Expression of Epidermal Growth Factor Receptor (EGFR) in the Bronchial Epithelium of Patients with Chronic Obstructive Pulmonary Disease (COPD)

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ABSTRACT

BACKGROUND AND PURPOSE: Chronic obstructive pulmonary disease (COPD) is a major health problem, it is caused predominantly by cigarette smoking. In COPD, the airway epithelium undergoes alterations, which are partially attributed to activation of the epidermal growth factor receptor (EGFR). The aim of this study was to study the expression of EGFR in the bronchial epithelium of patients with COPD and in smokers without COPD in comparison to its expression in normal bronchial epithelium, in a trial to investigate its role in the pathogenesis of COPD, and in the epithelial alterations that characterize this disease.

SUBJECTS AND METHODS:

This study included 50 subjects, who where admitted in Chest Department, Tanta University Hospital. They were classified into 3 groups: group I (apparently healthy non-smoking control volunteer subjects), group II (apparently healthy smoker volunteers), and group III (patients with COPD). Group III was further subdivided into: subgroup III_A (current smokers with COPD) and subgroup III_B (ex-smokers with COPD). The following was done for all of the studied subjects: thorough history taking and full clinical examination, chest X-ray, pulmonary function tests, and fiberoptic bronchoscopy. Bronchoscopic biopsies from all the studied subjects were subjected to histopathological, histochemical and immunohistochemical staining using antibody against EGFR.

RESULTS:

Non-COPD current smokers and COPD patients showed evident epithelial alterations compared to the normal bronchial epithelium of the control subjects. These alterations were significantly exaggerated in COPD patients compared to non-COPD smokers ($P<0.05$), however, no significant differences were found between current and ex-smokers with COPD ($P>0.05$). The expression of EGFR was significantly higher in group II and III compared to group I ($P<0.05$). Moreover, group III showed significantly higher EGFR expression compared to group II ($P<0.05$). Subgroup analysis revealed that subgroup III_B "ex-smokers with COPD" displayed significantly higher ($P<0.05$) EGFR expression compared to subgroup III_A "current smokers with COPD". Statistically significant positive correlations were found between EGFR expression and both goblet cell hyperplasia, and bronchial epithelial hyperplasia in groups II and III ($P<0.05$), while there was a significant negative correlation between EGFR expression and FEV1 in groups II and III ($P<0.05$). There was a significant positive correlation ($P<0.05$) between EGFR expression and smoking index in group II.

CONCLUSION:

There is increasing evidence that EGFR may play an important role in the epithelial phenotypic alterations observed in the bronchial epithelium of COPD patients through active smoking, and that it has a significant role in regulating mucus production in airway epithelium and in the repair of epithelium after injury. Disruption of the EGFR cascade may provide a mechanism and a strategy for therapy in airway inflammatory (hypersecretory) diseases by blocking EGFR activation with subsequent inhibition of goblet cell production and reduction of mucus secretion, which is the main cause of airway limitation in COPD patients. However, further studies evaluating these new therapeutic modalities are required.

Key words: COPD, EGFR, Epithelial alterations, Cigarette smoking

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lung to noxious particles or gases. [(1)]

Cigarette smoking is the main risk factor for COPD. It induces bronchial epithelial damage and shedding. [(2)], in addition, it is thought to be involved in mucus hypersecretion which is the hallmark of COPD. [(3)]

In response to injury of the bronchial epithelium, there is an urgent requirement to initiate tissue repair and to restore barrier function. The immediate response involves migration of epithelial cells adjacent to the area of damage into the wound to form a temporary squamous barrier consisting of poorly differentiated and highly spread cells often associated with inflammatory cells. These cells are unlikely to perform the normal differentiated functions of the epithelium (seromucous secretion, cilial motility, etc.), there follows a period of cell division and redifferentiation leading to complete restoration of normal epithelial barrier function. [(4)]

In COPD, the airway epithelium undergoes alterations, including squamous cell metaplasia, goblet and basal cell hyperplasia. The mechanisms underlying these epithelial alterations are incompletely understood, however, the epidermal growth factor receptor (EGFR) cascade has been shown to be involved in mucin production and goblet cell hyperplasia, repair of damaged epithelium [(5)], as well as epithelial differentiation into mucin-containing goblet cells, thus it may contribute to the development of airflow limitation in COPD. [(6)]

Epidermal growth factor receptor (EGFR), also called HER1, is a 170-kDa membrane glycoprotein, which is expressed on the surface of various cells, and may be related to mucin production in stomach, urothelium, and other epithelia. It is a member of the transmembrane tyrosine kinase receptor family [(7,8)]. They are important proteins that orchestrate the epithelial repair process via induction of epithelial migration, proliferation, differentiation, and extracellular matrix synthesis. However,

excessive expression of these receptors may lead to squamous metaplasia or epithelial hyperplasia. [2]

This work was designed to study the expression of EGFR in the bronchial epithelium of patients with COPD and in smokers without COPD in comparison to its expression in normal bronchial epithelium, in a trial to investigate its role in the pathogenesis of COPD, and in the epithelial alterations that characterize this disease.

SUBJECTS AND METHODS

This study included 50 subjects, who where admitted in Chest Department, Tanta University Hospital. They were classified into the following groups:

(1) Group I: Included 10 male apparently healthy non-smoking control volunteer subjects.

(2) Group II: Included 10 male apparently healthy smoker volunteers.

The smoking index (= number of cigarettes/day × years of smoking). [9] of these subjects was calculated.

(3) Group III: Included 30 male patients with COPD. All of them had mild to moderate COPD according to GOLD criteria. [1]

According to their smoking status, this group was subdivided into:

A) *Subgroup III_A:* Included 19 current smokers with COPD.

B) *Subgroup III_B:* Included 11 ex-smokers with COPD.

Inclusion criteria of COPD patients [1]:

-Post-bronchodilator FEV_1/FVC less than 70.

-Evidence of irreversible airway obstruction: FEV_1 did not increase by more than 12% or 200 ml after inhalation of 400 µg salbutamol [10].

