs increasing in acute exacerbations [12]. The activated macrophages release inflammatory mediators,

chemotactic factors including pro inflammatory cytokines such as Tumour necrosis factor alpha, IL-6, IL-8, monocyte chemotactic peptide 1 and Leukotriene. Neutrophilia and eosinophilia in sputum is feature of COPD[12] Other processes involved with lung damage include oxidative stress produced by high concentrations of free radicals in tobacco smoke and released by inflammatory cells, and breakdown of connective tissue of the lungs by proteases that are insufficiently inhibited by protease inhibitors[12,13].

In practice, patient suffering from frequent exacerbations are treated with combination of long acting beta agonist (LABA) and inhaled corticosteroids (ICS) and in addition to tiotropium, long acting anti – muscarinic agents (LAMA). Despite treatment available, exacerbations in severe to very severe disease are common. Since inflammation is responsible for frequent exacerbations, it is logical to add anti-inflammatory drug to combination therapy to cause.

Roflumilast, oral phosphodiesterase PDE-4 inhibitor targets inflammatory cells involved in exacerbations of COPD. It is only PDE-4 inhibitor approved by US food and drug administration (FDA) and is available as 500microgram tablets[14].

Clinical trials have already demonstrated ability of Roflumilast to decrease the frequency of exacerbation and improve lung function in COPD while its biological action may result in potentially targeting the inflammatory process underlying COPD.

The study looks the effect of Roflumilast as an add-on therapy in airway inflammatory cells (Sputum Neutrophil, eosinophils), serum inflammatory marker (C- reactive protein). Similar studies have not yet been conducted in our country.

Objectives

To determine if Roflumilast when given as add - on therapy to standard treatment in COPD patients reduces the percentage of sputum neutrophils, sputum eosinophils and serum marker C- reactive Protein (CRP) when given for four months in a tertiary hospital in Kerala.

METHODOLOGY

Study design: It is comparative longitudinal study of 1.5years duration (February 2016 to August 2017). Treatment period was 4 months.

Study setting: Conducted in Department of Pulmonary Medicine in Azeezia Medical College, Kollam

Study Population: The study included all severe and very severe COPD patients.

Inclusion criteria

- All severe and very severe COPD patients (FEV1/FVC <70%)
- FEV1 (Post – bronchodilator) < or equal 50% of predicted
- Age above 40 years, both sex
- Patients on standard treatment for COPD
- Patient who had at least one exacerbation in previous year despite on optimised therapy

Exclusion criteria

- COPD patients who had exacerbations during study periods
- Smokers
- Diagnosis of asthma and / relevant lung disease
- Known alpha 1 anti-trypsin deficiency

Sample size: Total of 70 patients, 35 patients in each group.

Ethical consideration

Ethical clearance was obtained from Institutional Human Ethics Committee. A written, signed informed consent was obtained from the patient enrolled. Confidentiality and anonymity of the patient's information were maintained during and after study.

Study Procedure

Study was conducted after obtaining approval from Institutional Ethics Committee. A signed, written informed consent was obtained from all the patients included in study.

The physician after diagnosing severe and very severe COPD patients prescribe Tab Roflumilast 500µg OD. On the basis of observation of the prescription (written by treating physician), severe COPD (FEVI1/FVC < 70%; 30% < FEV1 < 50% predicted) and very severe COPD patients (FEV1/ FVC < 70%; FEV1 < 30% predicted) were separated into two groups.

Group A: Roflumilast + Standard treatment for 4 months
Group B: Standard treatment for 4 months

Standard treatment given

- Inhaler formeterol fumarate (6mcg) + Budesonide (200/400 mcg) 2puff twice daily
- Inhaler Tiotropium Bromide (9mcg) 1 puff once daily

GROUP A

Day 1: Following parameters were measured

- Pulmonary Function Test (FEV1 %, FVC%, FEV1/FVC %)
- Sputum examination: neutrophils, eosinophils (%)
- Serum C –reactive protein (CRP) mg/l

Standard treatment + Tab Roflumilast 500µg OD for 4 months follow up after 4 months. Parameters are

- Pulmonary Function Test
- Sputum Examination for neutrophils and eosinophils
- Serum C – Reactive Protein (CRP)

GROUP B

Day 1: Following parameters were measured

- Pulmonary Function Test (FEV1 %, FVC%, FEV1/FVC %)
- Sputum examination: neutrophils, eosinophils (%)
- Serum C –reactive protein (CRP) mg/l

Standard treatment only for 4 months

Follow up after 4 months. Parameters are

- Pulmonary Function Test
- Sputum Examination for neutrophils and eosinophils
- Serum C – Reactive Protein (CRP)

All data were entered in the proforma.

