Gastroesophageal Reflux and Idiopathic Pulmonary Fibrosis: A Prospective Study

Ahmed Fahim[1], Peter W. Dettmar[2], Alyn H. Morice[1], Simon P. Hart[1]
[1]*Division of Cardiovascular and Respiratory Studies, Castle Hill Hospital, Cottingham, United Kingdom,*
[2]*Technostics Limited, The Deep Business Centre, Kingston upon Hull, United Kingdom*

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Summary. *Background and Objective. Idiopathic pulmonary fibrosis (IPF) is the most common of the idiopathic interstitial pneumonias. There is evidence of the increased prevalence of gastroesophageal reflux disease in patients with IPF. The aim of this prospective study was to evaluate reflux in patients with IPF by analyzing the scores of the reflux cough questionnaire, measurement of pepsin in exhaled breath condensate (EBC) to detect extraesophageal reflux, and Helicobacter pylori serology to evaluate the prevalence of this stomach bacterium in patients with IPF.*

Material and Methods. The Hull airway reflux questionnaire (HARQ) was completed by 40 patients with IPF and 50 controls in order to evaluate reflux symptoms. EBC was collected from 23 patients (17 patients with IPF and 6 controls) for measurement of pepsin by the lateral flow technique. A prospective study of 57 subjects (34 patients with IPF and 23 controls) for H. pylori antibody detection by ELISA was performed.

Results. Significantly higher HARQ scores (maximum score, 70) were recorded in patients with IPF compared with controls (19.6 [SD, 12.4] vs. 3 [SD, 2.9], P<0.001). There was no significant difference in EBC pepsin positivity between patients with IPF and controls (2 of the 17 patients vs. none of the 6 controls, P=0.38). There was no significant difference in H. pylori serology between patients with IPF and controls (17 of the 34 patients vs. 14 of the 23 controls, P=0.42).

Conclusion. Patients with IPF had significantly increased scores of airway reflux symptoms. However, no objective evidence of extraesophageal reflux or H. pylori infection in patients with IPF was obtained in this study. The role of gastroesophageal and extraesophageal reflux in pathogenesis of IPF should be evaluated in a larger prospective study.

Introduction

Idiopathic pulmonary fibrosis (IPF) is the commonest of the idiopathic interstitial pneumonias and carries a poor prognosis with a median survival of 2.5–3 years after diagnosis (1). The pathogenesis of this devastating disease is not known. The inciting factor for the development of IPF is believed to be alveolar epithelial injury (2). The epithelial injury may by immunological (either antibody- or cell-mediated), chemical (e.g., reflux of gastric contents), microbial, or particulate (organic or inorganic). As IPF is a heterogeneous disease manifesting as areas of peripheral and basal reticulation with honeycombing interspersed with normal lung (3), it is plausible that the alveolar epithelial injury is secondary to recurrent episodes of reflux of gastric contents and microaspiration. The aim of this study was a comprehensive evaluation of gastroesophageal reflux symptoms, extraesophageal reflux, and *H. pylori* prevalence in idiopathic pulmonary fibrosis.

There is evidence of high prevalence of up to 90% of classic gastroesophageal reflux disease (GERD) in IPF in a number of studies (4, 5). Moreover, patients with scleroderma-associated lung fibrosis have significantly increased reflux episodes as compared with scleroderma patients without pulmonary fibrosis (6). Gastroesophageal reflux is common in general population. However, the annual incidence of IPF is relatively low being 10 per 100 000. In order to study the possible susceptibility factors, we hypothesized that patients with gastroesophageal reflux and *H. pylori* infection may be at a significantly greater risk of developing pulmonary fibrosis. The likely pathways of *H. pylori*-induced pulmonary interstitial injury could be direct injury to alveolar epithelial lining in a similar way to injury to gastric mucosal barrier or immune-mediated injury after the aspiration of stomach contents. Aspiration would be more likely since the *H. pylori*-associated achlorhydria would decrease stimulation of cough reflex.

Correspondence to A. Fahim, Division of Cardiovascular and Respiratory Studies, Castle Hill Hospital, Castle Road, Cottingham, HU16 5JQ, United Kingdom
E-mail: ahmedfahim@doctors.org.uk

Adresas susirašinėti: A. Fahim, Division of Cardiovascular and Respiratory Studies, Castle Hill Hospital, Castle Road, Cottingham, HU16 5JQ, United Kingdom
El. paštas: ahmedfahim@doctors.org.uk

Exhaled breath condensate (EBC) measurement of pepsin is a reliable noninvasive technique for the detection of extraesophageal reflux in upper airways at the time of reflux episodes (Strugala et al., personal communication). Indeed, a relationship between pulmonary aspiration and increased pepsin concentration in bronchoalveolar lavage has been demonstrated in animal and human studies (7–15) and is associated with pulmonary inflammation (16). Hence, it is plausible to suspect that alveolar epithelial injury and fibrosis is a manifestation of toxic effects of gastric enzymes, such as pepsin, and bile salts.

