JOURNAL OF ADVANCED MEDICAL AND PHARMACEUTICAL RESEARCH

Research Article

A Pilot Study Comparing the Effectiveness of Three Combined Therapeutic Regimens in Egyptian Patients with Moderate to Severe Chronic Obstructive Pulmonary Disease

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ABSTRACT

Objective: We aimed at comparing the effectiveness of long-acting β-agonist+long acting muscarinic antagonist (LABA+LAMA) versus both LABA+inhaled corticosteroid (ICS) and LAMA+ICS in non-asthmatic patients with moderate to severe COPD. Besides, we aimed at assessing the changes that occur in plasma concentrations of TNF-α, fibrinogen, and IL-6 with the disease activity. **Methods:** In this pilot study, 45 non-asthmatic patients with moderate to severe COPD were randomized into three groups; group I (LABA+ICS) received Formoterol/Budesonide, group II (LAMA+ICS) received Tiotropium/Budesonide and group III (LABA+LAMA) received Formoterol/Tiotropium for twelve weeks. The patients were assessed at baseline, four and twelve weeks after therapeutic intervention through evaluating the changes occur in FEV1% predicted, mMRC dyspnea scale, and plasma concentrations of TNF-α, fibrinogen, and IL-6. **Results:** At baseline, the study groups were statistically concerning the demographic data and disease characteristics. All study therapeutic options produced an improvement in FEV1% predicted and mMRC dyspnea scale which was associated with a reduction in plasma concentrations of the inflammatory markers. The effects produced by the three therapeutic combinations on FEV1% predicted, plasma TNF-α, IL-6 and fibrinogen concentrations were statistically similar (four weeks after treatment; p=0.358, p=0.284, p=0.155, p=0.155 respectively) and (twelve weeks after treatment: p=0.710, p=0.773, p=0.240, p=0.076 respectively). **Conclusion:** In non-asthmatic patients with moderate to severe COPD, the three therapeutic combinations showed similar effectiveness. Furthermore, the results of this pilot study suggest that inflammatory markers can be used to follow the disease activity.

Keywords: COPD, ICS, LABA, LAMA, TNF-α.

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is associated with abnormal inflammatory response of the lungs towards noxious particles and gases, usually from cigarette smoke. Symptomatic COPD patients can be managed with one of the following inhaled medications; long-acting beta-agonists (LABA), long-acting muscarinic antagonists (LAMA), and inhaled corticosteroids (ICS).[1] Administration of two or more medications from different classes seems beneficial when the disease cannot be controlled adequately with LAMA or LABA monotherapy.[1] LAMAs dilate the airway by selectively blocking acetylcholine M3 receptors.[2] LABAs are

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β2-agonists, which provide smooth muscle relaxation by stimulating β2-adrenergic receptors.[3]

Furthermore, LAMAs and LABAs were reported to exert anti-inflammatory activity.[2,4-5] Although there is still little evidence for their clinical benefit and increasing evidence that the high doses currently recommended are harmful and costly, ICS are grossly overprescribed secondary to successful marketing.[6-7] ICSs were reported to be effective in bronchial asthma whereas eosinophils play a key role and they seem poorly effective in patients with COPD since neutrophils play a critical role. Pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 appear to amplify inflammation in COPD through activation of the transcription factor, nuclear factor (NF)-κB, thereby leading to increased expression of multiple inflammatory genes. TNF-α was reported to be involved in airway inflammation during COPD.[9] Fibrinogen is an acute phase soluble plasma glycoprotein, which regulates inflammation in many diseases and its plasma concentration may represent a promising biomarker to indicate the disease severity.[10] Additionally, it was postulated that IL-6 plays a considerable role in the systemic inflammatory response during COPD.[11]