Data analysis

Data were entered in MICROSOFT EXCEL 2017 and analysis done. Using EXCEL 2017 and SPSS Version 24.0 statistical software. To compare effect of Roflumilast on Sputum inflammatory cells and serum inflammatory markers, two statistical methods used.

- Unpaired Student t test: compareS between Group A and Group B (INTER GROUP VARIATION) which is done at
  - At baseline before giving treatment
  - After 4 months of treatment

- Paired Student t test: Compares within group A after giving Roflumilast for 4 months (INTRA GROUP VARIATION).

Intra group analysis was done only in Group A as add -on therapy (Roflumilast) was given only in that group.

RESULTS

From January 2016 to June 2017, 70 patients were recruited. Patients were divided into 2 groups with 35 patients in each. Group A received Roflumilast with standard regimen and group B received only standard regimen. The demographic profiles of the patients were studied. The effect of roflumilast was assessed by comparing the parameters in group A and Group B. The parameters assessed:

- Pulmonary Function Test
- Sputum neutrophils and eosinophils
- Serum CRP

DEMOGRAPHIC PROFILE

Table-1: Baseline Demographic characteristics in Group A & Group B

Characteristic	Group A* (N = 35)	Group B# (N = 35)
Age (years)		
Mean	64.3±7.9	61.2+ 6.4
Age Groups (years)		
41-50	1(2.5%)	2(5%)
51-60	13(37.5%)	15(42.5%)
61-70	13(37.5%)	16(45%)
71-80	8(22.5%)	2(7.5%)
Gender – (%)		
Male	20(57.1%)	22(62.8%)
Female	15(42.8%)	13(37.1%)
BMI – (%)		
<18.5	16(45.71%)	18(51.42%)
18.5 – 25	10(28.6%)	13(37.14%)
25 – 30	5(14.29%)	2(5.71%)
>30	4(11.42%)	2(5.71%)

* - Group A receiving Roflumilast + Standard treatment

- Group B receiving Standard treatment only

Data expressed in mean with ± SD

The demographic profile of patients included in study is age, sex, Body Mass Index (BMI). The data obtained is presented in table 1.

Effect of Roflumilast in Inflammatory Cells in Sputum

Percentage of Neutrophils and Eosinophils in Sputum

Inflammatory markers analysed were percentage number of neutrophil and eosinophil in sputum. Data expressed in mean with +/- SD.

Table-02: Percentage of sputum neutrophil and eosinophil in group A and Group B -Baseline value

Characteristic	Group A* (N = 35)	Group B# (N = 35)	P value
Number of inflammatory cells in Sputum (%)	70.05 ±12	71.97 ±15	0.55
Neutrophil			
Eosinophil	0.8 ±13.2	0.7 ±11	0.98

Data expressed in mean with ± SD

P value calculated using unpaired student t test. P value < 0.05 is statistically significant

* - Group A receiving Roflumilast + Standard treatment

- Group B receiving Standard treatment only

From table 02, on comparing the absolute number of neutrophil in sputum in two groups at baseline, there is no statistically different result (P value – 0.55). Similar findings in the absolute count of Eosinophil between two groups (P value – 0.98)

Table-3: Percentage of sputum neutrophil and eosinophil between Group A and Group B – after giving treatment for 4 months

Inflammatory mediators in sputum	Group A* (N = 35)	Group B# (N = 35)	P value
Percentage of Neutrophils in sputum (%)	51.9 ± 28	74.4 ± 21	0.002
Percentage of eosinophils in sputum (%)	0.4 ± 5.6	0.8 ± 0.78	0.001

Data expressed in mean with ± SD

P value calculated using unpaired student t test. P value < 0.05 is statistically significant

* - Group A receiving Roflumilast + Standard treatment

- Group B receiving Standard treatment only

From table 3, it is observed that the inflammatory cell load in airways is lower in group A (taking Roflumilast) and higher in Group B (not taking roflumilast). Roflumilast treatment was associated with a significant decrease in neutrophil numbers in sputum (p = 0.002). Roflumilast treatment also reduced sputum eosinophil significantly (p = 0.001).