Material and Methods

Patients. The study population comprised patients with IPF diagnosed according to the ATS/ERS criteria (3) attending the Interstitial Lung Disease Clinic at the University Hospital. The control group included patients with interstitial lung disease of known cause, patients without interstitial lung disease, and normal healthy subjects. The aim was to compare IPF population with potentially matched controls. As patients with rheumatoid-associated interstitial lung disease and chronic obstructive pulmonary disease are frequently encountered in clinical practice, these groups comprised the predominant controls. However, there were patients with other conditions such as sarcoidosis and respiratory infections included in the control population.

Ethical approval was obtained by the Hull and East Riding Research and Ethics Committee (LREC No. 08/H1304/54), and written consent was obtained from all participants.

Study Design. It was a prospective cross-sectional study of subjective evaluation of GERD symptoms and objective evaluation of *Helicobacter pylori* prevalence and extraesophageal reflux by measurement of pepsin concentration in EBC.

Hull Airway Reflux Questionnaire Study. It has been previously shown that a symptom complex associated with airway reflux can be identified (17). Based on these symptoms, a validated questionnaire has been developed, which is a sensitive and reproducible tool to assess this symptom complex (18). As part of investigation of gastroesophageal reflux in IPF, a cohort of 90 age-matched participants were asked to complete the Hull Airway Reflux Questionnaire (HARQ) to assess subjectively the symptoms of gastroesophageal reflux.

Helicobacter Pylori Study. According to the standard protocol, 6 mL of peripheral blood was obtained from 57 patients. The serum was stored at –80°C immediately after centrifugation (1500 RCF at 20°C for 10 minutes). The presence of *H. pylori* antibody (IgG) was detected by a rapid immunoassay (qualitative ELISA) in serum.

Exhaled Breath Condensate Study. A total of 23 EBC samples were obtained by patients breathing into an automated collection device made of polypropylene tubing connected to a Dreschel flask immersed in crushed ice. The collection of exhaled breath was performed according to the guidelines recommended by the ERS/ATS task force (19). The patients were asked to breath into the device (normal tidal breathing) for 10 minutes. Approximately 1 mL of EBC was obtained, and 30 μL was tested for the presence of pepsin after centrifugation. The samples were analyzed by a rapid lateral flow (LF) test (Peptest, RD Biomed, UK) using two unique monoclonal antibodies specific to human pepsin. The EBC was evaluated once for each patient.

Statistical Analysis. The statistical analysis was performed using SPSS (version 13, Chicago IL). Normally distributed data were analyzed with the unpaired *t* test, and skewed data were analyzed by the Mann-Whitney *U* test. Categorical data were analyzed by the Pearson chi-square test. An α value of 5% was considered statistically significant.

Results

The main demographics of study participants in HARQ, *H. pylori*, and EBC studies are shown in Tables 1, 2, and 3, respectively.

Hull Airway Reflux Questionnaire. The airway reflux questionnaire was completed by 90 participants (40 patients with IPF and 50 controls) for symptom evaluation. The questionnaire is a symptom-based assessment of gastroesophageal and extraesophageal reflux with 14 items, which are scored from 0 to 5. The upper limit of normal for the total score is 13 with a mean of 4. There was a significant difference in the symptom scores on the questionnaire between IPF and control groups (19.6 [SD, 12.4] and 3 [SD, 2.9], respectively; unpaired *t* test, $P<0.01$) (Fig.). In fact, 68% of patients with IPF had a score above the upper limit of normal.

Helicobacter Pylori Study. As shown in Table 4, there was no significant difference in the proportion of patients with IPF and controls in terms of positivity for *H. pylori* antibody (Pearson chi-square, $P=0.419$).

Exhaled Breath Condensate Study. The analysis of EBC pepsin was performed for 23 subjects. The study population included 17 patients with IPF and 6 controls. Although pepsin was detectable in EBC from two patients with IPF and none of the controls, there was no significant difference in exhaled breath pepsin between the IPF and control groups (2 of the 17 patients with IPF and none of the 6 controls; Pearson chi-square test, $P=0.379$).

