

ASSESSMENT OF BRONCHOALVEOLAR LAVAGE PEPSIN LEVEL IN IDIOPATHIC PULMONARY FIBROSIS

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ABSTRACT

Background: Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause. Recently, there is increasing evidence supporting a relationship between gastroesophageal reflux, microaspiration, and IPF, since the diagnostic gold-standard for microaspiration remains unknown. So, **the aim of this study** is to assess the presence of chronic microaspirates by bronchoalveolar lavage pepsin level in these patients.

Patients and method: A case-control study was conducted at Chest Department and outpatient clinics Faculty of Medicine, Zagazig University hospitals in the period from (February 2013 till January 2016) on thirty patients with stable interstitial lung diseases (ILD). They were divided into two groups: 15 patients (**group I**) with stable idiopathic pulmonary fibrosis (IPF), another 15 patients (**group II**) stable ILD other than IPF. Both groups underwent pulmonary function tests (PFTs), Pulmonary HRCT and Bronchoscopy. Measurement of bronchoalveolar lavage pepsin level was done for all patients. **Results:** 80% of IPF patients complained from gastroesophageal reflux with a statistically significant decrease in PaO₂, FEV₁ and FVC than in non IPF patients. Bronchoalveolar lavage pepsin level was (43.7±11.4ng/ml) in IPF group, which was significantly higher than non IPF group (32.7±10.5 ng/ml). Also total cell count in IPF patients were increased significantly including neutrophils, alveolar macrophages and red blood. Increased reticulation on HRCT for IPF patients group were associated with higher BAL pepsin levels (**p <0.01**). Regression coefficient revealed the most powerful predictor of IPF severity were; HRCT findings: reticulation score, honeycombing score [(Odds ratio(95%CI)=5.3 (1.9 to 14.9), 7.8 (2.7 to 22.3) respectively], pepsin level [(Odds ratio(95%CI)=4.6 (1.9–11.4)], FEV₁ and FVC [(Odds ratio(95%CI)=3.7 (1.6 to 8.9), 3.2 (1.4 to 7.5) respectively], while gastroesophageal reflux symptom [(Odds ratio(95%CI)=1.04(0.94-1.14)] was the least powerful predictor.

Conclusion: Gastroesophageal reflux or microaspiration is common in patients with IPF, associated with elevated levels of pepsin in BAL fluid.

KEYWORDS: Idiopathic pulmonary fibrosis; bronchoalveolar lavage pepsin level; gastroesophageal reflux.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is the commonest cause of lung fibrosis [1]. There is a bad prognosis presented with a median survival rate 2–3 years in patients probably diagnosed as IPF [2]. Unfortunately, no documented treatment for IPF was detected as the main causes are not known till now, despite the fact that numerous precipitating factors including smoking, viral infection, and gastroesophageal reflux (GER) with microaspiration have been declared [3].

There are many exciting purposes for detecting the relationship between GER, microaspiration and IPF. First, there is wide presentation of GER in IPF patients [4]. Second, age, smoking and male gender playing a role as a shearing risk factors in both IPF and GER [5]. It was declared that GER and microaspiration play a crucial role in pathogenesis of IPF, either by alteration in disease progression, acute exacerbation, or life span of these patients [6].

In many researches, it was detected that heartburn, regurgitation and dysphagia as

common symptoms of GER aren't detected in many IPF patients. So, screening of abnormal GER in IPF patients by using the symptoms only is not appropriate method [7].

There is no accurate diagnostic testing for microaspiration and GER as declared by many researches. However by using of 24-hour pH monitoring and/or impedance monitoring in diagnosis of presence or absence GER, but cannot emphasize presence of microaspirates [8].

The diagnosis of many aspiration events was reported in many barium swallow researches, but with limitations like aspiration must occur at the time of study and chronic microaspirates are not accurately detected[9]. One of more sensitive researches than barium swallow is pulmonary scintigraphy. However, more limitations presented that make it unused widely, like exposure to radiation and more expensive test [10]. Lastly, the valuable diagnosis of microaspiration was done by detecting the levels of bile acids and pepsin in Bronchoalveolar lavage (BAL)[11].

It was declared that pepsin and bile salt, acts as biomarkers of microaspiration specific to gastrointestinal tract (GIT), have been measured in respiratory tract. Normally there is no pepsin in lower respiratory tract and not always GER patients have elevated pepsin level in their BAL fluid[12].

