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Original Article

Gastro-esophageal reflux induced cough with airway hyperresponsiveness

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Abstract: To investigate the characteristics of gastroesophageal reflux induced cough (GERC) with airway hyperresponsiveness (AHR). Compared to patients with GERC alone and healthy subjects, cough sensitivity, multi-channel intraluminal impedance combined with pH monitoring and airway inflammation were evaluated in patients with GERC and AHR. 23 patients were definitely diagnosed as acid reflux induced GERC, 9 patients developed AHR concomitantly. When compared with GERC patients, patients with AHR had significantly increased number of proximal extent episodes (21.5 (28.6) vs. 7.5 (1.8), Z = -2.038, P = 0.042) and increased proportion of proximal extent episodes to total refluxes episodes (24.5 (13.5)% vs. 4.2 (7.3)%, Z = -2.138, P = 0.032), and the level of IL-8 in the airway of these patients was significantly higher than that in healthy subjects (71.1 (64.0) vs. 24.3 (35.2) pg/ml, Z = -2.013, P = 0.044). Gastroesophageal reflux may cause neutrophilic airway inflammation due to the acid reflux into the airway, which results in AHR. However, AHR is not definitely able to cause chronic cough. Thus differential diagnosis is required in clinical practice.

Keywords: Gastroesophageal reflux, airway hyperresponsiveness, neutrophilic airway inflammation

Introduction

Gastroesophageal reflux induced cough (GERC) is a clinical syndrome manifested predominantly with chronic cough caused by the backflow of gastric acid or other gastric contents into the esophagus [1]. GERC has been regarded as one of common causes of chronic cough with an estimated prevalence of 10-40% patients with chronic cough in western countries and 6-20% in China [2-4]. With the alteration in dietary habit and improvement in test facility, the incidence of GERC is increasing [5].

Studies have shown that GERC patients usually have other co-morbid condition, such as upper airway cough syndrome (UACS), cough variant asthma (CVA) and non-asthmatic eosinophilic bronchitis (NAEB) [5-7]. However, our clinical experience reveals the concomitant state in GERC patients is not always related to cough. To elucidate this, we retrospectively reviewed 9 patients with GERC and airway hyperresponsiveness (AHR) aiming to investigate the characteristics of this disease and provide evidence for the differentiation diagnosis of GERC.

Materials and methods

Subjects

A total of 23 patients with GERC due to acid reflux were recruited from our respiratory clinic between July 2009 and December 2012. The criteria for diagnosis of acid reflux induced GERC were as follows: 1) cough lasting for ≥8 weeks, with or without the typical upper gastrointestinal symptoms such as regurgitation, heartburn and chest pain, 2) multi-channel intraluminal impedance combined with pH monitoring (MII-pH) confirmed abnormal acid reflux, as shown by DeMeester score of ≥14.72 and/or syndrome association probability (SAP) for acid reflux of ≥95% [3, 8]; 3) cough relieved obviously or resolved completely with the treatment of standard pharmacologic anti-reflux treatment consisting of omeprazole 20 mg, twice daily, plus domperidone 10 mg, three times a day [8]. This study was approved by the

Ethics Committee of Tongji Hospital and registered with Chinese Clinical Trials Register (http://www.chictr.org/) number ChiCTR-ONC-13003066. All the subjects gave informed consent before entering the study and were divided into three groups.

Group A: Patients with acid GERC and AHR were designated as group A. Inclusion criteria: MII-pH results showed abnormal acid reflux and histamine bronchial provocation tests revealed AHR (PD_{20} FEV1<7.8 umol/L). There was no history of exposure to environmental irritants or use of angiotensin-converting enzyme inhibitors, and abnormal findings suggesting other causes for cough were absent; these included results from plain CXRs, pulmonary function testing, induced sputum cytology, sinus imaging, HRCT of the chest, fibre-optic bronchoscopy. Exclusion criteria: Patients had a history of smoking or the duration of smoking cessation was shorter than 2 years; patients did not undergo laboratory examinations; patients developed upper respiratory tract infection within past 2 months; patients had other causes of chronic cough; patients were lost to follow up. Although these 9 patients had AHR, they did not response to anti asthma therapy (200 mg diprophylline plus 2.5 mg terbutaline three times a day or oral prednisone 25 mg daily for one week). GERC was confirmed when cough controlled or improved after at least an 8-week course of standard anti-reflux treatment.

