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Original paper

The role of intestinal protozoa in chronic obstructive pulmonary disease exacerbation

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ABSTRACT. Chronic obstructive pulmonary disease (COPD) is a common respiratory disease with episodes of exacerbation. Variable factors including infectious pathogen can predispose for this exacerbation. The aim of this study is to evaluate the role of intestinal protozoa in COPD exacerbation. A total of 56 patients with COPD were included in this study. Patients were categorized into two groups based on the frequency of exacerbation during the last 6 months: those with ≤1 exacerbation (32 patients) and those with ≥2 exacerbations (24 patients). Stool specimens from each patient were collected two times (one week interval) examined for intestinal parasite. In univariate analysis, rural residence and parasitic infection were more common among patients with frequent exacerbation (45.83% and 33.33%, respectively) than patients with non-frequent exacerbation (18.75% and 9.38%, respectively) with significant differences. However, in multivariate analysis, only body mass index >25 kg/m^2 (OR=4.59, 95%CI=1.18–17.86, *P*=0.028) and parasitic infection (OR=5.51, 95%CI=1.01–30.18, *P*=0.049) were independently associated with COPD exacerbation. These data indicate a significant association between frequent COPD exacerbation and infection with some intestinal protozoa. However, the cause-effect relationship is debatable.

Keywords: chronic obstructive, pulmonary disease, parasitic disease, multivariate analysis

Introduction

Chronic obstructive pulmonary disease (COPD) is a common illness that is associated with persistent respiratory symptoms and airflow limitation due to abnormalities of the airway or alveoli or both [1]. COPD exacerbation is an important clinical event in the course of the disease that have profound impact on patients' health status, functional capacity, and lung function [2]. The severity of exacerbations varies, and the clinical definition of a severe exacerbation is not always uniform [3].

Patients who survive severe exacerbations are likely to experience significantly impaired quality of life, are at increased risk of further exacerbations [4], and represent a major contributor to the overall health-care costs associated with COPD [5]. Therefore, understanding the factors that lead to an exacerbation can greatly help in both prevention of exacerbation and management of those patients.

There are many factor associated with COPD exacerbation, among which microbial infection and pollution are the most common. Previous studies have indicated that 70% of exacerbations are caused by infections. These include bacteria (40–60%), viruses (about 30%) and atypical bacteria (5–10%) [6,7]. Some studies even isolated fungi from those patients [8,9]. However, in all these studies, the infection primary involves the respiratory system and, thus, the role of these microbes is justifiable. On the other hand, the potential impact of extra respiratory pathogen on COPD was rarely investigated. Therefore, the present study aimed to evaluate the role of intestinal protozoa in COPD exacerbation. Illustration of such a role can greatly help the clinician for better management of COPD patients with frequent exacerbations may be simply through using anti-protozoal medications.

Materials and Methods

Study populations

This is a cross sectional study which included a total of 56 patients with COPD who were attending

the outpatient clinic at Baghdad Teaching Hospital during the period from January to August, 2021. The diagnosis of COPD was based Global Initiative for Chronic Obstructive Lung Disease guidelines if the ratio of post-bronchodilator forced expiratory volume in 1s to forced vital capacity (FEV1/FVC) was <0.7 under the supervision of specialist. An exacerbation of COPD was defined if there was acute deterioration of the patient's respiratory symptoms beyond normal day-today variations, with additional steroids or antibiotics. Inclusion criteria included all adult patients with COPD from both sexes. Exclusion criteria were patients with autoimmune diseases, pregnant women, and patients with malignancy.

A written consent from each participant was obtained prior to data collection after explaining the aim of study. Each patient was given the complete unconditioned choice to withdraw anytime from the study. The confidentiality of data throughout the study was guaranteed and the patients were assured that data will be used for research purpose only.