Interestingly, the highly specific and sensitive method for diagnosing GER -associated with respiratory tract aspiration is measurement of pepsin level in BAL. Since the gold-standard for diagnosis of microaspiration remains undetected, so the assessment of pepsin and bile salt presence to the gastrointestinal tract makes this diagnostic technique incredibly attractive [13].

In general, adjusting the response of fibrogenesis after the injury is used mainly for recent treatment in IPF, not on prevention of injury itself. It was considered that microaspiration is a cause of frequent damage in idiopathic pulmonary fibrosis and may be controlled by medical and/or surgical therapy [3].

So, the objective of this study is to assess the presence of chronic microaspirates by measuring the pepsin level among idiopathic

pulmonary fibrosis diagnosed patients with aiming at combining treatment of microaspiration with anti inflammatory and anti fibrotic agents, which may give a crucial combined approach for prevention of more injury to the lung from microaspiration and monitoring the deterioration of the disease in IPF patients.

PATIENTS AND METHOD

The current study was a case -control one conducted at Chest Department and outpatient clinics Faculty of Medicine, Zagazig University hospitals in the period from February 2013 till January 2016. Written informed consents were obtained from all patients.

Patients:

Sample size was 30 patients who selected randomly from stable interstitial lung diseases (ILD) attended to outpatient clinic and admitted at chest department for evaluation to be enrolled in this study.

Fifteen patients (**group I**) with stable idiopathic pulmonary fibrosis(IPF) were diagnosed according to high resolution computed tomography (HRCT) findings of usual interstitial pneumonia (UIP) and after exclusion of other known causes of ILD by history taking , clinical examination and blood tests for collagen vascular diseases and hepatitis C virus antibodies. This is according to the recently changed consensus criteria which made multiple explanation review for IPF as follows: it is a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults, limited to the lungs, and associated with the histopathologic and/or radiologic pattern of usual interstitial pneumonia (UIP) after exclusion of other forms of interstitial pneumonia including other idiopathic interstitial pneumonias and ILD associated with environmental exposure, medication, or systemic disease[14].

Another 15 patients (**group II**) with well-known stable ILD other than IPF were included in this study as a control group. They were 3 patients with sarcoidosis, 4 patients with systemic lupus erythematosus, 3 patients with mixed connective tissue disease, and 5 patients with hypersensitivity pneumonitis. The Histopathological results and specific

autoimmune markers were used for diagnosis of this group associated with the clinical and radiological picture of these patients.

Exclusion criteria:

IPF in acute exacerbation; defined by worsening of respiratory symptoms within 30 days, new bilateral ground glass opacities on HRCT, and no evidence of an alternative etiology [15].

Methods:

More than or equal to 30 days before starting the study, all patients had been requested to stop using acid suppressive therapy in form of proton pump inhibitors, H2 blockers and antacids.

All patients were submitted to:

1. Thorough medical history with stress on dyspnea scale [modified Medical Research Council (mMRC)] and symptoms of GER as regard to gastroesophageal reflux disease (GERD) questionnaire which consisted of six items, four positive predictors for GERD and two negative predictors [16].

The four positive predictors are:

- Heartburn.
- Regurgitation.
- Sleep disturbance because of these two reflux symptoms.
- The use of over-the-counter medication used in addition to that prescribed.

The two negative predictors are:

- Epigastric pain.
- Nausea.

2. Full clinical examination (general and local examination).

3. Chest X-ray (postero anterior and lateral views).

4. Arterial blood gases analysis (ABGs).

5. Coagulation profile (INR, PT, and PTT) was before doing bronchoscopy for all patients.

6. Blood tests for collagen vascular profile and HCV antibodies.

7. Spirometric ventilatory functions: was done by (wins piro PRO 5.0.0) and guided by the American Thoracic society (ATS) standards. If the lung volumes were < 80% of the predicted, the results were considered abnormal [17].

8. Pulmonary high resolution computed tomography (HRCT): it was done according to standard protocols to become aware of the abnormal radiological findings in IPF [14]. The images were evaluated blindly as regard to the clinical forms of all patients by a radiologist. The calculation semi quantitatively for pulmonary fibrosis severity in HRCT were done by measuring the percent of affected lung by fibrosis (i.e. reticular abnormality and/or honeycombing) to the nearest 5% in three zones for each lung according to **Best et al.** [18]. The net radiological fibrosis score were done detecting the average numbers using a 4-point scale (0= no involvement, 1 =1–25% involvement, 2=26–50% involvement, 3= 51–75% involvement and 4 = 76–100% involvement) [19].