Group B: One control group enrolled acid GERC patients without AHR. Patients with acid reflux induced GERC and normal airway responsiveness were recruited as group B. The inclusion and exclusion criteria were identical to those described above.

Group C: Healthy subjects recruited from staff and medical students in the hospital were designated as another control group (group C). None of these subjects had a history of chronic respiratory, gastrointestinal or allergic disease, or currently had a cough.

Laboratory investigations

Reflux related symptoms were scored by a Chinese version of gastroesophageal reflux diagnostic questionnaire (GerdQ) provided by the designer [9]. Cough sensitivity to inhaled capsaicin was detected according to the method described previously [10]. Cough threshold was defined as the lowest concentration of capsaicin required for the induction of ≥2 (C2) and ≥5 coughs (C5). Sputum was induced and processed as described previously [4, 5]. Briefly, the subjects inhaled a 4% hypertonic saline solution through an ultrasonic nebulizer (YS9801, Yisheng Corp, Shanghai, China) and every 5 min, after blowing their noses and rinsing their mouths, expectorated sputum into a sterile pot placed in ice. Sputum with minimal salivary contamination was immediately selected, mixed with four volumes of 0.1% dithiothreitol by gentle aspiration and further mixed on a bench rocker for 20 min. The suspension was filtered through 48-mm gauze and centrifuged at 3000 rpm for 10 min. The cell-free supernatant was removed and stored at -80°C until analysis. The cell pellet was resuspended in 1 mL of PBS, smeared on glass slides, and a total cell count was performed using a standard hemocytometer. The air-dried preparations were stained with H&E. A differential cell count was performed on 400 nucleated cells according to standard morphological criteria.

MII-pH was performed as previously described by us [11, 12]. In brief, subjects were fasted for more than 10 h and the lower esophageal sphincter was located by esophageal manometry. Then, 6-channel impedance electrode catheter (K6011-E10632, MMS, Switzerland) and pH electrode catheter (819100; MMS, Netherland) which was pre-adjusted with buffers (pH = 4.0 and 7.01) were inserted into the esophagus via a nose. The impedance electrodes were placed at 3 cm, 5 cm, 7 cm, 9 cm, 15 cm and 17 cm above the lower esophageal sphincter, and the pH electrode at 5 cm above the lower esophageal sphincter. The reference electrode was fixed at middle to lower segment of the sternum and connected to the MII-pH monitor (Ohmega; MMS, Netherland) followed by recording. During the period of monitoring, the daily schedule and dietary habit remained unchanged, and the beginning and ending time of eating, supine position and symptoms. The acid food and alcohol were avoided. Monitoring was done for 24 h and the data were input into a computer and analyzed with specialized software (Database soft, 8.7 version, Medical Measurement System B.V., Netherlands). On the basis of impedance values, the reflux was divided into liquid reflux, gas reflux and mixed

reflux. Liquid reflux: the adjacent 2 MII channels show retrograde liquid reflux as impedance falls >50% from baseline which continued for at least 3 s; gas reflux: the increase in impedance in at least two electrodes is higher than 5000 Ω in a short time (3 kΩ/s); mixed reflux: There is gas reflux before or during the liquid reflux. On the basis of pH value, liquid reflux was divided into acid reflux (pH<4.0), weak acid reflux (pH 4.0-7.0) and weak base reflux (pH>7.0). The number of reflux, proximal extent (refluxate reached the 15-cm impedance site above the lower esophageal sphincter), bolus exposure (time of reflux to total time of monitoring [%]), time of bolus clearance (from the presence of bolus to the clearance of bolus) and time of acid clearance (from the acid reflux to the pH value of ≥4.0) were detected. On the basis of the proportion of time of pH<4.0 to total time of monitoring, the proportion of time of pH<4.0 to total time of monitoring in the orthostatic position, the proportion of time of pH<4.0 to total time of monitoring in the supine position, total number of reflux, total number of reflux sustaining for more than 5 min, and the longest time a reflux sustained were employed to calculate the DeMeester score. The DeMeester score of ≥14.72 was defined as abnormal acid reflux [13]. The above parameters were finally used to calculate the acid SAP and SAP≥95% was defined as a threshold [14].