There are discrepancies about the most effective combined therapeutic strategy for patients with COPD.[12] Besides, FEV1% predicted was reported to be poorly correlated with both symptoms and other measures of the disease progression; therefore, there is an urgent need for other biological biomarkers to follow the disease activity.[10,13] Furthermore, the current concepts suggest that an abnormal inflammatory response causes disease progression and many inflammatory cytokines were reported to induce airway inflammation and to be correlated with the severity of the disease.[9-11] In this context, we aimed at comparing the effectiveness of long acting β2-agonist+long-acting muscarinic antagonist (LABA+LAMA) versus both LABA+ inhaled corticosteroid (ICS) and LAMA+ICS in patients with moderate to severe COPD through evaluating the changes occur in FEV1% predicted and mMRC dyspnea scale. Also, we aimed at assessing the changes that occur in plasma concentrations of inflammatory markers (TNF-α, fibrinogen, and IL-6) with the disease activity.

2. METHODS

2.1. Study design

The design of this pilot study was a randomized double-blind prospective parallel study, which included 45 adult Egyptian patients of both sexes with moderate to severe COPD according to GOLD guidelines. All patients were recruited from Chest Disease Department, Tanta University Hospital, Tanta, Egypt, between November 2016 and December 2018. The patients were randomly categorized into three groups. Group I (LABA+ICS) received Formoterol/Budesonide combination 4.5/160 mcg, 2 inhalations BID. Group II (LAMA+ICS) received Tiotropium18 mcg inhaled capsule OD plus Budesonide 200 mcg, 2 inhalations BID. Group III (LABA+LAMA) received Formoterol 4.5 mcg inhaled

capsule BID plus Tiotropium18 mcg inhaled capsule OD. The treatment with the study medications was done after a washout period of two weeks from the entry medications and the only allowed medication during the washout period was salbutamol. The treatment duration was twelve weeks for all groups. Enrolled patients were randomized in a 1:1:1 ratio using a computer-generated code according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines. The double blindness included the investigator (physician) and the patients. The main investigator (the physician) was provided with a sealed randomization code for each available treatment generated by an independent researcher. The investigator remained blinded all over the study period and till the completion of laboratory analyses. The three therapeutic regimens were similar in route of administration, taste, and smell.

The study was approved by National Research Ethics Committee (CP00011), Tanta University, Egypt, and was registered retroactively as a clinical trial at ClinicalTrials.gov identifier: NCT04520230. Eligible patients gave their written informed consent. Inclusion criteria were patients with COPD aged ≥50 years old, FEV1/FVC < 0.70 and FEV1≥30% and<80% predicted and both sexes. Exclusion criteria were patients with very severe COPD (FEV1< 30% predicted), patients with chronic respiratory failure or recent chest infection, and patients with an exacerbation in the past 6 weeks. Patients with a history of asthma, other inflammatory diseases, and patients with clinically significant conditions such as unstable ischemic heart disease, uncontrolled hypertension, and diabetes were also excluded. The primary outcome was the measure of the effectiveness of the three combinations through evaluating the changes that occur in the FEV1% predicted and mMRC dyspnea scale. The secondary outcome was evaluating the changes in plasma concentrations of inflammatory markers.

2.2. Demography

All participants were submitted to demography (age, sex, and smoking habits), physical examination and measurement of weight, height, and calculation of body mass index (BMI).

2.3. Assessment of pulmonary function and dyspnea

Pulmonary function including FEV1% predicted was assessed by Spirometry (Chest® Spirometer, Model hl-101, Code 1113088, El-Radwan Company, Egypt). At baseline and twelve weeks after treatment, the modified Medical Research Council scale (mMRC dyspnea scale) was used for the assessment of dyspnea.

2.4. Sample collection and laboratory analyses

Blood samples were collected at baseline, four and twelve weeks after treatment. Plasma was separated and immediately stored at -80° C until biochemical analyses of plasma tumor

necrosis factor-alpha (TNF-α), plasma fibrinogen, and plasma interleukin 6 (IL-6) concentrations using the commercially available Enzyme-Linked Immunosorbent Assay kits (Assaypro, LLC Biotechnology company USA: Catalog No. ET2010-1, EF1040-1 and EI1006-1 respectively) using Tecan plate reader infinite F 50 (Tecan Group Ltd., Switzerland). All laboratory analyses were carried out at the laboratory of bioequivalence and pharmaceutical service unit, Faculty of Pharmacy, Tanta University.