Table-4: Percentage of sputum neutrophil and eosinophil within Group A- before and after treatment

Inflammatory Cells in sputum	Group A* (Before treatment) (N = 35)	Group A* (After 4 months of treatment) (N = 35)	P value
Percentage of Neutrophil in sputum	70.05 ± 12	51.9 ±28	0.0008
Percentage of Eosinophil in sputum	0.8 ± 0.78	0.4 ± 5.6	0.056

Data expressed in mean with ± SD

P value calculated using paired student t test. P value < 0.05 is statistically significant

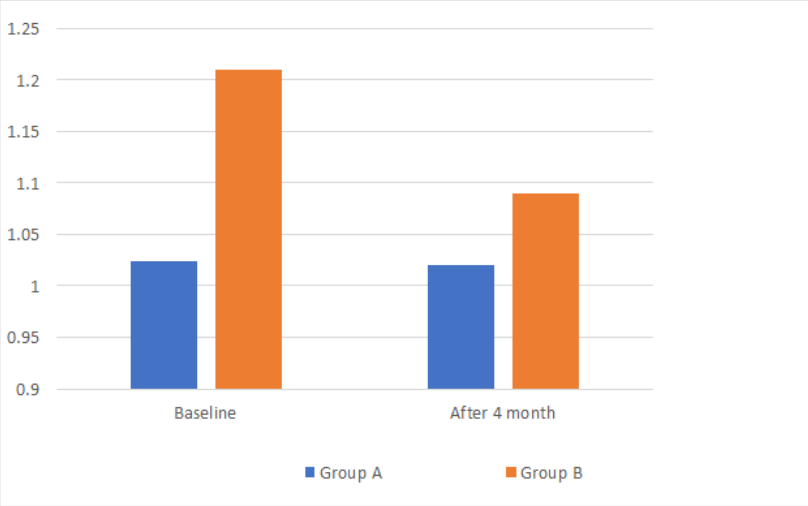
* - Group A receiving Roflumilast + Standard treatment

Table 4 shows the cell count of inflammatory cells in sputum before and after treatment with Roflumilast in Group A patients for intra group analysis. Effect of Roflumilast within Group A was analysed by paired student's t – test. From analysis of table 4, it is observed that there is significant decrease in the absolute neutrophil count in Roflumilast treated group when given for four months (P value – 0.0008). When comparing the absolute count of eosinophil in Roflumilast treated group, there is no statistical difference seen (P value 0.056).

Effect of roflumilast on serum CRP

The inflammatory marker which was analysed was C- reactive protein (CRP). CRP was analysed between group A and group B by unpaired student's t – test to determine the effect of roflumilast. Table 5 shows the level of CRP in Group A and Group B before starting treatment. It was observed that, there is no significant difference in CRP level before starting of treatment between Group A & Group B (P value - 0.37).

Graph 01: Comparison in level of CRP in Group A and Group B at baseline & after 4 months of treatment



Graph 01 shows the difference in the level of CRP between Group A and Group B at baseline and after 4 months

Table-5: Level of CRP in Group A and Group B

Inflammatory Markers CRP (mg/L)	Group A* (N = 35)	Group B# (N = 35)	P value
Baseline	1.024 + 0.15	1.21 ± 0.13	0.37[a] 0.42[b]
After 4 months of treatment	1.020 ± 0.12	1.04 ± 0.12	0.41[a]

Data expressed in mean with ± SD

P value calculated using student t test. P value < 0.05 is statistically significant

a – P value calculated using unpaired student t test between group A and B

b – P value calculated using paired student t test for intra group A variation.

* - Group A receiving Roflumilast + Standard treatment

- Group B receiving Standard treatment only

From table 5, it was observed that there is no statistically significant reduction in the level of CRP in Roflumilast added group (Group A) when given for four months with a P value of 0.41 on comparing with group B

Table 5 also shows the level of CRP in Group A before and after giving Roflumilast. Effect of roflumilast in CRP level in group A before and after giving was analysed by paired student's t-test. There was decrease in the level of CRP in the COPD patients after receiving Roflumilast for 4 months in Group A. The p value is 0.42 which is not statistically significant.