Pulmonary function and histamine bronchial provocation tests were performed according to the guidelines set out by the American Thoracic Society and the Respiratory section of the Chinese Medical Association, respectively, using an Aerosol Provocation System and MasterScreen Diffusion (Jaeger Toennies, Hochberg, Germany) [10, 15]. Subjects were defined as showing AHR if the cumulative provocative dose of histamine causing a 20% fall in FEV1 (PD_{20} EV1) was <7.8 mmol.

Substance P (SP), eosinophil cationic protein (ECP), mast cell tryptase (MCT) and interleukin-8 (IL-8) were measured with ELISA according to the manufacturer's instructions. (SP, ECP and MCT: R&D Systems, Minneapolis, MN, USA; IL-8: ADL Corporation, USA) The intra-assay difference was <5% and the inter-assay difference was <10%. The sensitivity of each kit was as follows: SP: 0.01 ng/ml; PGD2: 0.01 pg/ml; ECP: 1.5 ng/ml; MCT: 7.8 pg/ml; IL-8: 7.8 pg/ml.

Procedures

On the first hospital visit, initial assessments included the collection of patients' general information and the recording of reflux symptom score, cough sensitivity to capsaicin, examination of induced sputum, lung function and histamine bronchial provocation test. Patients received anti-asthma therapy with positive histamine bronchial provocation. If these patients did not response to anti-asthma therapy, they received MII-pH monitoring. Patients with abnormal findings in MII-pH received standard anti-reflux therapy. GERC with AHR were finally diagnosed when cough relieved obviously or resolved completely. Once patients negative in the histamine bronchial provocation test, further MII-pH monitoring was performed. If abnormalities were present in MII-pH results, GERC alone was definitely determined if cough of the patients controlled or improved with standard anti-reflux therapy.

Statistical analysis

Data with normal distribution were expressed as mean ± SD, or as median (25%-75% interquartile) if skewed distribution. Cough thresholds C2 and C5 were log transformed to normalize the data and then expressed as geometric means ± SEM. Comparisons across the three groups and between two groups were performed using the Kruskal-Wallis test followed by the Mann-Whitney U test for data with skewed distributions, or one way analysis of variance (ANOVA) followed by Newman-Keuls test for normally distributed data. Differences in gender distribution among the three groups were analyzed using the chi-square test. Correlation analysis was performed using Pearson correlation coefficients. Software (SPSS 17.0, Chicago, IL, USA) was used for statistical calculation. A value of $P<0.05$ was considered statistically significant.

Results

General information of the patients

A total of 529 patients with chronic cough were enrolled during the study period, of whom 133 received cytological examination of induced sputum, bronchial provocation test and MII-pH monitoring. A total of 42 patients were finally diagnosed with GERC and 19 patients with

Table 1. General information of the patients in three groups

Items	GERC+AHR	GERC	Healthy subjects
Subjects (male)	9 (4)	14 (5)	9 (5)
Ages (yr)	55.0 ± 7.8	51.9 ± 22.3	36.2 ± 17.1
Cough duration (month, range)	14 (16.0)	9 (8.5)	-
C2	0.98 (4.9)	0.98 (9.6)	31.2 (25.3)
LogC2	-0.01 (-0.60)*	-0.01 (1.05)*	1.49 (0.30)
C5	7.8 (32.5)	7.8 (15.1)	31.2 (60.6)
LogC5	0.89 (1.32)*	0.89 (1.05)*	1.49 (0.61)
FEV1, % predicted	95.8 ± 4.4	88.4 ± 14.1	89.6 ± 7.4
FVC, % predicted	98.2 ± 8.3	91.5 ± 8.7	93.6 ± 7.9
FEV1/FVC%	84.5 ± 7.6	88.1 ± 9.6	86.4 ± 7.6
PD_{20} FEV1	4.32 ± 1.83	--	--

GERC: Gastroesophageal reflux-induced cough; FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity; PD_{20} FEV1: Cumulative dose of histamine which caused a 20% fall in the one second forced expiratory volume. *P<0.05 compared with healthy subjects. --: not available.

