

GERD related micro-aspiration in chronic mustard-induced pulmonary disorder

Rasoul Aliannejad¹, Seyed-Mehdi Hashemi-Bajgani², Asharaf Karbasi³, Mahvash Jafari⁴, Jafar Aslani⁴, Maryam Salehi⁴, Mostafa Ghanei⁴

¹Respiratory Diseases and TB Research Center of Guilan University of Medical Science, Razi Hospital, Rasht, ²Department of Pulmonology, Kerman University of Medical Sciences, Kerman, ³Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical sciences, Tehran, ⁴Research Center of Chemical Injuries, Baqiyatallah University of Medical Sciences, Tehran, Iran

Background and Aim: Bronchiolitis obliterans (BO) is the main pulmonary involvement resulting from sulfur mustard (SM) gas exposure that was used against Iranian civilians and military forces during the Iran-Iraq war. The present study aimed to investigate the prevalence of gastro-esophageal reflux (GER) and gastric micro-aspiration in SM gas injured patients with chronic pulmonary diseases and recurrent episodes of exacerbations. **Materials and Methods:** This cross-sectional study was done at Baqiyatallah University of Medical Sciences, Tehran, Iran. Gastric micro-aspiration and GER were assessed in the enrolled patients by assessing bile acids, pepsin and trypsin in their bronchoalveolar lavage fluid. **Results:** Our result showed that bile acids were found to be high in 21.4% patients, and low in 53.6% of patients. Only in 16% patients, no bile was detected in the BALF. Trypsin and pepsin were detected in BAL fluid of all patients. **Conclusion:** Most of BO patients after exposure to SM suffer GER, while none the etiologic factors of GER in post lung transplant BO are present. It would be hypothesized that GER per se could be considered as an aggregative factor for exacerbations in patients. Further studies will provide more advances to better understanding of pathophysiological mechanism regarding GER and BO and treatment.

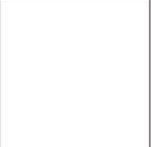
Key words: Bronchiolitis obliterans, gastro-esophageal reflux, sulfur mustard

INTRODUCTION

The association between chronic pulmonary diseases and gastro-esophageal reflux disease (GERD) has been recognized for a long time.^[1,2] In fact GERD is shown to be highly prevalent in patients with a variety of lung diseases; in particular, patients with chronic cough, asthma, Chronic obstructive pulmonary disease, cystic fibrosis, and idiopathic pulmonary fibrosis.^[3-5] Furthermore, GER disease is suggested to be a risk factor for acute exacerbation of COPD.^[6] In fact it is shown that COPD patients with GERD symptoms have more COPD exacerbations and subsequent hospitalization and drug usage.^[6-8] The pathophysiology of the associations between GERD and various pulmonary diseases including COPD is not clear. The proposed mechanisms are microaspiration of the gastric contents leading to airway irritation and increased resistance, vagally mediated bronchoconstriction from

esophago-bronchial reflex, and increased bronchial reactivity to other stimuli induced by esophageal acid exposure. Recent study indicated that abnormal swallowing reflexes would frequently occur in COPD patients and predisposes them to exacerbation and this abnormality might be affected by comorbidity of GERD.^[9] Disrupted breathing–swallowing coordination and impaired swallowing response could increase the risk of aspiration, and may contribute to exacerbations of COPD. Cvejic et al.^[10] demonstrated the aspiration of liquid material using sub-mandible video fluoroscopy during swallowing in COPD patient.

Sulfur mustard (SM) was used against Iranian civilians and military forces during the Iran-Iraq war; it leads to the development of acute and long term pulmonary complication. Previous studies have shown that bronchiolitis obliterans (BO) is the most frequent and the main pulmonary involvement resulting from SM gas exposure.^[11] Anecdotal evidence indicates that the prevalence of GERD in mustard-gas exposed patients is higher than that in the normal population.^[12] In this study we have aimed to investigate the prevalence of gastric micro-aspiration in SM gas injured patients with chronic pulmonary diseases as the cause of recurrent episodes of exacerbations, while they are under the treatment of GERD by proton pump inhibitor, by

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Address for correspondence: Dr. Mostafa Ghanei, Research Center of Chemical Injuries, Baqiyatallah University of Medical Sciences, Tehran, P.O. Box: 19945-546, Iran. E-mail: mghaneister@gmail.com

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assaying bile acids, pepsin, and trypsin in bronchoalveolar lavage fluid (BALF).