Sample collection and examination

Stool specimens from each patient were collected two times (one week interval) examined for intestinal parasite. In each time, about one gram of the faeces was transported by a wooden stick into a clean and dry slide. A drop of Lugol's iodine solution (Home Training Tools, Ltd., USA) was added and mixed with the faeces and spread by to a suitable thickness. A cover slip was put on the smear and examined microscopically. The entire wet mounts were examined initially by using a low-power (10×) objective and then again by using a high-power (40×) objective of the compound microscope (Olympus/Japan). The remained part of faecal sample was concentrated using the procedure of zinc-sulphate flotation technique and iodine stained slides were prepared and examined microscopically [10]. One gram of the stool specimen was emulsified in 10 parts of tap water and it was strained through a wire gauze. The filtrate was collected in a Wassermann tube and centrifuged at 2,500 round per minute (rmp) centrifuge (Eppendorf/Germany). The supernatant was discarded and the sediment was re-suspended in water. This step was repeated till the supernatant became clear. Three ml of 33% zinc-sulphate solution was added to the sediment which was was mixed well and filled with $ZnSO_4$ solution, about half an inch of the rim. Several loopfuls of the supernatant fluid were removed with a loop into a sterile slide. The slide was covered with a cover slip and examined microscopically as in the direct smear method.

Data regarding age, gender, body mass index (BMI), smoking status, residence, the frequency of exacerbation during the last six months, the presence of comorbidity and disease duration were collected through direct interview with each patient.

Statistical analysis

All statistical analyses were performed using SPSS statistical software, version 25 (IBM Corporation, USA). Quantitative variables were presented as mean ± standard deviation (SD), while categorical variables were expressed as counts and percentages. The association of different demographic and clinical characteristics (including parasitic infection) with COPD exacerbation was evaluated in univariate and multivariate analyses. In univariate analysis, Student t-test Chi square/Fisher exact test were used to compare quantitative and categorical data, respectively. In multivariate analysis, logistic regression test was used to calculate the odds ratio (OR) and its corresponding 95% confidence interval (CI). For all tests, a significant level of statistics was considered when $P<0.05$.

Results

Demographic characteristics of the study population

Mean age of the patients was 57.39±9.45 years (range 42–82 years). Males represented 82.14% of the patients with male:female ratio of 4.6:1. The mean BMI of the patients was 26.27±4.25 kg/m^2. About two-third of the patients had at least one comorbidity with hypertension was the most common comorbidity encountered in half of the patients, while type 2 diabetes mellitus (T2DM) was reported in 24 patients (42.86%). The mean duration of the disease was 4.18–4.21 years. The vast majority of the patients (89.29%) were either ex-or current smokers. More than two-third of the patients (69.64%) were urban residents (Tab. 1).

Intestinal parasitic infections

Laboratory investigation revealed three types of intestinal protozoa. *Entamoeba histolytica/E. dispar*, *Giardia intestinalis* and *Blastocystis hominis*. The infection rate for these parasites were 6 (10.71%), 3 (5.36%) and 3 (5.36%), respectively.

Table 1. Demographic and clinical characteristics of the patients (n=56)

Variables	Value
Age, years	
Mean±SD	57.39±9.45
Range	42–82
Gender	
Male	46 (82.14%)
Female	10 (17.86%)
BMI, kg/m^2	
Mean±SD	26.27±4.25
Range	19.3–35
Comorbidity	
None	19 (33.93%)
Hypertension	28 (50%)
T2DM	24 (42.86%)
Duration of the disease, years	
Mean±SD	4.18±4.21
Range	1.5–14
Smoking status	
Never	6 (10.71%)
Ex/current	50 (89.29%)
Residence	
Urban	39 (69.64%)
Rural	17 (30.36%)

BMI: body mass index, T2DM: type 2 diabetes mellitus, a patient can have more than one comorbidity

Mixed infection with E. *histolytica/E. dispar* and *B. hominis* was reported in one patients. Thus, the total infection rate was 11 patients (19.64%). There was no indication for helminth infection i.e. neither larval, ova or adult parasite was recovered.

Frequency of COPD exacerbation

Based on the definition of COPD exacerbation, patients were categorized into two groups according to the frequency of exacerbation during the past six months: those with ≤1 exacerbation 32 patients (57.14%) and those with ≥2 exacerbations, 24 patients (42.86%).