Table 2. Comparison of variables of MII-pH between three groups

Items	GERC+AHR	GERC	Healthy subjects
GerdQ score	8.4 ± 1.5	8.0 ± 2.7	--
DeMester score	30.5 (15.3)#	28.5 (23.2)#	4.1 (6.2)
SAP (%)	77.2 (29.9)	75.3 (40.2)	--
Reflux events (n)	56.0 (62.2)	27.0 (55.0)	30.0 (86.5)
Acidic reflux (n)	41.0 (35.2)	36.0 (30.2)	7.0 (31.5)
Weakly acidic reflux (n)	20.0 (19.0)	18.0 (12.5)	26.0 (61.5)
Weakly alkaline reflux (n)	0 (7.5)	1.0 (5.0)	0 (1.5)
Liquid reflux (n)	13.8 (31.0)	9.0 (13.0)	10.0 (29.0)
Gas reflux (n)	31.0 (29.3)	28.0 (96.5)	49.0 (27.3)
Mixed reflux (n)	55.5 (35.8)	41.5 (40.2)	17.0 (27.5)
Bolus exposure (%)	1.0 (1.2)	0.6 (0.5)	0.8 (1.5)
Time of bolus clearance (s)	13.3 (5.3)	7.0 (2.0)*	7.5 (4.0)*
Proximal reflux episodes (n)	21.5 (28.6)	7.5 (1.8)*	3.0 (3.5)*
Proportion of proximal reflux episodes (%)	24.5 (13.5)	4.2 (7.3)*	1.3 (9.0)*

*P<0.05 compared with AHR+GERC; #P<0.05 compared with healthy subjects; --: not available.

non-acid reflux induced GERC were excluded. In the present study, 23 patients with acid reflux induced GERC were recruited including 9 males and 13 females with a mean age of 53.4 ± 15.1 years (range: 19~69 years). The median course of cough was 12 months (range: 3~240 months) (**Table 1**).

Of these patients, 9 patients (Group A) were positive in bronchial provocation test including 4 males and 5 females, 5 subjects of them reviewed bronchial provocation test which showed negative after anti-reflux therapy; 14 subjects (Group B) were negative in bronchial provocation test including 5 males and 9 females; 9 healthy subjects were enrolled as group C. There were no significant differences in the general characteristics among three groups. No marked differences were noted in the logC2 and logC5 between Group A and B. When compared with healthy subjects, GERC patients had dramatically reduced logC2 (H = 10.45, P = 0.005) and logC5 (H = 5.58, P = 0.045) (**Table 1**).

Findings of MII-pH results in three groups

Patients in Group A and B had markedly increased DeMeester score as compared to the healthy subjects (Z = -3.004, P = 0.003 and *Z* = -2.745, P = 0.006, respectively). However, there were no marked differences in the DeMeester score and SAP between Group A and B. The time of bolus clearance in Group A was longer than that in Group B and healthy subjects (Z = -2.470, P = 0.014 and Z = -2.252, P = 0.024, respectively). In addition, the number of proximal reflux episodes and the proportion of proximal extent episodes to total refluxes in Group A were markedly higher than those in Group B (Z = -2.038, P = 0.042 and Z = -2.138, P = 0.032, respectively). Moreover, these two parameters in Group A were also significantly higher than that in the healthy subjects (Z = -2.273, P = 0.023 and Z = -2.345, P = 0.019, respectively) (**Table 2**).