-Mild COPD: FEV1/FVC ratio<70%, FEV1≥80% with or without symptoms.

-Moderate COPD: FEV1/FVC ratio <70 %, 50% ≤ FEV1 < 80% with or without symptoms.

Exclusion criteria:

-Upper or lower respiratory tract infection during last two months preceding the study.

-Therapy with inhaled or oral corticosteroid or other immunosuppressive agents within a month prior to the study.

-FEV_1 < 50% of predicted and or FEV_1 <1L

-Patient with history of atopy or other chronic respiratory diseases.

The following schedule was done for all subjects:

1-Thorough history taking and full clinical examination.

2-Chest X-ray postero-anterior and lateral views to exclude other pulmonary diseases (e.g. bronchiectasis).

3-Pulmonary function tests: Using computerized spirometry apparatus (Spiropro) including: Forced expiratory volume in the 1st second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, peak expiratory flow rates (PEFR) and forced expiratory flow at 25-75% of FVC ($FEF_{25–75\%}$).

4-Fiberoptic bronchoscopy (FB): FB was performed by Olympus BF type P10, Tokyo, Japan using a standardised protocol according to recent recommendations [11]. Smokers were requested to refrain from smoking on the day of the bronchoscopy.

Patients received premedication (400 µg inhaled salbutamol by metered dose inhaler). Intramuscular 1mg atropine sulphate, to reduce airway secretions and diminishing the chance of reflex vasovagal phenomenon such as bronchoconstriction and bradycardia and intramuscular 10 mg diazepam were administered 15 minutes before bronchoscopy to sedate the patient during the procedure. Local anaesthesia was done by lidocaine.

Six macroscopically adequate bronchial biopsy specimens were taken from subsegmental carinae in the right lower lobe.

5-Tissue specimens:

Bronchoscopic biopsies taken from the studied subjects were fixed with 10% buffered formalin and embedded in paraffin. The blocked tissues were processed to 3-5 μm sections and subjected to:

Haematoxylin and eosin (H&E) staining: For routine histopathological evaluation. A subjective semi-quantitative microscopic evaluation of the changes in the bronchial epithelium of the studied groups was done with score (+) for mild changes, (++) for moderate changes and (+++) for severe changes.

Periodic acid-Schiff (PAS) stain: For demonstration of goblet cells. The procedure of PAS staining was conducted according to Totty [(12)].

Immunohistochemistry:

For immunohistochemistry, sections were deparaffinized in xylene for 30 minutes and rehydrated with graded alcohol series. Sections were then subjected to antigen retrieval by boiling the tissue sections in 10mM citrate buffer, pH 6.0 (Lab Vision catalog # AP-9003), for 10 minutes followed by cooling at room temperature for 20 minutes and rinsing with phosphate buffered saline (PBS) for one minute. Endogenous peroxidase was blocked by immersion of the sections in 3% hydrogen peroxide solution for 10 minutes, then washing them in PBS. Immunohistochemical staining was performed using the UltraVision Detection Kit (TP-015-HD, Lab Vision, USA) according to the manufacturer's protocol. Sections were incubated for 10 minutes with Ultra V block to prevent non-specific background staining, followed by rinsing the sections with PBS. Afterwards, an overnight incubation was done in a humidity chamber with monoclonal primary antibody against epidermal growth factor receptor "EGFR" (Ab-23, Clone EGFR.113, Cat. #MS-1868-R7, Ready to use, Lab Vision, USA), followed by washing in PBS. Sections were then covered with 4-5 drops of UltraVision biotinylated goat anti-polyvalent secondary antibody, incubated at room temperature for 10 minutes, then washed in PBS, followed by incubation with streptavidin peroxidase solution for 10 minutes at room temperature, then rinsing with PBS. Sections were then covered for 15 minutes by adding one drop of 3-3`- diamino-benzidine-tetra-hydrochloride (DAB) chromogen mixed with 2 ml of DAB substrate. Finally, sections were counterstained with Mayer's haematoxylin, dehydrated in alcohol and mounted in di-n-butyl-phthalate-polystyrene-xylene (DPX).

Sections from a case of squamous cell carcinoma were used as positive controls, while negative controls were prepared by omission of the primary antibody.

Interpretation and quantification of EFGR expression:

Positivity for EGFR was indicated by membranous and/or cytoplasmic staining. A semiquantitative analysis of EGFR expression was performed using an arbitrary visual scale with grading scores of 0, 1, 2, and 3 representing absence of staining, weak, moderate, and intense staining respectively. [(13)]

Statistical analysis: By using Minitab 12.1 for windows. Data concerning patient characteristics were presented as mean ± standard deviation. The probability of significant differences among dual means of groups was determined by Student's t-test. Correlation between EGFR expression and other findings in the studied groups was tested using correlation coefficient. Statistical significance was defined as $P<0.05$.

RESULTS

I- Patient characteristics:

This study was conducted on 50 subjects who were divided into 3 groups:

Group I: Included 10 apparently healthy non-smoker subjects (control group); all of them were males, their ages ranged between 45 and 59 with a mean of 53.40 ±5.910 years.

Group II: Included 10 apparently healthy smoker volunteers, all of them were males with smoking index ranged from 280 – 440 with a mean of 361±56.460, their ages ranged between 48 and 58 with a mean of 55.70±3.497 years.

Group III: Included 30 patients with COPD, all of them were males, their ages ranged between 48 and 66 with a mean of 56.10±6.590 years.

This group was subdivided according to their smoking status into:

Subgroup III_A: Included 19 current smokers with COPD, their smoking index ranged from 300-470, with a mean of 399±52.376. Their ages ranged between 49 and 66 with a mean of 56.50±6.819 years.

Subgroup III_B: Included 11 ex-smokers with COPD; all of them stopped smoking for more than two years ago, their ages ranged between 48 and 66 with a mean of. 57.50±6.204 years.

Comparison between the spirometric data of the three studied groups and between the spirometric data of subgroups III_A and III_B were illustrated in tables 1 and 2 respectively.