MATERIALS AND METHODS

Subjects

This cross-sectional study was done at Baqiyatallah University of Medical Sciences, Tehran, Iran between January 2009 and December 2010. The studied populations were the sulfur mustard gas injured patients with chronic pulmonary disease who experienced pulmonary exacerbation more than twice per year or had persistent pulmonary symptoms in spite of receiving full medical treatment. The exclusion criteria were as follows: patients with accompanied chronic lung disease rather than SM intoxication, patients with the history of smoking, patients with liver disorders and raised level of serum bilirubin, patients who were high risk to undergo bronchoscopy. All patients were interviewed to take a complete medical history regarding gastro-esophageal reflux symptoms. Pulmonary function test (PFT) was performed without bronchodilator administration and patients were classified according to Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease (updated 2009). Chest high resolution computerized tomography (HRCT) was obtained from all patients and their findings were registered.

Bronchoscopy

All included patients underwent bronchoscopy. Subjects were pre-medicated with intravenous midazolam. 4% lignocaine was applied topically to the nose, pharynx, and larynx and below the vocal cords in 1-ml aliquots, as required, up to a maximum dose of 7 mg/kg body weight. Bronchoscopy was carried out with patients in a semi-reclined position. Bronchoalveolar lavage was done with oxygen saturation routinely measured during the procedure. The bronchoalveolar fluids were collected and split to assess the clinical microbiology and differential cell counts on Giemsa-stained cyto-centrifuge preparation. Aliquots of the BAL fluid were also collected and snap frozen by immersion in liquid N₂, stored at -80°C. The samples were clarified by centrifugation at 1,500 rpm for 10 minutes. The resulting supernatant was assayed for bile acids. The samples were also tested for IL-8 and IL-15.

The study was approved by the Research Ethics Committee of Baqiyatallah Medical Science University and informed consent was obtained from every patient after presenting enough information about the study.

Bile acids assay

The bile acid determination in BALF was performed with a spectrophotometric enzymatic assay. The assay was

conducted according to a commercially available kit (Sigma Diagnostics, Inc, St Louis, Mo). BALF bile acids levels were considered high when they were greater than 8 µmol/L, lower if they were between 0 to 8, and absent when they were equal to 0.

Statistical analysis

The statistical analysis was carried out using the statistical package for the Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA). Deviations from Gaussian distribution were tested using the Kolmogorov-Smirnov test. T student test, kruskal-wallis test and Mann-whitney test were used as needed. A P-value under 0.05 was considered to be statistically significant.

RESULTS

Samples from 25 patients (mean age, 43.19; age range, 30-59) were assayed and included in the analysis. Of the 25 patients, 92% were male and only 2 patients (8%) were female. In 92% patients, pulmonary function was successfully tested using spirometry. Sixteen percent of patients were smoker and none of them were alcohol drinker. The findings are revealed in Table 1. According to GOLD guideline, updated 2009, patients were divided into 4 groups: 3 patients stage I, 7 patients stage II, 9 patients stage III and 4 patients stage IV. In all patients chest HRCT was performed, air trapping was the most frequent finding of HRCT in the patients; 52% had 6-12% air trapping, 40% had 1-5% and 8% had 13-24% air trapping in their HRCT. Other HRCT findings of the patients are summarized in Table 2. The mean bile acid concentration detected in BALF of patients was 5.3 µmol/L, range 0-14 µmol/L. The average levels of bile acids quantified within the BALF were found to be high (more than 8 µmol/L) in 21.4% patients, low (greater than 0 and less than 8) in 53.6% of patients. In 16% of patients, no bile was detected in the BALF. Trypsin (4.55 ± 0.24 ng/ml) and pepsin (21.75 ± 7.62 ng/ml) were detected in BAL fluid of all patients.