Table 3. Comparison of total and differential cell counts in induced sputum of subjects between three groups

Items	GERC+AHR	GERC	Healthy subjects
Total cell number (*10^6/ml)	3.5 (2.4)#	2.0 (4.2)#	0.9 (1.4)
Neutrophils (%)	39.7 (35.6)	9.0 (40.4)	0.5 (2.0)
Macrophages (%)	51.5 (12.1)#	62.5 (25.3)	79.5 (7.6)
Lymphocytes (%)	10.0 (25.6)	14.5 (20.9)	20.5 (6.9)
Eosinophils (%)	0 (0)	0 (0.5)	0 (0.5)

#P<0.05 compared with healthy subjects.

Table 4. Comparison of mediator levels in induced sputum supernatants between three groups

Items	GERC+AHR	GERC	Healthy subjects
MCT (pg/ml)	42.9 (37.9)	32.8 (13.3)#	22.0 (11.9)
ECP (ng/ml)	5.7 (4.5)	5.6 (1.5)	5.3 (1.5)
SP (ng/ml)	0.5 (0.4)	14.7 (236.0)#	0.1 (0.4)
IL-8 (pg/ml)	71.1 (64.0)#	26.9 (37.1)	24.3 (35.2)

MCT: Mast cell tryptase; SP: P substance; IL-8: Interleukin 8; #P<0.05 compared with healthy subjects.

Comparisons of airway inflammation in three groups

The number of cells in the induced sputum in Group A and B was dramatically increased when compared with Group C (Z = -2.352, *P* = 0.019 and Z = -2.293, *P* = 0.022, respectively). In addition, the neutrophils in induced sputum were 39.7%, which were much higher in Group A than that in the Group B (9.0%) and healthy controls (0.5%) (**Table 3**). Whilst the macrophages in induced sputum was dramatically reduced in Group A as compared to healthy subjects (Z = -2.574, P = 0.010). Moreover, the levels of MCT (Z = -2.379, P = 0.017) and SP (Z = -2.745, P = 0.006) in Group B were markedly higher than those in healthy subjects; the level of IL-8 in Group A was significantly higher than that in healthy subjects (Z = -2.013, P = 0.044) (**Table 4**). Furthermore, in Group A, the IL-8 level was positively related to the number of proximal reflux (r = 0.954, P = 0.046).

Discussion

In the present study, our results demonstrated that some patients with GERC might develop AHR concomitantly. AHR was not the direct cause of chronic cough as cough resolved with anti-reflux therapy, rather than anti-asthma treatment. GERC patients with AHR had obvious proximal reflux and the airway inflammation was characterized by increased IL-8 in induced sputum. However, patients with GERC alone mainly presented increases in SP and MCT.

GERC is one of common causes of chronic cough. Mechanisms whereby gastroesophageal reflux events can trigger coughing include i) direct stimulation of the nerve terminals may be responsible for evoking mechanosensitive or chemosensitive cough receptors in the laryngeal pharynx and lower airways [16]; and ii) indirect stimulation by activation of vagal nerves in the airways may trigger an esophageal-tracheobronchial reflex [15, 17]. The close relationship between GERC and rhinitis, nasosinusitis, chronic pharyngitis and other pulmonary diseases has been demonstrated in some studies [16, 18-20]. CVA is another important cause of chronic cough [5, 21]. Palombini et al found that one or more of CVA, GERD and UACS were the causes of chronic cough in 93.6% of patients, and they proposed these three diseases were the pathological triad of chronic cough [6]. The above findings demonstrated the important and close relationship between GERC and CVA.

Bronchial provocation test is an effective tool to detect the AHR and also a required method in the diagnosis of CVA [5]. However, this test has poor specificity. That is, not all patients with AHR could be diagnosed as asthma. A study has revealed that only 60~88% of chronic cough patients with positive bronchial provocation were responsive to anti-asthma therapy [22]. Other research showed, 33~74% of allergic rhinitis patients without cough or asthma presented AHR [23, 24]. In the present study, 9 GERC patients presenting AHR were non-responsive to anti-asthma therapy, while cough resolved after anti-reflux therapy and then 5 of them showed normal airway responsiveness. Thus, we confirmed that these patients were diagnosed with GERC alone, and the AHR might be attributed to the gastroesophageal reflux. In clinical practice, we should identify patients

with both GERC and AHR to avoid over-treatment and unnecessary side effect.