Table (1): Comparison between the spirometric data of the three studied groups:

	Group I Mean± SD	**Group II Mean± SD**	**Group III Mean± SD**	**t (P)**
FVC % of predicted	102±5.715	93.60±2.913	79±16.448	t_1=4.14 *(<0.01) t_2=8.15 *(<0.01) t_3=7.32 *(<0.01)
FEV_1 % of predicted	100.70±7.334	97.50±3.027	72.1±14.63	t_1=1.28 *(<0. 01) t_2=8.15 *(<0. 01) t_3=32.3 *(<0.001)
$FEF_{25-75\%}$ % of predicted	104.10±7.1562	100.30±6.074	74.30±27.090	t_1=-1.28 *(<0.01) t_2=17.4 *(<0.01) t_3=17.5 *(<0.01)
PEFR % of predicted	95.13±17.040	65.72±8.660	59.52±24.120	t_1=-8.09 *(<0.01) t_2=7.4 *(<0.01) t_3=1.75 *(<0.01)
FEV_1/FVC Actual value	86.90±3.541	72.10±4.254	60.70±10.762	t_1=-8.09 *(<0.01) t_2=7.4 *(<0.01) t_3=1.75 *(<0.01)

*Significant

t_1= group I Vs group II.

t_2= group I Vs group III.

t_3= group II Vs group III.

Table (2): Comparison between the spirometric data of subgroups III_A and III_B:

	Subgroup III_A Mean± SD	**Subgroup III_B Mean± SD**	**t (P)**
FVC % of predicted	60.70±12.266	91.90±2.024	t=-5.71 *(<0.01)
FEV_1 % of predicted	57.40±2.503	81.70±4.667	t=-14.5 *(<0.001)
$FEF_{25-75\%}$ % of predicted	50.20±6.713	99±5.637	t=-17.6 *(<0.001)
PEFR % of predicted	57.54±25.900	64.72±12.280	t=-1.08 *(<0.01)
FEV_1/FVC Actual value	51.80±8.148	66.8±2.936	t=-5.48 *(<0.01)

*Significant

II-Histopathological and histochemical findings:

Histopathological examination of bronchial biopsies taken from the studied subjects revealed that non-COPD current smokers and COPD patients showed evident epithelial alterations compared to the normal bronchial epithelium of the control subjects. These alterations were significantly exaggerated in COPD patients compared to non-COPD smokers (P<0.05), however, no significant differences were found between current and ex-smokers with COPD (P>0.05).

The most prominent alterations were bronchial epithelial hyperplasia, in which the number of the bronchial epithelial cells was markedly increased (Fig. 1&2), and goblet cell hyperplasia, in which goblet cells were increased in number and evenly distributed along the airway epithelium (Fig. 3). Goblet cell hyperplasia was confirmed by PAS staining (Fig. 4). Subepithelial inflammatory infiltrate was also seen (Fig. 1).

In addition, minor alterations, in the form of areas of epithelial shedding, were also observed.

Comparison between groups II and III, and between subgroups III_A and III_B regarding the severity of these epithelial alterations was illustrated in tables 3 and 4 respectively.

Table (3): Comparison between group II and group III regarding the severity of the epithelial alterations:

Epithelial alterations / Groups	Goblet cell hyperplasia						Bronchial epithelial hyperplasia					
	(+)		(++)		(+++)		(+)		(++)		(+++)	
	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%
Group II (n=10)	6	60	3	30	1	10	5	50	3	30	2	20
Group III (n=30)	0	0	16	53.3	14	46.7	0	0	13	43.3	17	56.7
	t = 3.99 P = 0.0018*						**t = 3.14 P = 0.0094***					

*Significant

Table (4): Comparison between subgroup III_A and subgroup III_B regarding the severity of the epithelial alterations:

Epithelial alterations / Groups	Goblet cell hyperplasia						Bronchial epithelial hyperplasia					
	(+)		(++)		(+++)		(+)		(++)		(+++)	
	N.	%	N.	%	N.	%	N.	%	N.	%	N.	%
Subgroup III_A (n=19)	0	0	10	52.6	9	47.4	0	0	8	42.1	11	57.9
Subgroup III_B (n=11)	0	0	6	54.5	5	45.5	0	0	5	45.5	6	54.5
	t = 0.1 P = 0.92						**t = 0.17 P = 0.87**					

III-Immunohistochemical findings:

Group I:

Immunohistochemical analysis of EGFR expression in normal lung tissue of the control specimens revealed weak positive "grade 1" EGFR expression detected in 9 cases (90%), while one case (10%) showed moderate "grade 2" EGFR expression. The expression was limited to the basal cells of the bronchial epithelium (Fig. 5).

Group II:

In current smokers without COPD, the EGFR immunoreactive cells were not restricted to the basal compartment but extended to the intermediate cells. In this group, 4 cases (40%) showed weak "grade 1" EGFR expression, 4 cases (40%) showed moderate "grade 2" EGFR expression (Fig.6), and 2 cases (20%) showed intense "grade III" EGFR expression.

Group III:

In COPD patients, the expression of EGFR was also observed both in the basal as well as the intermediate cells. In this group, one case (3.3%) showed weak "grade 1" EGFR expression, 16 cases (53.3%) showed moderate "grade 2" EGFR expression (Fig. 7), and 13 cases (43.3%) showed intense "grade III" EGFR expression (Fig. 8).

The expression of EGFR was significantly higher in group II and III compared to group I ($P<0.05$). Moreover, group III showed significantly higher EGFR expression compared to group II ($P<0.05$).

Subgroup analysis revealed that subgroup III_B "ex-smokers with COPD" displayed significantly higher ($P<0.05$) EGFR expression (Fig. 8) compared to subgroup III_A "current smokers with COPD" (Fig. 7).

Comparison between the studied groups and between subgroups III_A and III_B regarding EGFR immunoreactivity was illustrated in tables 5 and 6 respectively.

Correlation between EGFR expression and other findings in group II and group III:

Statistically significant positive correlations were found between EGFR expression and both goblet cell hyperplasia, and bronchial epithelial hyperplasia in groups II and III ($P<0.05$), while there was a significant negative correlation between EGFR expression and FEV1 in groups II and III ($P<0.05$). There was a significant positive correlation ($P<0.05$) between EGFR expression and smoking index in group II (Tables 7 and 8 respectively).