Discussion

The present study of investigation of gastroesophageal and extraesophageal reflux in idiopathic

Table 1. Baseline Characteristics of Participants of Hull Airway Reflux Questionnaire Study

Characteristic	IPF Patients (n=40)	Controls (n=50)	*P* Value*
Age, years	70 (12)	68 (15)	0.210
Gender, M/F, n	31/9	31/19	0.221
HARQ score	19.6 (12.4)	3.0 (2.9)	<0.01
FVC, % predicted	88 (16)	NA	...
TL_{CO}, % predicted	56 (19)	NA	...
Medications, n (%)			
Prednisolone	11 (27)	6 (12)	0.055
N-acetylcysteine	3 (6)	0 (0)	0.084
Azathioprine	6 (15)	2 (4)	0.074
PPI	14 (35)	5 (10)	0.004
Diagnosis in control group, n			
Healthy	NA	40	...
Chronic obstructive pulmonary disease	NA	6	...
Rheumatoid lung	NA	4	...

Data are presented as mean (SD) unless stated otherwise. IPF, idiopathic pulmonary fibrosis; PPI, proton pump inhibitors; FVC, forced vital capacity; TL_{CO}, total gas transfer for carbon monoxide; NA, not applicable.
*Ellipses indicate *P* value not computed.

Table 2. Baseline characteristics of *Helicobacter pylori* study participants

Characteristic	IPF patients (n=34)	Controls (n=23)	*P* Value*
Age, years	71 (10)	67 (13)	0.032
Gender, M/F, n	29/5	9/14	0.145
FVC, % predicted	81 (21)	81 (23)	0.988
TL_{CO}, % predicted	44 (20)	64 (28)	0.212
Medications, n (%)			
Prednisolone	11 (32)	6 (26)	0.419
N-acetylcysteine	1 (29)	0	0.596
Azathioprine	5 (17)	3 (13)	0.590
PPI	15 (44)	6 (26)	0.047
Diagnosis in control group, n			
Chronic obstructive pulmonary disease	NA	8	...
Rheumatoid lung	NA	5	...
Sarcoidosis	NA	2	...
Respiratory infection	NA	4	...
Miscellaneous	NA	4	...

Data are presented as mean (SD) unless otherwise indicated. IPF, idiopathic pulmonary fibrosis; PPI, proton pump inhibitors; FVC, forced vital capacity; TL_{CO}, total gas transfer for carbon monoxide; NA, not applicable.
*Ellipses indicate *P* value not computed.

Table 3. Baseline Characteristics of Participants of Exhaled Breath Condensate Study

Characteristic	IPF Patients (n=17)	Controls (n=6)	*P* Value
Age, years	72 (7)	52 (15)	0.004
Gender, M/F, n	10/7	4/2	0.717
FVC, % predicted	86.2 (18.8)	97.6 (20)	0.932
TL_{CO}, % predicted	63.2 (19.5)	62 (17)	0.520
Medications, n (%)			
Prednisolone	3 (17)	1 (17)	0.730
N-acetylcysteine	1 (5)	0	0.739
Azathioprine	2 (12)	0	0.538
PPI	6 (35)	1 (17)	0.382
Pepsin positivity, n (%)	2 (12)	0 (0)	0.379

Data are presented as mean (SD) unless otherwise indicated. IPF, idiopathic pulmonary fibrosis; PPI, proton pump inhibitors; FVC, forced vital capacity; TL_{CO}, total gas transfer for carbon monoxide.