The overall mean level of IL-8 within the BALF of patients was 298 pg/ml with a range of 193- 797 pg/ml. There was not any significant statistical difference considering the quantity of IL-8 measured in patients with high levels of bile acids within the BALF (mean, 346 pg/ml; range, 240-797), compared with patients with low or absent bile acids (mean,

Table 1: Results of the pulmonary function test (PFT) in 23 patients

PFT	Result (mean ± SD)
FEV1	53.10 ± 19.26
FVC	60.76 ± 21.77
FEV1/FVC	67.11 ± 14.47
MMEF	37.42 ± 25.90

FEV1 = Forced expiratory volume in one second, FVC = Forced vital capacity, MMEF = Mean maximum expiratory flow

283 pg/ml; range, 193-557 pg/ml), although the mean level of IL-8 was higher in patients with the high levels of bile acids ($P=0.642$). No statistically significant difference was detected comparing the levels of bile acids among four different groups of GOLD classification ($P=0.946$) but the mean level of bile acid in stage III was the highest. In both groups, smoker and nonsmoker, there was not any significant relationship between bile acid, pepsin, trypsin, and IL8 of BALF and smoking.

DISCUSSION

In the present study, we found that a considerable number of patients with chronic pulmonary disease and frequent exacerbations had gastro-duodenal micro-aspiration showed in their BAL fluid. As we found, in 21.4% of patients the level of bile acid in BALF was more than 8 $\mu\text{mol/L}$ and also in 53.6% of patients, bile acid was present in BAL fluid with a level of less than 8 $\mu\text{mol/L}$. Only in 16% of patients, bile acid was not present in BAL fluid. Also, trypsin and pepsin were detected in BAL fluid of all patients. Air trapping was the most frequent finding of HRCT in the studied patients. It has been noted that air trapping is the most sensitive and accurate radiological indicator of BO.^[13] Considering the HRCT findings of the studied patients, BO is the main underlying pathology in these patients. This study is the first one in evaluation of the frequency of gastro-duodenal micro-aspiration in patients with chronic pulmonary disease and frequent exacerbations due to sulfur mustard gas inhalation. Unfortunately we did not any control group to compare our result.

Aguilar et al. in a study on COPD patients concluded that patients who have COPD and reflux symptoms simultaneously are more likely to have an increased number of COPD exacerbations compared to patient who are either asymptomatic or have reflux symptoms less than once a week.^[8] In another study by Rogha et al. they have found that patients who had daily or weekly symptoms of GERD not only were more likely to experience acute exacerbations of COPD but they showed more severe COPD, increased number of hospitalizations, and increased drug usage.^[7] Although the exact mechanism of COPD exacerbations by GERD is not clear but damage to the pulmonary tree following direct exposure to acid refluxate (reflux theory), or through bronchial constriction as a result of stimulation of vagal nerve endings in the esophagus (reflex theory) is suggested.^[14] In the present study, although all patients were under treatment of GER disease using proton pump inhibitors, they had experienced pulmonary exacerbation more than twice per year or had persistent pulmonary symptoms in spite of receiving full medical treatment. Previous studies showed that aspiration secondary to GER has been suggested to be a potential contributor to lung

Table 2: Chest HRCT findings of 25 patients were the sulfur mustard gas injured patients with chronic pulmonary disease

HRCT findings	Number (%)
Centri-lobular nodules	3/25 (12%)
Thracheomalacia	2/25 (8%)
Bronchomalacia	2/25 (8%)
Esophageal wall thickening	3/25 (12%)
Bronchiectasis	4/25 (16%)
Emphysema	1/25 (4%)

allograft dysfunction and the development of BO. In the D'Ovidio et al. study,^[15] elevated BALF bile acids were measured in 17% of 120 patients. They also demonstrated an association between the presence of bile acids in BALF and the onset of BOS. Blondeau et al.^[16] in a cross-sectional study showed that gastric aspiration occurs frequently in lung transplantation patients, as shown by the presence of pepsin in BALF of all patients and bile acids in BALF of 50% of the patients. The studied population of the mentioned articles was lung transplant patients. In lung allograft patients, significant damage to vagal innervation of the gastrointestinal tract would occur. Consequently, defense mechanisms, such as cough reflex and mucociliary activity, are frequently altered in these patients. Furthermore, mucociliary clearance is reduced in up to 15% of normal values in this population, a fact that can explain why even short exposure of the graft to gastric content can result in an intense inflammatory reaction and fibrosis.^[17] In addition, the use of immunosuppressant drugs (cyclosporin and tacrolimus) in lung transplant patients would reduce gastric motility and LES dysfunction.^[18] Mentioned mechanisms are not responsible for occurring GER in SM gas injured patients. A variety of respiratory symptoms are associated with GER because the impaired swallowing reflex perturbs the inspiratory expiratory transition during deglutition in COPD patients. As a result, GER-related symptoms and aspiration may be increased in these patients.^[19] Cvejic et al. in a study using sub-mandibular video fluoroscopy during swallow of graduated volumes of barium demonstrated convincing aspiration of liquid material in stable COPD.^[10] It is supposed to be due to altered airway protective mechanisms in COPD patients possibly through reduced coordination of breathing with swallowing. The prevalence of abnormal swallowing reflexes and its relationship with COPD exacerbation was assessed in a study by Terada et al. They concluded that abnormal swallowing reflexes frequently occurred in COPD patients and predisposed them to exacerbations.^[9] Gastroesophageal reflux and silent microaspiration has been suggested as a cause of chronic bronchiolar and interstitial lung disease. Recently, a strong association between gastroesophageal reflux and idiopathic pulmonary fibrosis has been reported but there are no direct data demonstrating that microaspiration leads to pulmonary fibrosis in humans. It has been suggested