Effect of roflumilast on the lung function

Pulmonary function was assessed by spirometry.

Table-6: Baseline Characteristics in Group A & Group B

	Group A* (N =35)	Group B# (N = 35)	P value
Pulmonary Function Test (% of predicted)			
FEV_1	44.11 ± 9.29	44.86 ± 6.55	0.62
FVC	46.54 ± 11.71	42.26 ± 14.88	0.18
FEV_1/ FVC	52.74 ± 9.43	52.53 ± 9.57	0.92

Data expressed in mean with ± SD
P value calculated using unpaired student t test. P value < 0.05 is statistically significant
* - Group A receiving Roflumilast + Standard treatment
# - Group B receiving Standard treatment only

Baseline PFT parameters are presented in table 6. There is no statistically significant difference in two groups before starting treatment.

Table-7: Value of PFT parameters in Group A and Group B – after giving treatment for 4 months

Pulmonary Function Test (%)	Group A* (N = 35)	Group B# (N = 35)	P value
FEV_1 %	47.14 ± 6.33	45.74 ± 6.49	0.34
FVC %	51.26 ± 11.05	45.26 ± 14.88	0.059
FEV_1/ FVC %	53.86 ± 8.24	50.34 ± 8.32	0.07

Data expressed in mean with ± SD
P value calculated using unpaired student t test. P value < 0.05 is statistically significant
* - Group A receiving Roflumilast + Standard treatment
# - Group B receiving Standard treatment only

Table 7 shows the PFT parameters in Group A and Group B after giving treatment for 4 months. There is improvement seen in the value of FEV_1, FVC and FEV_1/FVC in Group A on comparison with Group B, but not statistically significant.

Table-8: Value of PFT parameters in Group A before and after giving Roflumilast for four months

Pulmonary Function Test	Group A* (N = 35)	Group A* (after 4 months) (N = 35)	P value
FEV1 %	44.11 ± 9.29	47.14 ± 6.33	0.01
FVC %	46.54 ± 11.71	51.26 ± 11.05	0.04
FEV1 / FVC %	52.74 ± 9.43	53.86 ± 8.24	0.02

Data expressed in mean with ± SD
P value calculated using paired student t test. P value < 0.05 is statistically significant
* - Group A receiving Roflumilast + Standard treatment

DISCUSSION

This observational study is to examine the anti-inflammatory and pulmonary effects of oral PDE4 inhibitor Roflumilast in patients with COPD. In this study Roflumilast is given as an add – on therapy to the severe and very severe COPD patients. It showed the effect of Roflumilast in decreasing the sputum neutrophil and eosinophil levels when treated with Roflumilast. When there is decrease in the inflammatory mediators in sputum, the frequency of exacerbations in the COPD will decrease thereby improving the lung function.

The study type is merely observational. We had no role in allocating the patients into each group. The bias of the treating physician in the decision to prescribe Roflumilast has not been mitigated in the current study. Although all the assessed base line characteristics were similar, randomisation would have reduced bias in group allocation.

Demographic data analyzed were age distribution, gender distribution and distribution based on Body Mass Index. Mean age of the patients was 62.8 years. Maximum COPD patients were in the age groups 51-70 years. This was same in Group A and Group B. The findings of this study were similar to study by Halbert *et al.* [70]. They found out that prevalence of physiologically defined COPD in adults > 40 years was around 20% among the COPD patients. 60% of the study population was males and 40% were females. Tobacco smoking in form of beedi was the predominant smoke exposure exposure in males, whereas smoke from biofuel burning was the predominant exposure in

females. The findings of our study were similar to the study by Jaindal *et al*. [71]. The study showed gender related differences do exist in COPD patients. Jindal *et al*. indicated a higher prevalence of COPD among beedi smokers than cigarette smokers (8.2% v/s 5.9%).

It was found out that 45.71% of patients from Group who received roflumilast and 51.42% from Group who received only standard drug have low body mass index <18.5. The causes of cachexia in COPD patients are multifactorial and include decreased oral intake, the effect of increased work of breathing due to abnormal respiratory mechanics, and effect of chronic systemic inflammation. Our findings were similar to the study by Daniel A K *et al*. where the nutritional aspects of COPD patients were evaluated[72]. Landbo and coworkers studied a cohort of 2,132 patients with COPD in the Copenhagen City Heart Study[73]. As in smaller studies, they found increased mortality in patients with low BMI compared with subjects of normal weight.