Table (5): Comparison between the studied groups regarding EGFR immunoreactivity:

EGFR expression / Groups	Grade 0 EGFR expression		Grade 1 EGFR expression		Grade 2 EGFR expression		Grade 3 EGFR expression	
	N.	**%**	**N.**	**%**	**N.**	**%**	**N.**	**%**
Group I (n=10)	0	0	9	90	1	10	0	0
Group II (n=10)	0	0	4	40	4	40	2	20
Group III (n=30)	0	0	1	3.3	16	53.3	13	43.3

I vs II: t = -2.60 P = 0.024*
I vs III: t = -9.06 P = 0.00001* II vs III: t = -2.22 P = 0.046*

*Significant

Table (6): Comparison between subgroups III_A and III_B regarding EGFR immunoreactivity.

EGFR expression / Groups	Grade 0 EGFR expression		Grade 1 EGFR expression		Grade 2 EGFR expression		Grade 3 EGFR expression	
	N.	**%**	**N.**	**%**	**N.**	**%**	**N.**	**%**
Subgroup III_A (n=19)	0	0	1	5.3	13	68.4	5	26.3
Subgroup III_B (n=11)	0	0	0	0	3	27.3	8	72.3
t	-2.77							
P	0.011*							

*Significant

Table (7): Correlation between EGFR expression and other findings in group II:

Correlation between intensity of EGFR staining and	**r**	**P**
Goblet cell hyperplasia	0.96	0.001*
Bronchial epithelial hyperplasia	0.93	0.001*
FEV1	-0.95	0.001*
Smoking index	0.95	0.001*

*Significant

Table (8): Correlation between EGFR expression and other findings in group III:

Correlation between intensity of EGFR staining and	**r**	**P**
Goblet cell hyperplasia	0.94	0.001*
Bronchial epithelial hyperplasia	0.96	0.001*
FEV1	-0.93	0.001*
Smoking index	0.425	0.294

*Significant

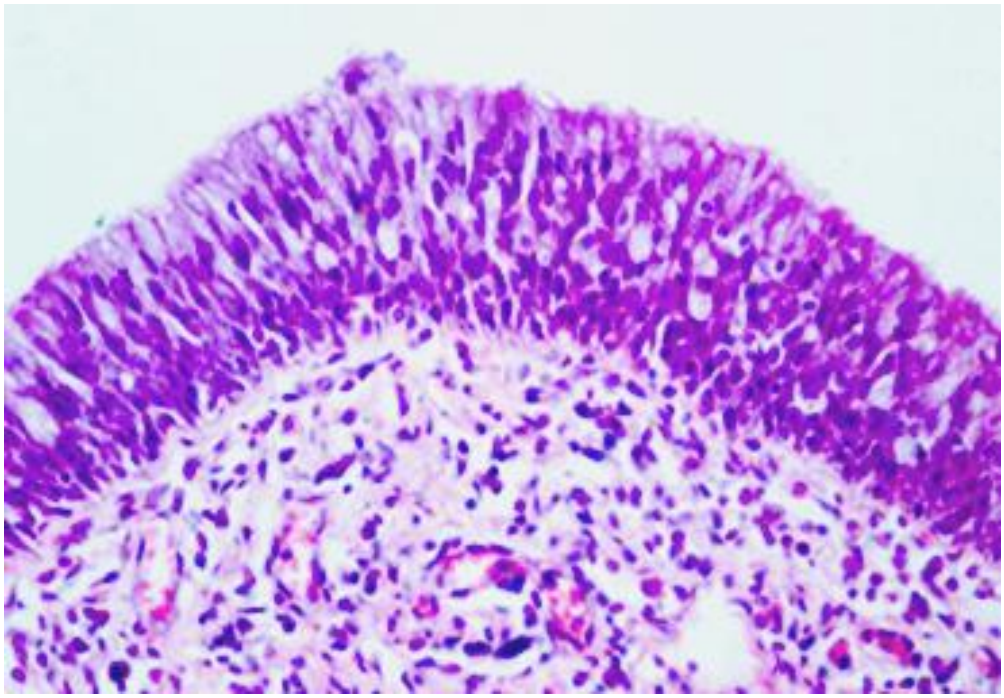

Fig. (1): Bronchoscopic biopsy of a COPD patient showing hyperplasia and stratification of the bronchial epithelial cells, as well as subepithelial inflammatory infiltrate (H&E X400).