2.5. Subjective data analysis

Through weekly phone calls and biweekly direct meetings, patients were followed-up to assess their adherence to the study medication and to report any adverse effects. The patient was considered non-adherent when he/she underused, overused, or discontinued the study medications. Patients' adherence to the study medication was assessed by counting the empty inhalers and through the medications refill rate. Non- adherent participant was excluded from the study as illustrated in Figure 1.
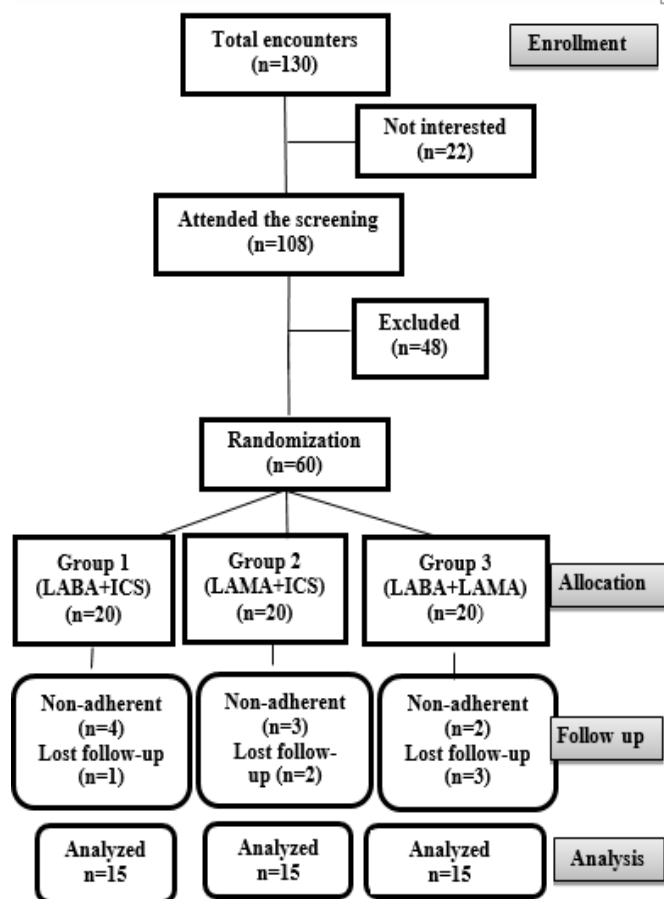

Figure 1: Flow-chart illustrates the participants screening, enrollment, and randomization.

2.6. Statistical analysis

The collected data were statistically analyzed using Statistical Package for the Social Sciences (SPSS), version 16 (Inc. Chicago, IL, USA). Quantitative data were presented as a range, mean ± SD, and median. Qualitative data were expressed as number and percent. For comparison between more than two means of parametric data (normally distributed data), the F value of the ANOVA test was calculated. Scheffe test was used to compare between each two means if F value was significant. Paired t-test was also used. For comparison between more than two means of non-parametric data (non-normally distributed data), Kruskal-Wallis (χ2) was used. Chi-square test was used for categorical variables. Correlation between variables was evaluated using Pearson's correlation. The significance level was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

The total encounters, screening, randomization, and follow-up procedures of the study participants are illustrated in Figure 1. At baseline, the study groups were statistically similar concerning demographic (age, male sex, weight, height, BMI), smoking (duration of smoking, past smokers, current smokers, non-smokers), and disease characteristics (Table 1). There was no group or treatment change during the follow-up of all patients.

Group II showed a significant increase in FEV1% predicted and significant improvement in the mMRC dyspnea scale twelve weeks after treatment as compared to baseline data (p=0.002 and p=0.0001 respectively). Instead, Group I and group III showed a significant improvement in the mMRC dyspnea scale twelve weeks after treatment (p=0.0001 and p=0.0001 respectively) which was associated with non-significant elevation of FEV1% predicted four and twelve weeks after treatment as compared to baseline data (p>0.05) as illustrated in Tables 2,3.