9. Bronchoscopy:

Premedication were taken to all studied patients (intravenous midazolam, and 4% lignocaine was applied topically to the nose, pharynx, and larynx). Besides, 4% lignocaine was applied below the vocal cords in 1-ml aliquots with maximum dosage 7 mg/kg body weight. Bronchoscopy was done with patients in a semi recumbent position through the nasal route. Oxygen supply was given and oxygen saturation monitored by oximetry [13]. Instillations of at least 100 ml of sterile saline were done in a sub-segment of the right middle lobe or lingual for performing BAL. After that keeping BAL fluid on ice were done and processed within one hour of collection then frozen at -80°C. Measurements of total and differential cell counts in BAL fluid were done. Also, detection of pepsin in BAL fluid was done by using ELISA (USCNLife Science, Wuhan, China) [20].

Statistical analysis

Statistical analysis was performed with Epi Info_ version 7 and the SPSS version 19 statistical software package (SPSS Inc., Chicago, IL, USA). T test, the Chi-squared or Fisher's exact test and Binary logistic regression were used for comparing the differences in proportions. The correlation was calculated using Spearman's correlation coefficient by rank. P-value < 0.05 was considered significant and P-value < 0.01 considered highly significant.

RESULTS

Table (1) showed that this study included 30 patients with stable interstitial lung diseases (ILD) with mean age (62.6 ± 3.3 years), 19 males and 11 females. They were divided into; 15 patients (**group I**) with stable idiopathic pulmonary fibrosis (IPF) with mean age (66.41 ± 9 years), and Another 15 patients (**group II**) with well known stable ILD other than IPF with mean age (57.70 ± 6 years). There was very high statistical significant difference between both groups as regard age ($p < 0.001$).

The two groups were matched for sex and smoking habit ($P > 0.05$), while a significant difference was detected between both groups as regard presence of GER symptoms ($p < 0.05$). There was a statistically significant decrease in PaO_2 , $\text{FEV}_1\%$ and FVC in (**group I**) than in (**group II**) ($p < 0.05$).

Table (2) detected that there was a statistical significant increase in pepsin concentration, total cell count, neutrophils and red blood

cells in bronchoalveolar lavage fluid among patients with stable IPF (**group I**), associated with a higher significant increase in alveolar macrophages at BAL fluid of (**group I**) patients comparing to patients in (**group II**) ($P < 0.05$ and $p \leq 0.001$, respectively).

Table (3) illustrated that increased reticulation on HRCT for IPF patients group were associated with higher BAL pepsin levels ($p < 0.01$). Increased percentage of BAL lymphocytes was correlated with increased BAL pepsin level ($p < 0.05$), while there was a high negative correlation between $\text{FEV}_1\%$ and BAL pepsin levels ($p < 0.01$). Also, there were a negative correlation between BAL pepsin levels, FVC% and PaO_2 in IPF patients (group I) ($p < 0.05$).

Table (4) showed the predictors of IPF severity. HRCT findings and BAL pepsin levels were the most powerful predictor while presence of GER symptoms was the least powerful one. Parameters were ordered according to the regression coefficient.

Table (1): Demographic and clinical characteristics and laboratory investigations of the studied groups.

Parameter	Group I No = 15	Group II No = 15	P-value	test
				T
Age (years)	66.41 ± 9	57.70 ± 6	0.00	3.12
				χ^2
Sex: Male/Female	11/4	8/7	0.26	1.29
Smokers No. (%)	2 (13.3 %)	1 (6.7%)	0.37	0.54
Ex-smokers	7 (46.7%)	5 (33.3%)	0.56	0.46
Comorbidities				
Coronary vascular disease	4 (26.66%)	6 (40.00%)	0.44	0.06
Hypertension	5 (33.33%)	7 (46.66%)	0.46	0.56
Diabetes Mellitus	9 (60.00%)	8 (53.33%)	0.71	0.14
GER symptoms No. (%)	12 (80.0%)	4 (26.7%)	0.00	8.57
				T
ABGs				
pH	7.38 ± 0.02	7.38 ± 0.04	1.73	0.09
PaCO_2 (mmHg)	32.7 ± 6.8	33.6 ± 5.3	0.45	0.66
PaO_2 (mmHg)	57.1 ± 4.2	61.5 ± 3.4	3.89	0.04
PFTs				
FEV_1 (% pred.)	43.7 ± 11.3	51.2 ± 9.6	2.22	0.03
FVC (% pred.)	44.3 ± 10.5	53.4 ± 10.1	2.15	0.04
Dyspnea scale (mMRC)	2.8 ± 0.6	2.6 ± 0.7	1.26	0.22

No = number, χ^2 = Chi-squared test

Table (2): Criteria of BAL fluid in the studied patients.