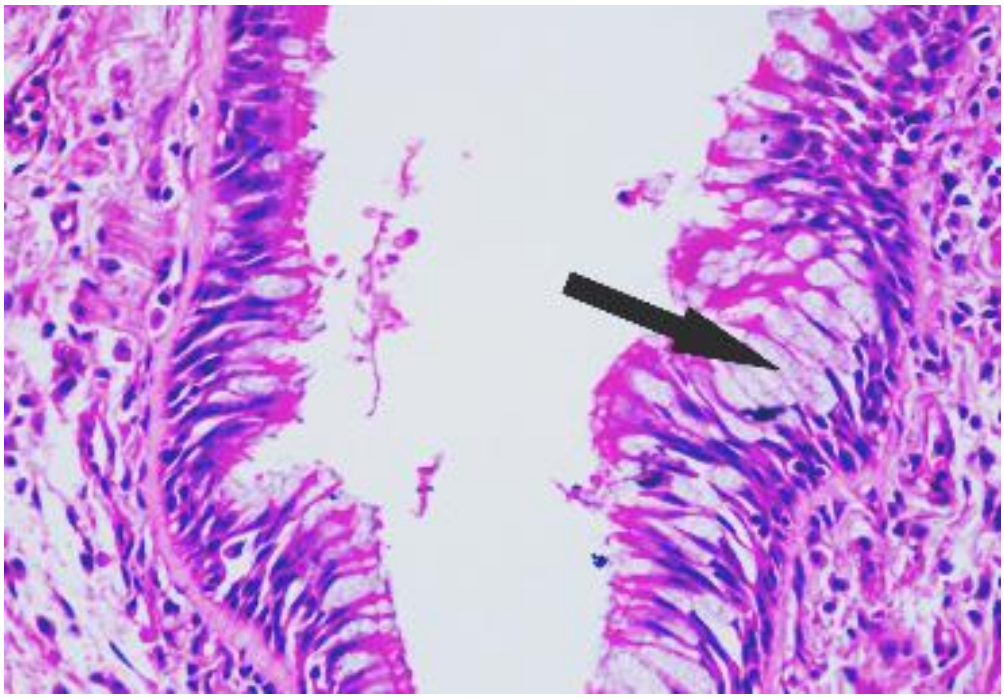

Fig. (2): Bronchoscopic biopsy of a non-COPD cigarette smoker showing hyperplasia and stratification of the bronchial epithelial cells as well as goblet cell hyperplasia "arrow", the severity of changes was less than observed in COPD patients (H&E X400).

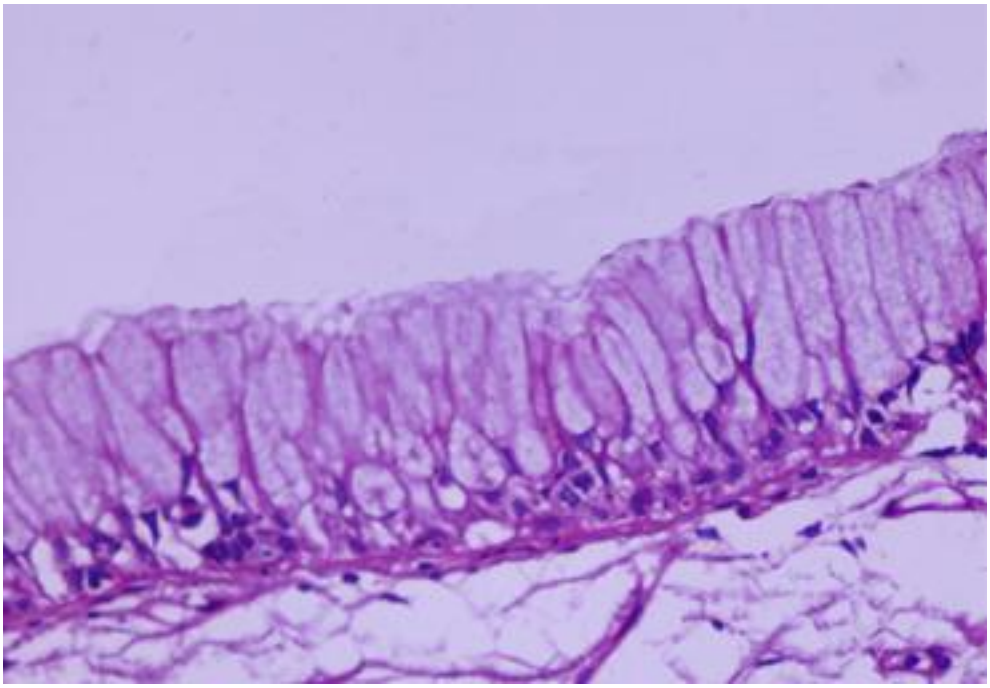

Fig. (3): Bronchoscopic biopsy of a COPD patient showing goblet cell hyperplasia, with numerous and evenly distributed goblet cells along the air way epithelium (H&E X400).

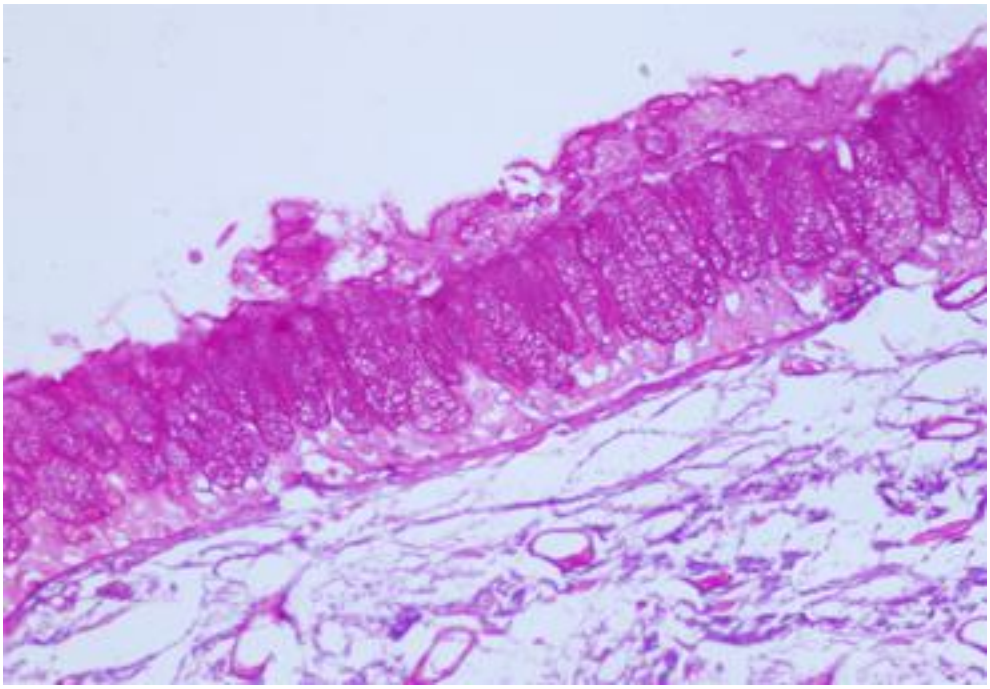

Fig. (4): Bronchoscopic biopsy of COPD patient showing marked goblet cell hyperplasia (PAS X400).

