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ORIGINAL ARTICLE

The influence of gastroesophageal reflux in the lung: A case–control study

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ABSTRACT

Background and objective: **Many researchers have investigated the pH of exhaled breath condensate but direct measurement of pH in the lung has not been performed *in vivo* in humans. We hypothesized that the pH measured directly in the lung would differ between healthy subjects and patients with gastroesophageal reflux disease (GERD). We also wished to determine whether an acidic environment in the lung influences pulmonary function and DL_{CO}, and whether microaspiration of gastric contents directly influences non-specific inflammation in the lung.**

Methods: **The patients were otherwise healthy individuals who had been newly diagnosed with GERD. The control subjects were mostly volunteers who underwent bronchoscopy for different reasons. For all subjects ($n = 63$) a medical history was taken, and physical examination, oesophagogastroduodenoscopy, fibreoptic bronchoscopy and pulmonary function testing were performed.**

Results: **In patients with GERD the average pH in the lung was 5.13 ± 0.43; this was significantly lower than the pH in the lung of controls 6.08 ± 0.39 ($P = 0.001$). Patients with GERD had lower FEV_1% ($P = 0.035$), PEF ($P = 0.001$), $FEF_{50\%}$ ($P = 0.002$) and $FEF_{25\%}$ ($P = 0.003$), while the differences in FVC% and $FEF_{75\%}$ were not significant. DL_{CO} ($P = 0.003$), as well as transfer coefficient of the lung ($P = 0.001$), was lower in patients with GERD. LDH levels in bronchoalveolar aspirate were higher in the patients with GERD ($P = 0.001$).**

Conclusions: **This study found evidence of cell and tissue injury in the lung, a lowering of pH and higher bronchoalveolar aspirate LDH levels in patients with GERD compared with healthy subjects. These findings suggest that pulmonary function, and especially DL_{CO}, should be evaluated in patients presenting with GERD.**

SUMMARY AT A GLANCE

The pH measured directly in the lung of patients with newly diagnosed gastroesophageal reflux disease was lower than that of healthy subjects. Microaspiration of gastric contents and an acidic environment in the lung influences pulmonary function and DL_{CO}, as well as causing non-specific inflammation in the lung.

Key words: bronchoscopy, gastroesophageal reflux disease, lactate dehydrogenase, pulmonary function test.

INTRODUCTION

Gastroesophageal reflux (GER) is the efflux of acidic gastric contents in to the oesophagus and, potentially, the upper and lower respiratory tract.[1] GER is known to be associated with a variety of respiratory disorders, including chronic cough,[2,3] asthma,[1,3,4] COPD,[5–7] sleep apnoea,[8] pulmonary fibrosis[9–11] and scleroderma.[12]

One potential mechanism by which GER affects pulmonary function is by aspiration of acid or gastric contents into airways and alveoli, causing chronic inflammation and damage to the alveolocapillary membrane, which may be detected by measurement of DL_{CO}. This damage may eventually lead to impairment of pulmonary function. The other potential mechanism involves a vagally mediated reflex triggered by acid within the oesophagus, leading to bronchial or laryngeal constriction and hyperresponsiveness.[13] In clinical practice, 24-h oesophageal pH monitoring has been mostly used to identify pathological GER in patients with unexplained or refractory respiratory symptoms.[1,13–15]

Many researchers have investigated the pH of exhaled breath condensate (EBC);[16–19] however, direct measurement of pH in the lung has not been performed *in vivo* in humans. Studies on the influence of

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acidic gastric contents on pH in the lung have been conducted on animals, including pigs,[20,21] cats[22] and rats.[23] These animal studies have shown that the continuous aspiration of acidic fluid into the lung causes inflammation and chronic pulmonary disease with or without pulmonary fibrosis.

In this study, pH was measured directly in the lung of healthy subjects and patients with newly diagnosed GER disease (GERD), based on the hypothesis that there is a difference in lung pH between these two groups. We also wished to establish whether an acidic environment in the lung influences pulmonary function and DL_{CO}, and whether microaspiration of gastric contents directly influences non-specific inflammation of the lung.