Comparing to baseline data, plasma TNF-α concentration showed a significant decrease four and twelve weeks after treatment in group I and group III (p=0.019, p=0.009 respectively for group I and p=0.036, p=0.007 respectively for group III). Concerning Group II, plasma TNF-α concentration showed significant decrease twelve weeks after treatment as compared to its baseline value (p=0.001) as shown in Table 4.

IL-6 plasma concentration showed a statistically significant decrease within group I four and twelve weeks after treatment versus baseline data (p=0.026, p=0.001respectively). Group II showed significant decrease in serum IL-6 concentration only twelve weeks after treatment as compared to its baseline data (p=0.043). Group III showed a non-significant decrease in IL-6 plasma concentration four and twelve weeks after treatment when compared to baseline data (p>0.05) as shown in Table 5.

Furthermore, group I showed a significant decrease in plasma fibrinogen concentration four and twelve weeks after treatment as compared to its baseline value (p=0.015, p=0.003 respectively). On the other hand, both group II and

Table 1: Baseline demographics, disease characteristics, pulmonary function test, and dyspnea scale.

Parameters	Group1 LABA+ ICS	Group LAMA+ ICS	Group 3 LABA+ LAMA	P value
Age (years)	63.5±9.19	62.5±7.04	64.9±9.38	0.74
Sex (Male)	10 (66.66%)	13 (86.66%)	11 (73.33%)	0.44
Weight (kg)	78.47±5.88	76.43±7.357	77.57±8.27	0.74
Height	171.33±5.31	170.27±6.03	172.40±4.12	0.54
BMI	26.75±2.36	26.64±2.71	26.33±3.03	0.97
Past Smokers	10 (66.66%)	12 (80%)	11 (73.33%)	0.72
Current Smokers	5 (33.33%)	7 (46.66%)	6 (40%)	0.76
Never Smoke	5 (33.33%)	3 (20%)	4 (26.66%)	0.71
Duration of Smoking (years)	23.9±9.02	21.83±7.25	22.27±8.3	0.83
GOLD Classification				
B	12 (80%)	8 (53.33%)	10 (66.66%)	0.30
D	3 (20%)	7 (46.66%)	5 (33.33%)	
FEV1% predicted				
Range	30.00-79.10	37.80-75.80	35.90-76.90	0.35
Mean±SD	61.62±15.14	54.53±11.57	60.24±15.59	
mMRC dyspnea scale				
Range	2.00-4.00	2.00-4.00	2.00-4.00	
Mean±SD	3.07±0.70	3.33±0.72	3.07±0.80	0.53
Median	3.00	3.00	3.00	
Entry Medications				
SABA	9(60.00%)	7 (46.66%)	7 (46.66%)	
SAMA	5(33.33%)	4(26.66%)	7(46.66%)	
Mucolytic	11 (73.33%)	8 (53.33 %)	10 (66.66%)	
Expectorant	7 (46.66%)	5 (33.33%)	8 (53.33%)	
Oral xanthines	5 (33.33%)	3 (20%)	4 (26.66%)	
LABA(Formoteol)	6 (40.00%)	8 (53.33%)	7 (46.66%)	
ICS(Budesonide)	2 (13.33%)	4 (26.66%)	2 (13.33%)	

The data are expressed as range, mean± SD, and median. LABA: Long acting β2-agonist, ICS: Inhaled corticosteroid, LAMA: Long-acting muscarinic antagonist, BMI: Body mass index, GOLD: Global Initiative for Chronic Obstructive Lung Disease, FEV1: Forced expiratory volume in 1 second, mMRC: Modified Medical Research Council, SABA: Short-acting B2-agonist, SAMA: short-acting muscarinic antagonist.

group III showed a non-significant decline in plasma fibrinogen concentration four and twelve weeks after treatment when compared to its baseline concentration (p>0.05) as demonstrated in Table 6.