When the effect of Roflumilast on the pulmonary function was analysed, it was found out that there is improvement in the values of FEV1%, FVC% and FEVI/FVC% in patients of receiving roflumilast and Group receiving only standard drug after treatment for 4 months (Table 7). Even though Roflumilast does not have bronchodilators effects, our patients showed improvement in lung functions. This may be because with use of Roflumilast, the inflammatory load has been reduced. Significant difference were also seen in the intra group analysis in Group receiving roflumilast patients, who were reassessed after using Roflumilast for 4 months (table 08). Peter MA *et al*. showed improvement the post –bronchodilatory FEV1 from baseline with use of Roflumilast, whereas deterioration was observed with placebo. The difference was present from 4 weeks and was maintained thereafter throughout the study ($p = 0.001$)[74].

In our study the effect of Roflumilast in level of C - reactive protein was not statistically significant between Group receiving roflumilast and Group receiving only standard drug after 4 months of treatment (table 5). There is decrease in CRP level in Group receiving roflumilast before and after treatment which is not statistically significant (table 5). The findings of our study not similar to the earlier studies conducted on CRP. Kseniia O *et al.* studied the effect of Roflumilast in CRP level of COPD patient of 180 days. There is significant difference in the treatment and placebo group of P value <0.05[75,76].

Regarding the sputum Neutrophil and eosinophil absolute count, the inflammatory cell load in airway is decreased in patients receiving roflumilast when compared to those receiving only standard drug with P value 0.002 (Absolute Neutrophil count) and P value <0.001 for eosinophil count (table 3). The reason for decrease may be due to decrease in frequency of exacerbation in Group a patients who were taking Roflumilast. But intragroup analysis of Group A patients who were on Roflumilast for 4 months (table 4) showed statistical significant reduction in the absolute count of neutrophils only. Absolute count of eosinophils decreased in intra group analysis of Roflumilast treated patients, but no statistically significant.

Our data extend the findings of Gamble et al who showed that treatment with the PDE4 inhibitor Cilomilast for 12 weeks significantly reduced inflammatory cell counts in bronchial biopsy specimens from the patients with COPD[77]. In the present study, as well as in the study of Gamble *et al*. treatment with a PDE4 inhibitor decreased the inflammatory cell counts.

From the literature search it was found out that main adverse event with use of Roflumilast was gastrointestinal symptoms which can lead to discontinuation of drug. In our study, 7 patients from the Group A complained diarrhea but did not lead to stoppage of drug. 5 patients complained of headache. All adverse events were transient and none of the patient taking Roflumilast experienced serious adverse event.

By our study, with use of Roflumilast the activity of several types of inflammatory cell was suppressed, as shown by large reductions in the levels of absolute count of Neutrophil and eosinophil count in sputum. With the decrease in the inflammatory mediators, the frequency of exacerbations seen in severe and very severe COPD patients has decreased significantly. Thereby the lung functions have improved in COPD patients using Roflumilast. But there was no much decrease seen in the CRP levels with use of Roflumilast.

Our data have important clinical implications. At present the recommended treatment for COPD consists of bronchodilators for symptom relief with the addition of inhaled corticosteroids for more severe disease. Inhaled steroids have only limited effects on airway inflammation and lung function in patients with COPD. Roflumilast as an add on therapy to the standard regimen improves not only lung function and health status, but also reduces the rate of frequent exacerbations. This may be associated with reduced airway inflammation observed in our study.

Main limitation of the study is the study duration. Roflumilast was given only for 4 months. It has been suggested recently that a window of at-least 6 months should be considered to evaluate the effect of treatment in COPD. Even though our patients treated for 4 months showed a clear treatment effect within 4 months except in the level of CRP.

Long term adverse event with use of the drug could not be monitored because of short duration of study. The effect of possible confounders such as smoking status and previous inhaled steroid usage – on roflumilast treatment could not be assessed because of limited sample size.

CONCLUSION

The following conclusions were made.

- Roflumilast when given as add on therapy in COPD patients have significantly reduced the sputum neutrophils and sputum eosinophils.
- Roflumilast when given as add on therapy in COPD patients has significantly improved lung function.
- Commonly reported adverse event was diarrhea and headache. No serious adverse event was reported which lead to discontinuation of drug.

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