METHODS

Patients and control subjects

This was a case–control study conducted over 30 months, from November 2006 to April 2009 at Split University Hospital. The patients were otherwise healthy individuals aged 18 years or more who had been diagnosed with GERD. The diagnosis of GERD was made on the basis of symptoms, including heartburn and food regurgitation, and on oesophagogastroscopic findings, as defined by Savary and Miller.[24] Heartburn, acid regurgitation and food regurgitation were considered to be typical symptoms of GERD. Heartburn was defined as a burning sensation or burning pain in the retrosternal area. Acid regurgitation was defined as bitter or sour-tasting fluid returning to the throat or mouth, and food regurgitation was defined as the return of swallowed food to the mouth. The patients included in the study had first-, second- or third-grade GERD, while those with fourth- or fifth-grade GERD were excluded on account of the severity of their illness. In the patients video-bronchoscopy (BSC) was performed after oesophagogastroduodenoscopy.

The control group consisted of six subjects with acute foreign body aspiration (a bone, a thread, a sour cherry pit, a needle, a pea and a pill), two subjects with suspected foreign body aspiration, two patients with primary brain tumours and five subjects who underwent BSC due to suspected traumatic rupture of bronchi after traffic accidents. The control subjects had no symptoms of GERD, or endoscopically proven GERD, or respiratory disease. In the control subjects oesophagogastroduodenoscopy was performed after BSC.

For all subjects a detailed medical history was taken, a physical examination was performed and BMI was assessed. The exclusion criteria for both groups were: obesity (BMI $\geq$ 30), malnutrition (BMI < 18.5), a history of heart disease or other diseases affecting pulmonary function, especially DL_{CO}, including musculoskeletal disease, collagen vascular disease, liver disease, long-term inhalation of toxic vapours and/or gases, anaemia and diabetes mellitus. A CXR was performed for all subjects, to exclude pulmonary disease. The influence of tobacco smoke on DL_{CO} and bronchial hyperresponsiveness was avoided by including in the study only non-smokers and smokers who had ceased smoking for a minimum of 10 years.

Measurement of lung pH and pulmonary function

Oesophagogastroduodenoscopy was performed with a flexible video gastroscope (Olympus-Evis extra II GIF Type H-80, Tokyo, Japan). The effects of GER were investigated by measuring pH in peripheral branches of the bronchi and LDH in bronchoalveolar aspirate obtained using a video-bronchoscope, as well as by measuring DL_{CO} and pulmonary function.

Bronchoscopy was performed under local anaesthesia with 2% lidocaine, using a video-bronchoscope (Olympus BF type 1T160, Tokyo, Japan). The pH in peripheral bronchi was measured semi-quantitatively by means of test strips (Multistix 10 SG, Bayer AG, Leverkusen, Germany), which are normally used to determinate the pH of other body fluids (urine, cerebrospinal liquid, pleural effusions). The range of pH values measured was 5.0–9.0. A small piece of test strip was placed in biopsy forceps, which were then wedged into one of the peripheral branches of the right lower lobe, most frequently DB9 or DB10, for 15–20 s. The forceps were then pulled out and the pH was read immediately. Aspirate from peripheral branches of DB9 or D10 was obtained by deep catheter aspiration using an 18 cm $\times$ 2 mm catheter, which is normally used for BSC and provided with the video-bronchoscope. When the lumen of the catheter was completely filled with aspirate, the catheter was rinsed with 1.5 mL of distilled water. LDH in the bronchoalveolar aspirate (BA-LDH) was measured within 1 h in an Architect C-8000 instrument (Clinical Chemistry, Abbott Park, Illinois, USA), located in close proximity to the endoscopy suite. Subjects whose aspirate was bloody, tinged with blood or pus (purulent, mucopurulent), or showed evidence of infection (positive biogram, mycobacterial culture), were excluded from the study because diseases resulting in such aspirates may influence BA-LDH values.

Pulmonary function testing was performed using a body plethysmograph (Jaeger Masterlab, Wurzburg, Germany). This included measurements of FVC, FEV_1, PEF, $FEF_{25–75\%}$, DL_{CO} and transfer coefficient of the lung (KCO). DL_{CO} was measured using a rapid carbon monoxide and methane analyser, which was calibrated before each measurement. Results were expressed as percentage of predicted values by comparison with reference values.[25,26]

Arterial blood gases were analysed using a blood gas electrolyte analyser (GEM Premier 300, model 5700, Instrumentation Laboratory, Lexington, MA, USA).