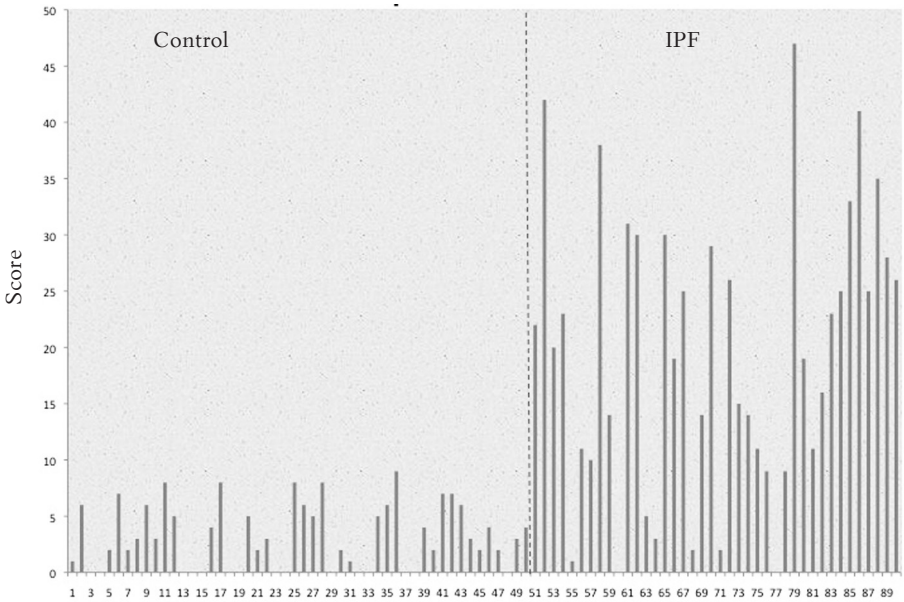

Fig. Hull Airway Reflux Questionnaire (HARQ) scores in patients with idiopathic pulmonary fibrosis (IPF) and control subjects
The bars show individual patients with height of each bar demonstrating the total score on the questionnaire for a particular participant. The data suggest a marked difference in airway reflux symptoms in the group of patients with idiopathic fibrosis with a mean score of 19.6 as compared with 3 in control population.

Table 4. Cross Tabulation of *H. Pylori* Status in Patients With Idiopathic Pulmonary Fibrosis (IPF) and Control Subjects

Group	*H. pylori* Status		Total
	Positive	Negative	
IPF	17	17	34
Control	14	9	23
Total	31	26	57

pulmonary fibrosis has shown significantly increased airway reflux symptoms in patients with IPF. However, there was no objective evidence of significant difference in *H. pylori* infection or extraesophageal reflux detected by exhaled breath pepsin analysis. The association of GERD and interstitial pulmonary fibrosis of obscure etiology had been documented long before the term IPF was even described (20). More recently, Tobin and colleagues (4) investigated 17 patients with biopsy-proven IPF and 8 control patients with other interstitial lung diseases in a prospective study. There was a significantly higher proportion of patients with IPF (16 out of 17) who had abnormal distal and/or proximal esophageal acid exposure as compared with controls (4 out of 8). Raghu et al. (5) evaluated 65 patients with IPF with 24-hour pH testing and compared them with 133 intractable asthmatics. There was a significantly increased prevalence of abnormal acid exposure in the IPF group (87% vs. 68%, P=0.014). Moreover, the odds of having IPF in patients with abnormal acid exposure were 3.19 (OR). In a prospective study of 28 consecutive patients with IPF, Bandeira et al. (21) evaluated the prevalence of GERD by esophageal manometry and pH studies. They divided the study population into two groups based on the presence of gastroesophageal reflux. In GERD-positive group, 77% had heartburn or regurgitation as compared with 33% in the GERD-negative group supporting the suggestion that esophageal symptoms alone are inadequate markers of gastroesophageal reflux. These studies suggest that gastroesophageal reflux is very common on objective measurement to assess GERD in patients with IPF. However, classic GERD symptoms are not as prevalent as gastroesophageal reflux on objective studies.

Our HARQ data show a marked difference in reflux scores between patients with IPF and control subjects. This questionnaire is designed to detect nonacid airway reflux (laryngopharyngeal reflux) that can be brief and occur at repeated intervals. This symptom assessment provides a comprehensive evaluation of both acid and nonacid reflux episodes.

The EBC pepsin measurement was taken in the outpatient clinic (single measurement for each patient), whereas the episodes of reflux over a period of 24 hours indicated by the HARQ may have been occurring infrequently and the EBC possibly missed them.

The prevalence of *H. pylori* infection varies with geographical location and the laboratory method to identify the infection. In an Australian study (22), a prevalence of 15% was reported, while a study of Italian villagers showed the prevalence to be as high as 58% (23). In our study, there were signifi-

cantly higher proportions of patients taking proton pump inhibitors (PPI) in the IPF group as compared with controls (44% vs. 26%). However, a subgroup analysis showed that being on PPI did not have a significant effect on *H. pylori* positivity. Hence, it is unlikely that patients' exclusion based on PPI usage would have had an effect on *Helicobacter pylori* status. Moreover, *H. pylori* infection can itself be associated with achlorhydria with a reduction in acid reflux and could potentially be an explanation for failure to find a difference in *H. pylori* status in patients with IPF and control subjects.