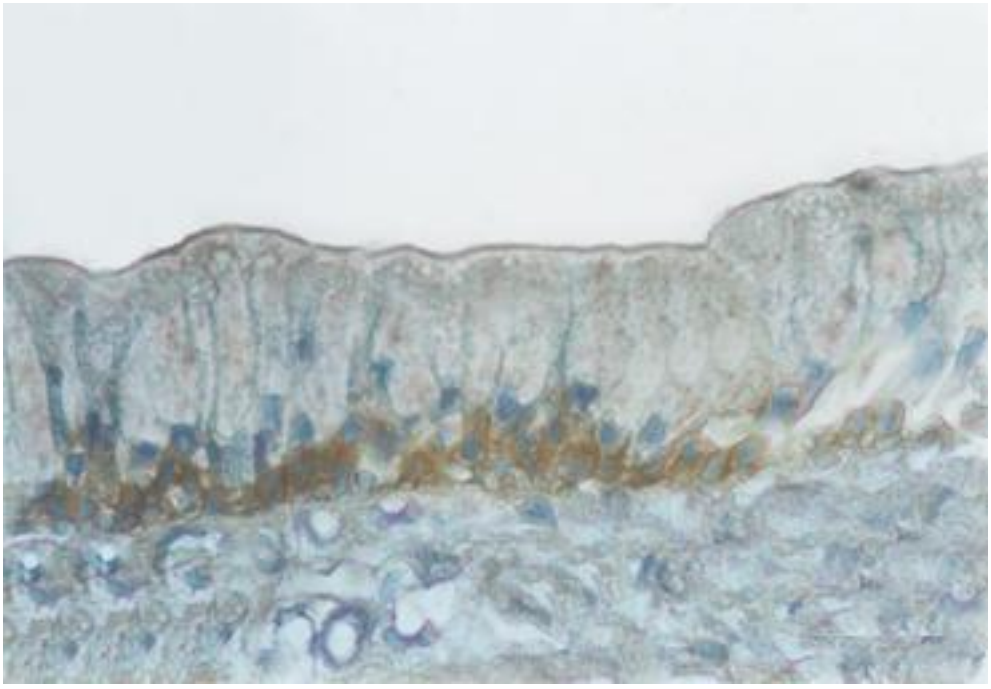

Fig. (5): Bronchoscopic biopsy of a normal control subject. The bronchial epithelium shows weak (grade 1) expression of EGFR restricted to the basal cells (Immunoperoxidase X400).

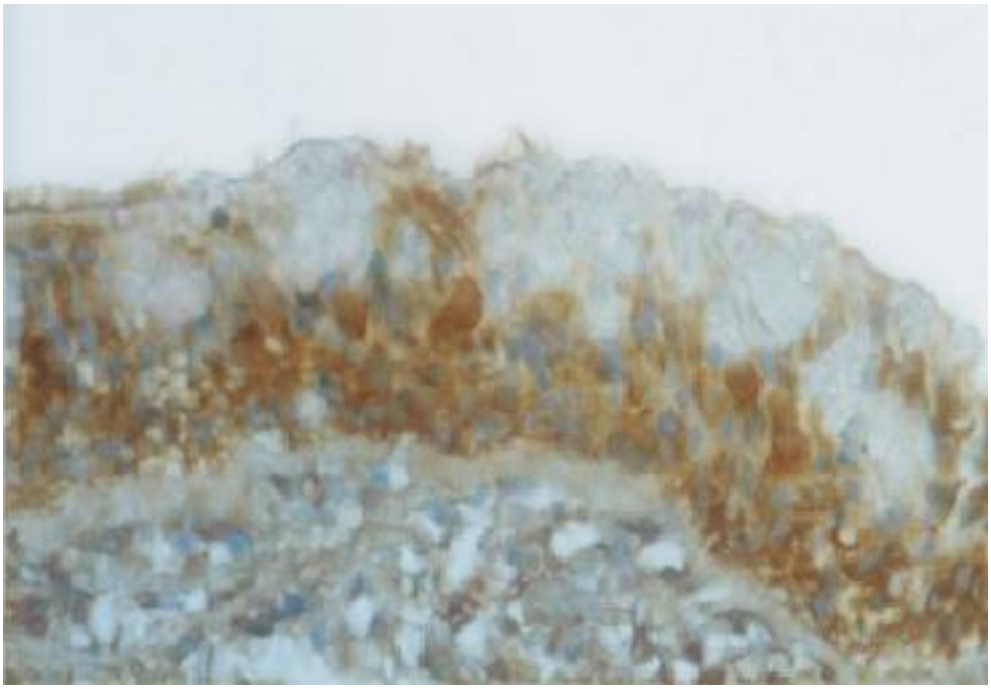

Fig. (6): Bronchoscopic biopsy of a non-COPD smoker showing moderate (grade 2) expression of EGFR extending to the intermediate compartment of the bronchial epithelium (Immunoperoxidase X400).