Proximal reflux episode refers to the retrograde reflux at more than 15 cm above the lower esophageal sphincter, and reflux at less than 15 cm above the lower esophageal sphincter is defined as distal reflux [25]. In the present study, MII-pH monitoring revealed that distal reflux accounted for 75~96% in 23 patients with GERC, the levels of SP and MCT, indicators of neurogenic inflammation were at a high level, which suggest that the distal reflux is a major cause of GERC and tracheoesophageal reflex may induce neurogenic inflammation resulting in cough [1]. As compared to patients with GERC alone, patients with both GERC and AHR had significantly increased number of proximal extent episodes and proportion of proximal extent episodes to total refluxes. Thus, we postulated that the AHR perhaps be attributed to the reflux of acid stomach contents into the airway. First, the refluxate may not only directly cause tissue injury, but also stimulate the release of inflammatory mediators by epithelium. Second, acid infusion of the esophagus has been found to result in vagally mediated reflexes leading to bronchoconstriction. Third, refluxate may stimulate the airway mucosa and cause neurogenic inflammation via tracheoesophageal reflex, which is also one of causes of AHR [20]. Combined, these 3 mechanisms may lead to an increase in vagal efferent impulses that can cause or augment airway hyperresponsiveness.

In the present study, detection of airway inflammation showed neutrophils in induced sputum of GERC patients with AHR was markedly higher than that of GERC patients without AHR. In addition, the level of IL-8 in the supernatant of induced sputum of GERC patients with AHR was markedly higher than that of healthy subjects. IL-8 is a potent chemokine of neutrophils, patients with non-acid reflux were excluded in our study, and the influence of difference in the nature of refluxate on airway inflammation was excluded. Thus, we postulated that GERC patients with AHR had reflux of acid infusion into the airway inducing neutrophilic airway inflammation via different ways. The neutrophilic airway inflammation may cause AHR via stimulating production of oxygen free radicals, elastase etc. or facilitating the excessive secretion of the airway. However, the AHR is mild and unlike to cause real asthma [26]. This has been demonstrated that intra-airway administration of acid substance in mouse model caused neutrophilic airway inflammation and increased the airway responsiveness [27]. In addition, cough itself could induce damage to the airway wall in guinea pigs resulting in neutrophilic airway inflammation [28]. Traditionally, asthma is an inflammatory airway disease characterized by eosinophil infiltration. To date, neutrophilic asthma is described as phenotype of asthma [29]. In our study, whether the gastroesophageal reflux induced neutrophilic airway inflammation and AHR may develop into variant asthma or bronchial asthma is required to be further studied.

There were several limitations to the present study. First, the conclusions should be accepted with caution, since the small numbers of patients with both GERC and AHR may have limited the power of the study. A recent multi-center survey has shown that GERC is relatively rare in China [30], not to mention GERC with AHR, which accounts merely for a small part of GERC. Therefore, it is difficult to enroll a large number of patients. In addition, this study was retrospectively designed, but the results of present study were consistent with those of the previous related reports. Finally, the differential cell counts in induced sputum varied greatly within and between groups. In our experience, heterogeneity in induced sputum cytology is frequent because similar results have been reported previously [11, 31].

Taken together, gastro-esophageal reflux may cause neutrophilic airway inflammation due to the acid reflux into the airway, which results in AHR. However, AHR is not definitely able to cause chronic cough. Thus, for patients with both GERC and AHR, therapy should be done targeting the cause one by one, which aims to avoid over-treatment and possible side effects. In future prospective studies with large sample size, it is necessary to investigate whether the GERC and AHR may develop into cough variant asthma or even bronchial asthma.

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Disclosure of conflict of interest

None.

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