Parameter	Group I No = 15	Group II No=15	t test	P-value
Pepsin concentration (ng/ml) mean±SD (range)	43.7±11.4 (20-87)	32.7±10.5 (15-53.2)	2.86	0.04
Total cell count cells/mL	198.4±43.9	180.3±53.7	2.53	0.02
-Neutrophils(mean±SD)	28.3±4.7	13.1±2.7	2.64	0.01
-Alveolar macrophages (mean±SD)	137.1±33.4 29.1±7.8	155.3±41.7 24.5±8.4	3.74 1.75	0.00 0.09
-Lymphocytes(mean±SD)	4.1±1.1	5.3±1.9	0.59	0.56
-Esinophils(mean±SD)	95.7±29.2	68.7±22.3	2.85	0.01
-Red blood cells (mean±SD)				

Table (3): Correlation analysis between bronchoalveolar lavage pepsin level and different studied parameters in stable IPF (group I) using spearman's rank correlation test.

Group Parameter	Stable IPF (Group I) No.= 15	
	r	p-value
HRCT:		
Reticulation score %	0.57	<0.01
Honeycombing score%	0.36	>0.05
FEV1%	-0.72	<0.01
FVC%	-0.59	<0.05
PaO ₂	-0.68	<0.05
BAL lymphocytes	0.56	<0.05
BAL neutrophils	0.07	>0.05
BAL red blood cells	0.13	>0.05
BAL alveolar macrophages	0.35	>0.05
BAL esinophils	0.13	>0.05

r= correlation

Table (4): Regression analysis of significant predictors of IPF severity

Variables value	Odds ratio(95%CI)	P-value
BAL pepsin level	4.6 (1.9–11.4)	<0.001
HRCT:		
Reticulation score %	5.3 (1.9 –14.9)	<0.001
Honeycombing score%	7.8 (2.7 –22.3)	<0.001
FEV ₁ %	3.7 (1.6 –8.9)	0.00
FVC%	3.2 (1.4 –7.5)	0.00
PaO ₂	2.8 (1.2 –6.5)	0.02
GER symptoms	1.04(0.94–1.14)	0.04

CI: confidence interval

DISCUSSION

Idiopathic pulmonary fibrosis (IPF) is a progressive disease characterized by chronic and irreversible interstitial pneumonia of unknown origin, which leads to lung fibrosis, respiratory failure, and finally death [21].

The role of hidden aspiration and gastroesophageal reflux in IPF patients became one of the crucial growing researches [3]. It was declared that hidden microaspiration was responsible for the progression and severity of IPF [22]. Besides, GERD was much more common among IPF patients than in patients suffering from cystic fibrosis, asthma, or chronic obstructive pulmonary disease [23].

So, the objective of this study is to assess the presence of chronic microaspirates by measuring the pepsin level among idiopathic pulmonary fibrosis diagnosed patients in BAL fluid.

As shown in **Table (1)**, IPF patients were older in age than non IPF patients with very high statistical significant difference. Similarly **Raghu** and **Meyer** [24] concluded from their results of study that older patients were detected in IPF. Many studies declared that changes in gene expression or genetic polymorphisms which occur in older age were the commonest cause for injury and fibrosis of aged lung [25].

Poh et al. [26] concluded that elder patients are always associated with decrease gastroesophageal motion with decreasing in the pressure of the upper sphincter of the esophagus, and increasing exposure to acids in esophagus. Also, **Patti et al.** [27] detected that 60% of individuals presented with hiatal hernias older than 60 yrs in which oesophageal reflux and erosive oesophagitis were increased.

In the current study smoking habit was more common in IPF patients (**group I**) than in non IPF patients (**group II**) but without statistical significance. In concordance with **Baumgartner et al.** [28], they detected that one of most important risk factor for development of IPF was smoking with the odds ratio (OR) ranging from 1.6 to 2.9 from different parts in the world.