Association of demographic and clinical factors with the frequency of exacerbation

Most included factors were comparable between patients with frequent exacerbation and those with non-frequent exacerbation. However, two factors were significantly associated with frequent exacerbation. Rural residents were more common among patients with frequent than non-frequent exacerbation (45.83% vs. 18.75%) with a significant difference. Likewise, 33.33% of patients with frequent exacerbation were positive for parasitic infection compared with only 9.38% of patients with non-frequent exacerbation who were positive for this infection with a significant difference (Tab. 2).

Multivariate analysis

In order to find if parasitic infection is independent risk factor for COPD exacerbation, multivariate analysis was used. For this analysis, all factors that showed a *P*-value lower than 0.250 in univariate analysis were entered the model. Furthermore, quantitative variable (BMI) was categorized into two categories. Surprisingly, BMI>25 kg/m^2 was significantly associated with frequent exacerbation (OR=4.59, 95%CI=1.18–17.86, *P*=0.028). Furthermore, parasitic infection still has significant association with COPD exacerbation (OR=5.51, 95%CI=1.01–30.18, *P*=0.049). On the other hand, residence lost it significant association (OR=2.68, 95%CI=0.67– 10.75, *P*=0.146) as shown in table 3.

Discussion

The prediction and prevention of COPD exacerbations are important goals in the management of COPD. According to the multivariate analysis, obesity was independent risk factor for frequent exacerbation in COPD patients (OR=4.59, 95%CI=1.18–17.86, *P*=0.028). This implies that obese patients have about 4.5-fold increase in the incidence of frequent exacerbation than normal weight patients. These results are in accordance with many previous studies.

A multicenter prospective cohort study conducted by Lambert et al. [11] found that obesity was associated with worse COPD-related outcomes, dyspnea during walk test, and severe exacerbations of COPD. In a large population-based study including 187,647 patients hospitalized for acute exacerbation of COPD, Goto et al. [12] found that

Table 2. Association of demographic and clinical factors with the frequency of exacerbation

Variables	≤1 exacerbation (n=32)	≥2 exacerbations (n=24)	*P*-value
Age, years	58.41±10.19	56.04±8.38	0.659
BMI, kg/m^2	25.68±4.57	26.97±3.74	0.243
Gender			
Male	26 (81.25%)	20 (83.33%)	0.840
Female	6 (18.75%)	4 (16.67%)	
Comorbidity			
None	9 (28.12%)	10 (41.67%)	0.238
Hypertension	16 (50%)	12 (50%)	1.00
DT2DM	16 (50%)	8 (33.33%)	0.212
Smoking status			
Never	4 (12.5%)	2 (8.33%)	0.691
Ex/current	28 (87.5%)	22 (91.67%)	
Residence			
Urban	26 (81.25%)	13 (54.17%)	0.029
Rural	6 (18.75%)	11 (45.83%)	
Parasitic infection			
Yes	29 (90.62%)	16 (66.67%)	0.041
No	3 (9.38%)	8 (33.33%)	

BMI: body mass index, T2DM: type 2 diabetes mellitus

obesity was significantly associated with a higher risk of prolonged hospital stay. The underlying mechanisms of obesity in COPD exacerbation are likely multifactorial. For example, the complexity of overlapping comorbidities among obese patients (e.g., congestive heart failure, gastroesophageal reflux) plays an important role in this link [11]. Furthermore, the degree of static lung hyper-inflation, indicated by functional residual capacity and residual volume, is decreased in overweight and obese patients with COPD compared with underweight and normal-weight patients [13]. Finally, obesity-associated restrictive ventilatory patterns and decreased residual volumes, altered gut microbiome that modulates host immune response [14], and systemic inflammation secondary to adipose-tissue-derived proinflammatory mediators [15].