There was a non-significant difference between the three therapeutic strategies four and twelve weeks after treatment regarding FEV1% predicted, plasma TNF-α, Il-6, and fibrinogen (four weeks after treatment; p=0.358, p=0.284, p=0.155, p=0.155 respectively) and (twelve weeks after treatment: p=0.710, p=0.773, p=0.240, p=0.076 respectively). Besides, twelve weeks after treatment, there was a non-significant difference in the mMRC dyspnea scale between the three therapeutic combinations (p=0.749). The comparison between the three groups is illustrated in Tables 2, 3, 4, 5, and 6.

For group I, there was a significant positive correlation between plasma TNF-α and IL-6 at baseline and twelve weeks after treatment (r=0.61, p=0.015 and r=0.562, p=0.029 respectively). Four weeks after treatment, TNF-α showed a significant positive correlation with plasma fibrinogen (r=0.874, p=0.0001). Concerning group II, a significant positive correlation was observed between plasma TNF-α and IL-6 at baseline (r=0.599, p=0.018). Additionally, four weeks after treatment, a significant negative correlation was observed between plasma TNF-α and FEV1% predicted (r= -0.678, p=0.006) as illustrated in Table 7.

Regarding drug-related adverse effects, mild and manageable adverse effects were reported whereas two patients in group I (13.33%) and one patient in group II (6.66%) showed mouth thrush. One patient in group III sho-

Table 2: Mean values of forced expiratory volume in one second (FEV1% predicted) among patients with moderate to severe COPD under three therapeutic options

Time of assessment	Mean values of FEV1 among the studied COPD patients under three options for treatment (n=45)			F value	P
	Group 1 (LABA+ICS) (n=15)	Group 2 (LAMA+ICS) (n=15)	Group 3 (LABA+LAMA) (n=15)		
Baseline:(a)					
Range	30.00-79.10	37.80-75.80	35.90-76.90	1.050	0.35
Mean±SD	61.62±15.14	54.53±11.57	60.24±15.59		
4 weeks after treatment: (b)					
Range	33.50-94.20	46.50-96.50	43.50-87.20	1.052	0.35
Mean±SD	75.29±21.22	66.10±14.21	70.85±15.89		
12 weeks after treatment: (c)					
Range	59.80-116.40	53.80-102.80	52.30-96.50	0.346	0.71
Mean±SD	77.61±21.34	72.60±13.31	73.83±15.97		
F value	2.962	7.349	3.060		
P	0.063	0.002*	0.057		
Scheffe test (P)		a vs b, P=0.064 a vs c, P=0.002* b vs c, P=0.404			

The data are expressed as range, mean± SD. FEV1: Forced expiratory volume in 1 second, LABA: Long acting β2-agonist, ICS: Inhaled corticosteroid, LAMA: Long acting muscarinic antagonist. *Significant (p<0.05) Scheffe test: Group II: Baseline significantly different from 12 weeks after treatment.

Table 3: m-MRC dyspnea scale at baseline and 12 weeks after treatment among the three studied groups.

Time of assessment	m-MRC values among the studied three groups			F value	P
	Group 1 LABA/ICS (n=15)	Group 2 LAMA/ICS (n=15)	Group 3 LABA/LAMA (n=15)		
Baseline:					
Range	2.00-4.00	2.00-4.00	2.00-4.00	0.644	0.53
Mean±SD	3.07±0.70	3.33±0.72	3.07±0.80		
Median	3.00	3.00	3.00		
12 weeks after treatment:					
Range	1.00-3.00	1.00-3.00	1.00-3.00	0.292	0.74
Mean±SD	1.73±0.80	1.73±0.80	1.93±0.88		
Median	2.00	2.00	2.00		
Paired t-test	6.33	12.22	8.50		
P	0.0001*	0.0001*	0.0001*		

The data are expressed as range, mean± SD, median. mMRC: Modified medical research council, LABA: Long acting β2-agonist, ICS: Inhaled corticosteroid, LAMA: Long acting muscarinic antagonist. *Significant difference with ANOVA test (p<0.05)

wed mild palpitation (6.66 %). There was a non-significant difference in the reported adverse effects among the three studied groups (P= 0.34 for mouth thrush and P = 0.36 for palpitation).