Each subject was tested using the same instruments and devices by the same investigator within 5 days. Written consent was obtained from all subjects after full explanation of the purpose, nature and risks of all procedures. The study was approved by the Ethics Committee of Split University Hospital and

was performed over a period of 2 years because of the strict inclusion and exclusion criteria.

Statistical analyses

Between groups differences in quantitative variables were compared using Student's *t*-test or analysis of variance with the Bonferroni post-hoc test, when the data were normally distributed, and the Mann–Whitney test when the data were not normally distributed. Pearson's correlation coefficient (*r*) was used to assess the correlation of pH and DL_{CO} with BA-LDH. *P*-values <0.05 were accepted as indicating statistical significance. Statistical analyses were performed using the Statistica 7 programme (StatSoft, Inc, Tulsa, Oklahoma, USA).

RESULTS

A total of 63 subjects were included in the study; 38 (60.3%) were men and 25 (39.7%) were women. There were 38 (60.3%) patients with GERD, of whom 25 (65.8%) were men. The median age of the patients with GERD was 55 years and their median BMI was 25 kg/m^2. There were 18 (47.4%), 12 (31.6%) and 8 (21.1%) patients with stage 1, stage 2 and stage 3 GERD, respectively. There were 25 (39.7%) subjects in the control group, of whom 15 (60%) were men. The median age of the control group was 56 years and their median BMI was 23.5 kg/m^2. The differences in median age and BMI between the two groups were not statistically significant (Table 1).

Pulmonary function

There were no significant differences in FVC% ($P = 0.087$) or $FEF_{75\%}$ ($P = 0.074$) between the two groups. However, FEV_1% ($P = 0.035$), PEF ($P = 0.001$), $FEF_{50\%}$ ($P = 0.002$) and $FEF_{25\%}$ ($P = 0.003$) were significantly lower in the GERD group (Table 1). Both DL_{CO} ($P = 0.003$) and KCO ($P = 0.001$) were also significantly lower in the GERD group (Table 2).

The mean value for BA-LDH in the GERD group was 368.1 ± 101 U/L, while in the control group it was 116.9 ± 71 U/L, which was a statistically significant difference ($P = 0.001$). BA-LDH in the lungs decreased with increasing pH ($r = –0.539$, $P = 0.001$). There was a

Table 1 Ventilatory pulmonary function parameters in patients with GERD and control subjects

	Patients with GERD ($n = 32$)	Control subjects ($n = 25$)	*P*-value
BMI (kg/m^2)	25.0 (19–28.8)	23.5 (18.5–29.3)	0.937†
Age (years)	55 (26–75)	53 (35–77)	0.829†
FVC%	97.2 (55.4–122)	102 (88.9–132)	0.087†
FEV_1%	92.8 (54.7–121)	101 (88.8–126)	0.035†
PEF%	80.6 (50–107)	99.4 (80.2–118)	0.001†
$FEF_{75\%}$	96.6 (56–117)	110.8 (85.3–132)	0.074†
$FEF_{50\%}$	87.0 (43–152)	112 (86–138)	0.002†
$FEF_{25\%}$	86.1 (58–144)	102 (89–127)	0.003†

Values are medians (range).
† Mann–Whitney test.
BMI, body mass index; GERD, gastroesophageal reflux disease.

Table 2 Lung pH, diffusing capacity, LDH and arterial blood gases in patients with GERD and control subjects

	Patients with GERD ($n = 32$)	Control subjects ($n = 25$)	*P*-value
Lung pH	5.13 ± 0.43	6.08 ± 0.39	0.001†
DL_{CO}, %	84.6 (62–121)	99.8 (86.2–123)	0.003‡
KCO, %	70.7 (59–122)	91.3 (77.6–117)	0.001‡
LDH in serum (U/L)	162.8 ± 22	158.2 ± 16	0.438‡
BA-LDH (U/L)	368.08 ± 101	116.85 ± 71	0.001‡
PaO_2 (kPa)	10.89 ± 1.03	12.2 ± 0.59	0.01†
$PaCO_2$ (kPa)	4.75 ± 0.18	4.76 ± 0.35	0.842†

Values are medians (range) or means ± SD.
† Analysis of variance.
‡ Mann–Whitney test.
BA-LDH, LDH in the bronchoalveolar aspirate; KCO, transfer coefficient of the lung; GERD, gastroesophageal reflux disease.

significant correlation between DL_{CO} and BA-LDH for all subjects, with BA-LDH values increasing with DL_{CO} ($r = 0.826$, $P < 0.001$) (Table 2).