In our study GER symptoms were presented among 80% of IPF patients with statistical

significant difference between two groups as shown in **table (1)**. The same results were revealed by **Sameh et al.** [29], their results of study by comparing IPF patients with non IPF patients detected that in IPF patients group there was an increasing in the frequency of GER episodes. Also, in patients with IPF as regard the degree of pulmonary fibrosis and the severity of GER there was a significant correlation between them. However in non IPF patients, they didn't find any significant correlation between the severity of GER and the degree of lung fibrosis, which indicated that more damage and excessive fibrosis occurred in IPF diagnosed patients having more severe GER.

However, **Sweet et al.** [7] illustrated that 25–65% was the range of the GER symptoms in their patients. They detected that the specificity of the presence of actual GER pathology was 71% for their patients. On the other hand **Pilotto et al.** [30] documented in their study that heart burn, epigastric discomfort and regurgitation were presented in GER and they detected the severity and frequency of symptoms, tend to become less in elderly which lead to the concept of "silent/occult GER and microaspiration".

However **Locke** [31] and **Raghu et al.** [32] observed from their studies, an apparent discordance between the prevalence of both GER and IPF diseases. They revealed this observation firstly, into variable degrees and duration of GER disease. Secondly, they clarified the presence of some other co-factor that pre-disposes to IPF pathogenesis such as (e.g. smoking, short telomere length, genetic predisposition). So, they confirmed that repeated aspiration in genetically susceptible patients could develop IPF.

Recently, the American Thoracic Society (ATS) guidelines had documented comprehensive, evidence-based perspective on the clinical importance of BAL cellular analysis in assisting and predicting nature of the ILD [33].

In the current study BAL fluid analysis as shown in **table (2)** revealed significant increase in pepsin concentration, total cell count, neutrophils and red blood cells in patients with stable IPF (**group I**), associated with a higher significant increase in alveolar

macrophages. In concordance with **Lee et al.[20]** who found that there were pepsin in BAL of IPF patients. Moreover they concluded that BAL pepsin and neutrophil percentages were significantly increased in acute exacerbations of IPF. On the same way, **Davis et al. [34]** concluded from their study that BAL fluid pepsin levels were higher in IPF patients than in patients with COPD, α 1-antitrypsin deficiency, or cystic fibrosis. Their study was based on measuring pepsin level in BAL fluid in 100 patients underwent lung transplantation.

As shown in **table (3)**, a significant correlation was found between degree of lung fibrosis detected by HRCT and BAL lymphocytes with BAL pepsin level. This is in agreement with **Lee et al. [20]** which revealed that increasing both fibrosis on HRCT and lymphocytic counts in BAL fluid were associated with increased BAL pepsin level ($p<0.01$). While in our study there was a high negative correlation between FEV1% and BAL pepsin levels in stable IPF group ($p<0.01$). These results are in contrary with the results of **Lee et al. [20]**, as they compared between exacerbated IPF and stable IPF groups and within the stable IPF, there was no correlation between BAL pepsin levels and percentage of predicted forced vital capacity (FVC) ($p=0.39$). The explanation for this different results is due to our stable IPF patients (**group I**) were more severe as regard pulmonary ventilatory function results than stable IPF patients in the previous study.

It was detected that HRCT findings in the form of reticulation score and honeycombing score, bronchoalveolar lavage pepsin level, FEV₁% and FVC were the most powerful predictor of IPF severity. While a significant correlation was found as regard the severity of both pulmonary fibrosis and GER in patients diagnosed IPF, as GER symptom has [(Odds ratio(95%CI)=1.04(0.94-1.14)] which was the least powerful predictor as shown in **table (4)**. These results are consistent with the results obtained by **Savarino et al.[35]**. In the same way **Sameh et al.[29]** enrolled that numbers of reflux episodes in proximal and distal part of esophagus were significantly correlate with the degree of lung fibrosis by HRCT scores in IPF diagnosed patients which

insure the crucial role of GER in pulmonary fibrosis development and progression.

Recent researches illustrated that lung parenchymal pathology mostly increased due to continual microaspiration of gastric contents which play an important triggering mechanism. These results postulated that treatment of GER could improve clinical and functional parameters of IPF patients partially **[36]**. Besides, **Lee et al.[37]** who declared that using treatment for GER decreased the lung fibrosis in HRCT and also considered as an independent predictor of longer life span in IPF patients.

CONCLUSION

Gastroesophageal reflux or microaspiration is common in patients with IPF, associated with elevated levels of pepsin in BAL fluid. Besides, there is a high positive correlation between increased reticulation on HRCT and increased BAL pepsin level in IPF patients. Also, BAL pepsin level was the most powerful predictor in assessment of IPF severity.

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