In this randomized double-blind pilot study, we aimed at comparing the effectiveness of long-acting β2-agonist+long-acting muscarinic antagonist (LABA+LAMA) versus both (LABA+ICS) and (LAMA+ICS) in non-asthmatic patients with moderate to severe COPD through evaluating the changes that occur in FEV1% predicted and mMRC dyspnea scale. In addition, we aimed at assessing the

changes that occur in plasma concentrations of inflammatory markers (TNF-α, fibrinogen, and IL-6) with the disease activity. The sample size used during the current study comes in accordance with the notion that; a sample size of 12 per group seems appropriate for a pilot study.[14] Furthermore, the sample size and the follow-up period of the current study were based on other previous studies conducted on patients with COPD.[15,16]

As compared to baseline data, the results obtained with group 1 (LABA+ICS) and group III (LABA+LAMA) four and twelve weeks after treatment revealed the presence

Table 4: Mean values of Tumor necrosis factor alpha (TNF-α) among patients with moderate to severe COPD under three therapeutic options.

Time of assessment	Mean values of TNF-α (Pg/ml) among the studied COPD patients under three options for treatment (n=45)			F value	P
	Group 1 (LABA+ICS) (n=15)	Group 2 (LAMA+ICS) (n=15)	Group 3 (LABA+ LAMA) (n=15)		
Baseline:(a)					
Range	3-44	18-55	18-76	0.601	0.55
Mean±SD	27.53±9.50	30.87±9.29	31.87±14.45		
4 weeks after treatment: (b)					
Range	10-30	12-48	14-31	1.297	0.28
Mean±SD	20.07±5.59	24.20±9.10	22.33±5.89		
12 weeks after treatment: (c)					
Mean±SD	13-30	10-35	11-30	0.259	0.77
	19.33±4.70	18.47±7.06	20.07±6.27		
F value	6.453	7.914	6.238		
P	0.004*	0.001*	0.004*		
Scheffe test (P)	a vs b, P=0.019*	a vs b, P=0.114	a vs b, P=0.036*		
	a vs c, P=0.009*	a vs c, P=0.001*	a vs c, P=0.007*		
	b vs c, P=0.959	b vs c, P=0.197	b vs c, P=0.816		

The data are expressed as range, mean± SD. TNF-α: Tumor necrosis factor alpha, LABA: Long acting β2-agonist, ICS: Inhaled corticosteroid, LAMA: Long acting muscarinic antagonist.*Significant (p<0.05) Scheffe test:Group I: Baseline significantly different from both 4 and 12 weeks after treatment. Group II: Baseline significantly different from 12 weeks after treatment. Group III: Baseline significantly different from both 4 and 12 weeks after treatment.

Table 5: Mean values of Interlukin-6 (IL-6) among patients with moderate to severe COPD under three therapeutic options.

Time of assessment	Mean values of Interlukin-6 (IL-6) (pg/ml) among the studied COPD patients under three options for treatment (n=45)			F value	P
	Group 1 (LABA+ICS) (n=15)	Group 2 (LAMA+ICS) (n=15)	Group 3 (LABA+ LAMA) (n=15)		
Baseline:(a)					
Range	3.50-13.10	0.33-19.05	3.60-22.50	1.270	0.29
Mean±SD	6.11±2.90	6.03±4.17	8.17±5.12		
4 weeks after treatment:(b)					
Range	2.15-7.30	1.30-7.60	3.10-21.70	1.953	0.15
Mean±SD	4.01±1.47	4.05±1.80	5.87±4.54		
12 weeks after treatment:(c)					
Range	1.50-6.30	0.50-7.10	2.20-13.50	1.477	0.24
Mean±SD	3.07±1.33	3.35±1.81	4.29±2.75		
F value	8.809	3.636	3.132		
P	0.001*	0.035*	0.054		
Scheffe test (P)	a vs b, P=0.026*	a vs b, P=0.172			
	a vs c, P=0.001*	a vs c, P=0.043*			
	b vs c, P=0.452	b vs c, P=0.792			