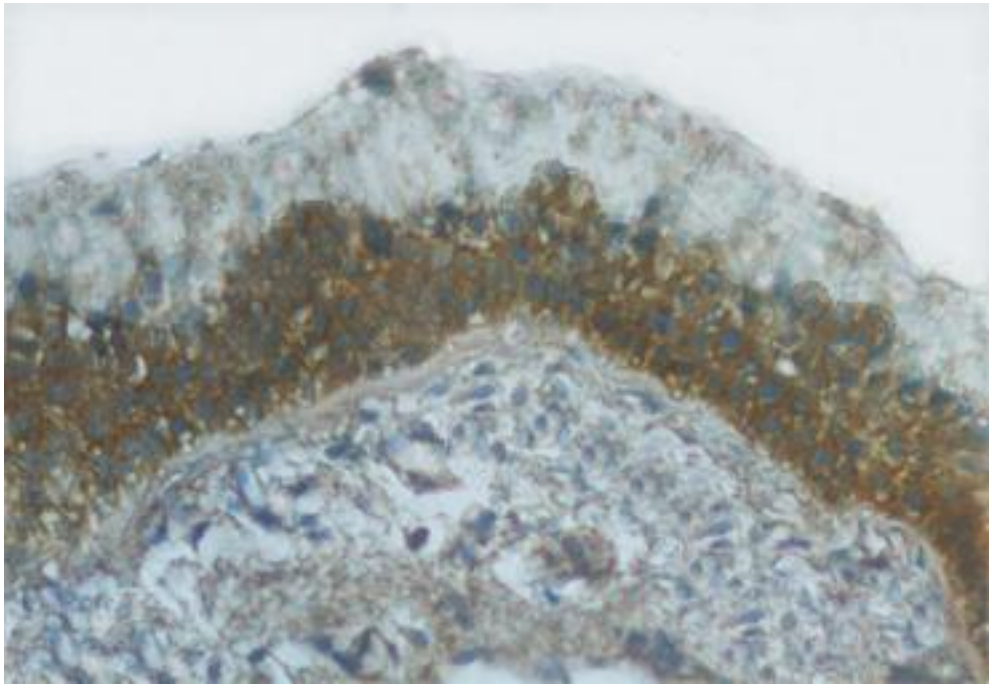

Fig. (7): Bronchoscopic biopsy of current smoker with COPD showing moderate (grade 2) expression of EGFR extending to the intermediate compartment of the bronchial epithelium (Immunoperoxidase X400).

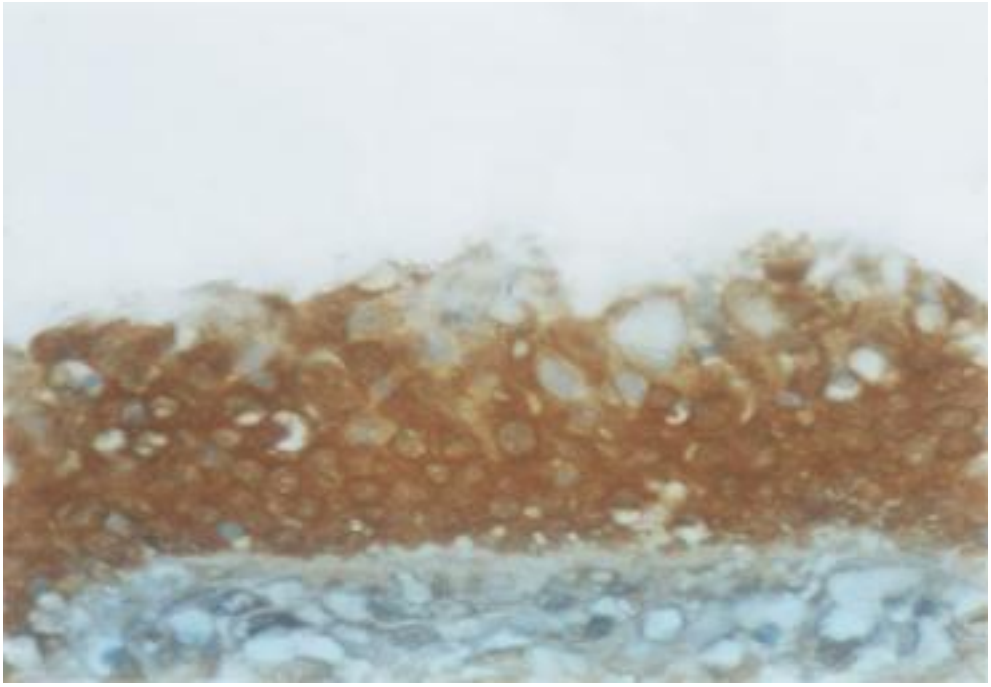

Fig. (8): Bronchoscopic biopsy of ex-smoker with COPD showing intense (grade 3) expression of EGFR involving the whole thickness of the bronchial epithelium (Immunoperoxidase X400).

DISCUSSION

Chronic obstructive pulmonary disease (COPD) is a major and increasing global health problem. It is predicted by the World Health Organization to become the third most common cause of death and the fifth most common cause of disability in the world by 2020. [14] One of the major causal factors is tobacco smoking. [15] Cigarette smoke exposure results in airway epithelial damage, squamous and goblet cell metaplasia, and bronchial epithelial hyperplasia. [2] These epithelial phenotypic alterations (also known as epithelial remodeling) are important for our understanding of the pathogenesis of COPD, since bronchial epithelial cells orchestrate an adequate maintenance of lung homeostasis by mucus production, ciliary beating, secretion of antimicrobial products and adequate immunological drive in response to noxious stimuli, however, they can be life threatening, especially goblet cell metaplasia, which is the foundation for hypersecretion, which is associated with morbidity and mortality in COPD. [5]

In the present study, these epithelial alterations were evaluated by examination of H&E-stained sections (and PAS-stained sections for demonstration of goblet cells), from bronchoscopic biopsies taken from the different groups included in this study. We observed that the bronchial epithelium of cigarette smokers without COPD as well as COPD patients (either current smokers or ex-smokers) displayed the same epithelial changes in the form of bronchial epithelial hyperplasia and stratification, as well as goblet cell hyperplasia. These observations were in accordance with other studies. [2]

In the present study, these epithelial alterations were significantly exaggerated in COPD patients than non-COPD smokers ($P<0.05$). This finding was in accordance with Luppi et al. [16], who reported that some of these epithelial features were more pronounced in COPD patients than in asymptomatic smokers, however they contrasted the findings of de Boer et al. [2] and Aarbiou et al. [17], who found no differences in the epithelial features between subjects with and without COPD, irrespective of current smoking status.

The present study revealed no significant differences regarding the severity of these epithelial alterations between current and ex-smokers with COPD ($P>0.05$), the same finding was reported by Aarbiou et al. [17]

A possible explanation of the latter finding is that these epithelial changes may be caused directly by cigarette smoke or indirectly by the inflammatory response induced by cigarette smoking. [18] This inflammatory process, and subsequently, the inflammation-induced epithelial changes, may persist even after smoking cessation for a prolonged time. [19-21]

On the other hand, Lapperre et al. [5] demonstrated that long-term ex-smokers with COPD had less bronchial epithelial mucin stores, proliferating cells, and squamous cell metaplasia than current smokers with COPD.