that occult aspiration may be a cause of acute exacerbations of idiopathic pulmonary fibrosis, as aspiration of gastric contents is a known cause of diffuse alveolar damage.^[20] As mentioned in previous studies, the best available method to demonstrate that GER disease may have a role in pulmonary diseases is to treat the GER and observe if the pulmonary disease improves.^[4] In a review by Schnatz *et al.*, 42 of 54 patients with chronic cough or asthma suspected to be due to reflux had abnormal acid reflux documented by dual channel, ambulatory pH monitoring. In 71% of these documented reflux patients, pulmonary symptoms relieved when treated with anti-reflux therapy.^[21] Recently, in a randomized, prospective study, authors demonstrated that treatment of GER with a proton pump inhibitor (PPI) significantly improved the quality of life and the symptom scores of asthma patients with GER.^[22] PPI only changes the acidity of the refluxate; they do not prevent reflux or micro-aspiration of gastric contents. Studies have suggested an increased risk of community-acquired pneumonia in association with current use of PPI. PPIs also have been associated with an increased risk of hip fracture.^[20] At this time, a diagnostic gold standard for micro-aspiration remains unknown. However, based on the current data, the specificity of pepsin and bile salt in the gastrointestinal tract makes this diagnostic approach highly appealing.

In an *in vitro* study, a component of bile acid, chenodeoxycholic acid, has been shown to induce TGF-beta production from human airway epithelial cells via a p38 MAP-kinase dependent pathway. Fibroblast cell proliferation also was increased with exposure to chenodeoxycholic acid.^[23] In a study by Ghanei *et al.*, they indicated that levels of TGF-beta 1 and TGF-beta 3 mRNAs were significantly higher in chemical gas-injured patients than non-injured group. The authors concluded that TGF-beta1 and TGF-beta3, but not TGF-beta2, secretion is a result of efficient efferocytosis in chemically injured patients, playing a protective role by improving airway remodeling and lung homeostasis in this group. These properties of TGF-beta are consistent with long-time survival of chemical-injured people suffering from BO.^[24]

The exact mechanism of gastric micro-aspiration and GER in patients exposed to SM gas is not explained but high acute dose-exposure may damage the gastrointestinal tract severely.^[12] It was shown that the frequency of GER and esophagitis was higher in the patients with BO due to SM exposure than the control group.^[12] It should be considered that we are encountering to BO in SM gas injured patients where patients suffer from GER disease but none the etiologic factors of GER in lung transplant patients such as immunosuppressant drugs and damage to vagal innervation are present. All together, it would be hypothesized that GER per se could be considered as an aggregative factor for exacerbations in BO and one of important cause of resistance

to therapy in such patients. It is of note that all patients were receiving proton pump inhibitor but it seems has no inhibitory effect on GER and related outcomes. Whether BO and GER have developed simultaneously after exposure to SM or one of them occurred at first and led to another is remained unknown. In conclusion, our study has shown that a considerable number of patients with chronic pulmonary disease and frequent exacerbations due to exposure to SM, have gastro-duodenal micro-aspiration studying their BALF. Gastric aspiration and GER may be not only an etiologic factor of their exacerbations and weak response to pulmonary disease treatment but also it could be a result of underlying pathophysiological mechanism behind BO. Further studies are needed to reveal if treatment of gastrointestinal disease in these patients might be able to improve their pulmonary symptoms. In addition, more studies in this setting will provide more advances to provide better understanding of pathophysiological mechanism regarding GER and BO, e.g., neurogenic inflammation after SM exposure.

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