The measurement of IgG antibody for *H. pylori* infection has limitations as it may persist for years after eradication of *H. pylori*. Conversely, a positive IgG result does not necessarily confirm the persistence of *H. pylori* infection. The sensitivity of IgG is 87% with a low specificity of 61% (24), and combination of *H. pylori* stool antigen and IgG may offer the best noninvasive method for the detection of *H. pylori* infection.

This study of investigation of gastroesophageal and extraesophageal reflux in patients with IPF demonstrates that they have a very high incidence of airway reflux symptoms. However, *H. pylori* infection or single EBC pepsin analysis is not helpful in the diagnosis of gastroesophageal reflux. As the measurement of EBC pepsin is dependent on the timing of reflux symptoms, repeated sampling with a portable device immediately after the symptoms of reflux may increase the diagnostic yield. A larger prospective study to evaluate the contribution of reflux by both detailed subjective and objective measurements including airway pH analysis might be able to address the association in greater detail.

Conclusions

In view of significantly increased airway reflux symptoms, the role of gastroesophageal and extraesophageal reflux in the pathogenesis of idiopathic pulmonary fibrosis should be further explored. Investigation of nonacid reflux may provide us with further insight into the mechanism of alveolar injury by gastrointestinal secretions.

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Statement of Conflict of Interest

Prof. Morice and Prof. Dettmar are coapplicants for a patent of an exhaled breath condensate device. There are no conflicts of interest for Dr. Fahim and Dr. Hart.

Gastroezofaginis refliuksas ir idiopatinė plaučių fibrozė (prospektyvusis tyrimas)

Ahmed Fahim[1], Peter W. Dettmar[2], Alyn H. Morice[1], Simon P. Hart[1]

[1]Castle Hill ligoninės Širdies ir kraujagyslių ir kvėpavimo tyrimų skyrius, Kotingemas, Jungtinė Karalystė,
[2]Verslo centras, „Technostics Limited", Kingstonas prie Halo, Jungtinė Karalystė

Raktažodžiai: idiopatinė plaučių fibrozė, gastroezofaginis refliuksas, iškvėpto oro kondensatas, *Helicobacter pylori*.

Santrauka. *Tyrimo tikslas.* Idiopatinė plaučių fibrozė (IPF) – tai dažniausiai pasitaikanti idiopatinė intersticinė plaučių pneumonija. Įrodyta, kad sergantieji IPF dažnai serga ir gastroezofaginio refliukso liga. Šio prospektyviojo tyrimo tikslas – įvertinti pacientų, sergančių idiopatine plaučių fibroze, refliuksą analizuojant klausimyno apie refliukso kosulį rezultatus, pepsino kiekį iškvėpto oro kondensate siekiant nustatyti ekstrastemplinį refliuksą ir *Helicobacter pylori* serologinį tyrimą, įvertinti šios skrandžio bakterijos paplitimą esant idiopatinei plaučių fibrozei.

Medžiaga ir metodai. Halo klausimynas apie ryklės–gerklų refliuksą pateiktas 40 pacientų, sergančių idiopatine plaučių fibroze, ir 50 kontrolinės grupės pacientų siekiant įvertinti refliukso simptomus. Iškvėpto oro kondensatas surinktas iš 23 pacientų (17 iš jų sirgo IPF, 6 – kontrolinės grupės pacientai), kad šoninės tėkmės metodu būtų įvertintas pepsino kiekis. Taikant ELISA metodą, *H. pylori* antikūnių tyrimas atliktas 57 pacientams.

Rezultatai. Lyginant su kontroline grupe, sergantiesiems IPF nustatyti gana aukšti Halo klausimyno rezultatai, kai maksimalus taškų skaičius 70 (sergantieji IPF 19,6±12,4; kontrolinės grupės pacientai – 3±2,9; $p<0,001$). Tačiau nenustatyta didelių teigiamo pepsino kiekio skirtumų tarp sergančiųjų IPF ir kontrolinės grupės pacientų (sergantieji IPF 2/17; kontrolinės grupės pacientai 0/6; $p=0,38$). *H. pylori* serologinio tyrimo skirtumai taip pat nežymūs (sergantieji IPF 17/34; kontrolinės grupės pacientai 14/23; $p=0,42$).

Išvados. Sergantiesiems IPF nustatyti žymiai aukštesni ryklės–gerklų refliukso simptomų rodikliai. Tačiau objektyvių ekstrastemplinio refliukso arba *H. pylori* infekcijos įrodymų, atliekant šį tyrimą, nepavyko aptikti. Gastroezofaginio ir ekstrastemplinio refliukso įtaka IPF raidai turėtų būti įvertinta atliekant platesnį prospektyvųjį tyrimą.

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