The most interesting finding in the present study was the significant association between intestinal protozoal infection and COPD exacerbation (OR=5.51, 95%CI=1.01–30.18, *P*=0.049). That means COPD patients with intestinal protozoa experience 5.5-fold increase in frequent exacerbation than those without such infection. To the best of our knowledge, this is the first study which addressed this issue. Previous studies showed a correlation between blood eosinophil count and incidence of exacerbation of the disease [16,17]. However, eosinophilia results from several etiologies such as parasitic infections, allergy, hematologic malignancies, and autoimmune diseases. The most common cause of eosinophilia in developing countries is parasitic infection [18].

The significant association between intestinal protozoa and COPD exacerbation can be explained through two paradoxical point of view. Firstly, the potential cause of protozoa as trigger for exacerbation. This may be due to provoking eosinophilia which is a cardinal manifestation for may parasitic infections. In this regard, Tsutsumi et al. [19] reported that eosinophilia is regularly found in the early inflammatory stages of experimental

Table 3. Multivariate analysis

Variables	≤1 exacerbation (n=32)	≥2 exacerbations (n=24)	*P*-value	OR(95%CI)
BMI, kg/m^2				
≤25	18 (56.25%)	8 (33.33%)	0.028	1.0
>25 14 (43.75%)	16 (66.67%)		4.59 (1.18–17.86)	
Comorbidity				
No	9 (28.12%)	10 (41.67%)	0.520	1.0
Yes	23 (71.88%)	14 (58.33%)		1.71 (0.33–8.86)
Diabetes				
No	16 (50%)	16 (66.67%)	0.500	1.0
Yes	16 (50%)	8 (33.33%)		0.57 (0.11–2.9)
Residence				
Urban	26 (81.25%)	13 (54.17%)	0.164	1.0
Rural	6 (18.75%)	11 (45.83%)		2.68 (0.67–10.75)
Parasitic infection				
No	29 (90.62%)	16 (66.67%)	0.049	1.0
Yes	3 (9.38%)	8 (33.33%)		5.51(1.01–30.18)

BMI: body mass index

amebic liver disease. Dos Santos and Vituri [20] demonstrated an association of eosinophilia with giardiosis in a Brazilian study, Chan et al. have suggested the possible role of *Giardia lamblia* allergens in provoking eosinophilia which in turn a risk factor for COPD exacerbation [21]

Secondly, frequent exacerbation is a cause rather than the result of intestinal parasitic infection. It is well known that COPD patients with frequent exacerbation episode use steroids to muffled these episodes. Steroids are immune suppressors and enhance parasitic infection in general [22]. Furthermore, aside from the influence of corticosteroids in the course of parasitic infections, it has been demonstrated that these medications directly influence parasite's growth. For instance, cortisol and dexamethasone increase the in vitro multiplication of the hemoflagellate *Cryptobia salmonistica*, possibly by their interaction with glucocorticoid receptor-like protein [23,24].

In the present study, rural residence was significantly associated with frequent exacerbation univariate but not in multivariate analysis.

Burkes et al. [25] observed that rural residence was independently associated with increased OR and incidence of COPD exacerbation that requiring treatment. Moreover, rural residence independently conferred a 46% increase in the incidence of total COPD exacerbation. Such association was attributed to multiple factors, including a lack of adequate diagnoses and treatments, differential access to healthcare centers, and limited availability of pulmonary specialists in rural areas [26].

Many other studies showed other risk factors for COPD exacerbation. For example, previous research has shown that cardiovascular, psychiatric, and metabolic comorbidity are highly prevalent in exacerbated patients, [27,28]. However, out study excluded those patients from the study.

The present study has many limitations. Firstly, because of the small size of population, some factors might not be obviously different between groups. Secondly, this study was is a single research center in Iraq, and the result might not be applicable to other ethnicities or countries. Lastly, due to limitation of direct stool exam and concentration method for parasites detection, some parasites, such as *Cryptosporidium*, might not have been detected.

In conclusion, these data suggested a reciprocal

role between frequent COPD exacerbation and infection with some intestinal protozoa. Furthermore, obese patients are more prone for exacerbation than their counterpart normal weight patients. Therefore, patients with frequent exacerbation may be in need for stool examination for possible detection of parasitic infection, which can be properly treated whether it is a cause or result of COPD exacerbation.

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