The data are expressed as range, mean± SD. LABA: Long acting β2-agonist, ICS: Inhaled corticosteroid, LAMA: Long-acting muscarinic antagonist. *Significant (*p<0.05) Scheffe test: Group I: Baseline significantly different from both 4 and 12 weeks after treatment Group II: Baseline significantly different from 12 weeks after treatment.

of statistically non-significant but clinically important improvement in FEV1% predicted. On the other hand, the results obtained with group II (LAMA+ICS) four and twelve weeks after treatment showed presence of significant improvement in FEV1% predicted. Twelve weeks after treatment, the three study groups showed significant improvement in the mMRC dyspnea scale as compared to their baseline data. The data obtained with the three studied groups can be explained on the basis that, LAMAs are muscarinic antagonists which block acetylcholine-mediated bron-

choconstriction by binding to M3 receptors in airway smooth muscles,[17] whereas LABAs are β2 agonists which provide smooth muscle relaxation by stimulating β2-adrenergic receptors.3 In addition, it was demonstrated that, LAMA and LABA combined therapy showed synergistic bronchodilator effect and improved symptoms in COPD patients.[17,18] Furthermore, it was postulated that, ICS may enhance the efficacy of LAMA.[19]

During the current study, group I showed a significant decrease in all inflammatory markers' concentrations while group III showed a significant reduction in TNF-α concentrations four and twelve weeks after treatment as compared to baseline data. On the other hand, the results obtained with group II showed a significant reduction in TNF-α and IL-6 concentrations twelve weeks after treatment. These results may be justified on the basis that, LAMA and LABA were reported to exert anti-inflammatory activity.[2, 4, and 5]. However, it was postulated that ICS monotherapy failed to reduce inflammatory markers in sputum or bronchial biopsies of COPD patients, 20 combining drugs with different modes of action may improve outcomes. Two-way synergistic activity between ICSs and LABAs has been demonstrated.[21-22] One of the cellular actions of ICSs is to translocate glucocorticoid receptors from the cytoplasm to the nucleus.[21] This action is enhanced in the presence of β-agonists and causes an anti-inflammatory effect greater than either drug alone.[22] In addition, ICSs activate β-receptor genes to produce more β-receptors, thereby enhancing the bronchodilator effect of LABA.[23] Therefore, the improvement in both FEV1% predicted and mMRC dyspnea scale may be attributed also to the reduction of plasma concentrations of inflammatory markers through the anti-inflammatory properties of the implicated therapeutic regimens.

The three therapeutic combinations showed similar efficacy, there was a non-significant difference between the four and twelve weeks after treatment regarding all measured parameters. In this context, the former result concerning the lack of significant effect of the combinations containing ICS over LAMA+LABA combination may be attributed to the notion that ICS seems more effective in patients with asthma-chronic obstructive pulmonary disease overlap syndrome.[24] Furthermore, this result may be related to smoking which increases airway inflammation and decreases corticosteroids responsiveness.[25] Our results seem in accordance with a former study demonstrated that, in real-world clinical practice setting of COPD treatment, combined LABA/LAMA inhalers appear to be as effective as combined LABA/ICS inhalers in preventing COPD exacerbations.[26] Additionally, our previous data come in agreement with a former study reported the absence of a significant difference in transition dyspnea index (TDI) focal score between LABA/LAMA and LABA/ICS treated groups.[27] In contrast, the data obtained with the current study seem incompatible with ENERGITO® study which proposed that dual bronchodilators can be considered to optimize lung function in patients with COPD who require maintenance treatment.[28] Our result seems in contradiction with other authors who reported that, compared to LABA+ICS, LAMA+LABA was associated with greater efficacy.[29,30]

Regarding the safety and tolerability of the study medications, the three therapeutic combinations were well tolerated which comes in agreement with a previously reported finding.[31] Besides, the three therapeutic combinations showed a statistically similar safety profile, a result seems in accordance with previously reported findings.[29]

Table 6: Mean values of fibrinogen among patients with moderate to severe COPD under three therapeutic options.