These structural alterations represent a repair process that occurs in response to epithelial injury aiming at restoration of the normal epithelial integrity [22], however, they may have a role in the development of airway limitation [23], and may considerably disturb the innate immune functions of the airway epithelium [24], especially goblet cell hyperplasia, that leads to mucus hypersecretion, which is the hallmark of COPD. [25]

Understanding the mechanisms implicated in the development and regulation of these structural changes will help to improve our knowledge of COPD pathogenesis. In this respect, many growth factors and their receptors have the potential to regulate processes involved in epithelial responses to

injury. Among these, the epidermal growth factor receptor (EGFR) occupies a prominent role as a primary regulator of epithelial cell function, as it is known to play a role in the regulation of cellular growth and differentiation. [26] It is known to be involved in epithelial repair in skin, the gastrointestinal tract, and animal models of acute lung injury. [4]

In the airway epithelium, activation of the EGFR cascade has been shown to be involved in mucin production and goblet cell hyperplasia [27-28], repair of damaged epithelium, as well as development of squamous cell carcinoma. [29]

Because of the critical involvement of EGFR in epithelial remodeling, the present work aimed at studying the expression of EGFR in the bronchial epithelium of patients with COPD and in smokers without COPD in comparison to its expression in normal bronchial epithelium, in a trial to investigate its role in the pathogenesis of COPD, and in the epithelial alterations that characterize this disease.

Comparison of the expression of EGFR between the different groups included in this study revealed significantly higher expression of EGFR in the bronchial epithelium of current smokers without COPD as well as COPD patients compared to its expression in the normal bronchial epithelium of the control group ($P<0.05$). Moreover, the expression of EGFR was significantly higher in COPD patients than in current smokers without COPD ($P<0.05$).

Subgroup analysis of EGFR expression within the COPD group revealed significantly higher expression of EGFR in ex-smokers with COPD compared to current smokers with COPD ($P<0.05$).

The increased EGFR expression in smokers compared to non smokers was also reported by de Boer et al. [2], O'Donnell et al. [30], and Mao et al. [31], however, they found no significant difference in the expression of EGFR between healthy smokers and smokers with COPD, irrespective of disease severity.

In accordance with the present study, de Boer et al. [2] found higher expression of EGFR in ex-smokers with COPD compared to current smokers with COPD, however, this finding contrasted those reported by Lapperre et al. [5], who found no difference in EGFR expression between current and ex-smokers with COPD.

The results of the present study as well as those of de Boer et al. [2] indicate that current smoking and the presence of airflow obstruction independently may increase the expression of EGFR, whereas current smoking in the presence of airflow obstruction inhibits the mechanisms leading to increased EGFR expression.

A possible explanation for this finding was reported by de Boer et al. [2], who suggested an inhibitory effect of cigarette smoke on the expression of mediators involved in the regulation of expression of epidermal growth factors (EGFs) and their receptors obscuring the intrinsically higher expression of these factors or by (oxidative) inactivation of these mediators.

This inhibitory effect of cigarette smoke on the expression of EGFR in the bronchial epithelium of current smokers with COPD may explain the discrepancy between the histological findings and EGFR in these patients.

The present study also revealed statistically significant positive correlations between EGFR expression and both goblet cell hyperplasia, and bronchial epithelial hyperplasia in groups II and III ($P<0.05$), while there was a significant negative correlation between EGFR expression and FEV1 in groups II and III ($P<0.05$). There was a significant positive correlation between

EGFR expression and smoking index in group II ($P<0.05$).

In accordance with our results, Bon et al. [32] found that EGFR in COPD patients showed a direct association with FEV_1. However, on the other hand, de Boer et al. [2], found no relation between EGFR scores and FEV1, and Lapperre et al. [5] found that the epithelial features were not associated with the degree of airflow limitation.

The mechanisms involved in the activation of EGFR require discussion. In vitro studies point to a central role for activation of the EGFR in the restoration of the bronchial epithelium following injury, since EGF is a mitogen for bronchial epithelial cells, mechanical damage induces rapid phosphorylation of the EGFR. [33]

Subsequently, EGFR tyrosine phosphorylation promotes its association with signaling proteins, leading to membrane-associated Ras activation, and initiates downstream signaling to the nucleus. Thus, EGFR activation can regulate gene transcription and subsequent protein synthesis. It has recently been reported that EGFR activation by its ligands leads to mucin synthesis and goblet-cell production in human bronchial epithelial cells [27], leading to exaggerated mucus production in conducting airways in COPD. [3]

A wide variety of stimuli can induce EGFR activation including cigarette smoke [28,29], which causes both mucus hypersecretion and increases the number of goblet cells. [3]

To induce EGFR activation by cigarette smoke, two different pathways have been reported. First, the binding of its ligands to EGFR activates the intrinsic receptor tyrosine kinase and induces tyrosine phosphorylation. [3] Second, tyrosine phosphorylation of EGFR can be activated by a ligand-independent mechanism "transactivation", which is known to occur with various stimuli such as oxidative stress, which can be produced by cigarette smoke [28], ultraviolet light, and osmotic stress, and with stimulation of G protein-coupled receptors by endothelin-1, lysophosphatidic acid, and thrombin. This implicates that cigarette smoke is an important regulator of epithelial cell differentiation that may result in abnormal induction of mucin-producing cells in airways. [3]

Takeyama et al. [34] reported that the cigarette smoke –induced epithelial changes could be inhibited by selective EGFR inhibitors and antioxidants.

Small molecule inhibitors of EGFR kinase, such as gefitinib and erlotinib, have now been developed for the treatment of non-small cell lung cancer and are worth exploring in COPD patients. [35]

In conclusion, there is increasing evidence that EGFR may play an important role in the epithelial phenotypic alterations observed in the bronchial epithelium of COPD patients through active smoking, and that it has a significant role in regulating mucus production in airway epithelium and in the repair of epithelium after injury.

Disruption of the EGFR cascade may provide a mechanism and a strategy for therapy in airway inflammatory (hypersecretory) diseases by blocking EGFR activation with subsequent inhibition of goblet cell production and reduction of mucus secretion, which is the main cause of airway limitation in COPD patients. However, further studies evaluating these new therapeutic modalities are required.

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