Time of assessment	Mean values of fibrinogen (µmol/L) among the studied COPD patients under three options for treatment (n=45)			F value	P
	Group 1 (LABA+ICS) (n=15)	Group 2 (LAMA+ICS) (n=15)	Group 3 (LABA+ LAMA) (n=15)		
Baseline:(a)					
Range	2.65-16.17	3.23-26.16	3.82-14.73	0.637	0.53
Mean±SD	7.14±4.2	9.03±6.14	7.61±3.38		
4 weeks after treatment:(b)					
Range	1.47-7.05	1.32-20.82	1.76-13.52	1.949	0.15
Mean±SD	4.08±1.7	6.94±5.7	5.91±3.59		
12 weeks after treatment:(c)					
Range	1.94-6.47	2.03-19.99	0.88-13.82	2.741	0.07
Mean±SD	3.44±1.38	6.82±5.32	5.23±3.99		
F value	7.771	0.697	1.692		
P	0.001*	0.504	0.197		
Scheffe test (P)	a vs b, P=0.015*				
	a vs c, P=0.003*				
	b vs c, P=0.822				

The data are expressed as range, mean± SD. LABA: Long acting β2-agonist, ICS: Inhaled corticosteroid, LAMA: Long-acting muscarinic antagonist. *Significant (*p<0.05). Scheffe test: Group I: Baseline significantly different from both 4 and 12 weeks after treatment

Table 7: Correlation between the measured parameters among the three studied groups

Time of treatment	Significant correlations			
	FEV1% predicted		TNF-α	
	r	P	r	P
COPD patients under treatment with LABA+ICS (n=15): Baseline:				
IL-6	-0.394	NS	0.61	0.015*
4 weeks after treatment:				
Fibrinogen	-0.200	NS	0.87	0.0001*
12 weeks after treatment:				
IL6	-0.272	NS	0.56	0.029*
COPD patients under treatment with LAMA +ICS (n=15): At Baseline:				
IL-6	-0.450	NS	0.59	0.018*
4 weeks after treatment:				
TNF-α	-0.678	0.006*		

LABA: Long-acting β2-agonist, ICS: Inhaled corticosteroid, LAMA: Long-acting muscarinic antagonist. *Significant difference with ANOVA test ($p<0.05$), r= correlation coefficient.

During the current study, TNF-α was negatively associated with FEV1% predicted which seems in accordance with previously reported findings.[32]

Finally, the change that occurred in plasma concentrations of inflammatory markers during the course of this pilot study can not only suggest their implication in following the disease activity but also can add to treatment selection. The latter suggestion is based on the current concepts which postulated that an abnormal inflammatory response causes disease progression and drugs with anti-inflammatory activities can modify the underlying disease mechanisms driving disease progression.[33]

4. CONCLUSIONS

The three therapeutic regimens implicated for the treatment of non-asthmatic patients with moderate to severe COPD showed statistically similar safety and efficacy in improving FEV1% predicted and mMRC dyspnea scale, and in reducing the concentrations of inflammatory markers. Furthermore, the changes that occurred in plasma TNF-α, fibrinogen, and IL-6 concentrations during the treatment course may focus the attention on the possibility of implicating these biomarkers to follow the disease activity.

STUDY LIMITATIONS

The small sample size represents the major limitation of our study. In this context, further large-scale studies are still needed.

ACKNOWLEDGMENT

We are so thankful to all participants and the physicians in the chest department at Tanta University Hospital for their recommendations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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