The difference in serum LDH between the GERD group (162.8 ± 22 U/L) and the control group (158.2 ± 16 U/L) was not significant ($P = 0.438$). LDH values were greater in bronchoalveolar aspirate than in serum of patients with GERD and the difference was significant ($P < 0.001$). PaO_2 at rest differed significantly between the groups ($P = 0.01$), whereas $PaCO_2$ did not (Table 2).

Lung pH

The average pH in peripheral branches of the bronchi in the GERD group was 5.13 ± 0.43, while in the control group it was 6.08 ± 0.39. This difference in lung pH between the two groups was statistically significant ($P = 0.001$) (Table 2).

DISCUSSION

This is the first study in which pH has been measured directly in the lung of humans *in vivo*, and the first study in which lung pH has been measured in patients with GERD. In contrast, many researchers have investigated pH in EBC, which was mostly between 6 and 8 in healthy subjects.[16,18,19,27,28]

The pH values measured directly in the lung of healthy subjects concur approximately with the EBC measurements in previous studies. This relatively large pH range is difficult to explain. In this study pH values were at the lower end of the range, probably because of the different locations at which pH was measured.

In this study the average pH value in the lung of subjects with GERD was 5.13, which is significantly lower than that in the control group. Such an acidic environment would undoubtedly alter local metabolism and damage local cells and tissues, causing significant narrowing of the lumen of small airways, due to oedema and/or destruction of the alveolocapillary membrane.

One of the indicators of cell injury is release of LDH, the value for which was 3.15 times greater in bronchoalveolar aspirate of patients with GERD compared with that of healthy subjects. In patients with GERD, the BA-LDH level was 2.26 times greater than the serum LDH level. This study also showed that BA-LDH values decreased with increasing pH and increased with the increase in DL_{CO}.

Lactate dehydrogenase in the bronchoalveolar aspirate was measured as a simple indicator of cell and tissue injury that can be assayed in most biochemical laboratories, does not require special preparations, materials or training of staff, and does not involve additional financial expenses for purchase of assays for the measurement of inflammatory markers such as tumour necrosis factor-α, IL-8 and other cytokines.[16,29] LDH was measured in bronchoalveolar aspirate and not in BAL fluid, because during BAL the aspired contents are considerably diluted, providing misleading information regarding local pathological changes. In order to obtain more accurate information on the degree of lung cell damage caused by microaspiration of acidic gastric contents, LDH was measured in bronchoalveolar aspirate, which was diluted with the minimum amount of distilled water necessary to displace the contents from the 18-cm long catheter. To our knowledge, this is the first time LDH has been measured in bronchial aspirate.

We were not able to identify similar research in the recent literature, with which the present results could be compared. However, Rydell-Tormanen *et al.*[30] recently reported that pulmonary inflammation in LPS-exposed mice was associated with increased levels of the pan-necrosis marker LDH in BAL fluid.

All patients with GERD had pulmonary function parameters that were within reference ranges. However, most values for these patients were significantly different from the values for the control group. $FEF_{50\%}$, $FEF_{25\%}$ and PEF, which indicate airflow in the small airways, were significantly lower in patients with GERD, while the other pulmonary function parameters were mostly within the reference ranges. Increased acidity in the lungs leads to oedema of the airways, with significant narrowing of the lumen of small airways and a consequent decline in airflow in these areas ($FEF_{25–75\%}$), as demonstrated in this study. Because pulmonary function was measured in patients with GERD who did not suffer from any other pulmonary disease, the results were not comparable with those from other studies, which mostly investigated pulmonary function in patients with miscellaneous pulmonary diseases, who were also diagnosed with GERD.[1,2,4,6,8,9]

The results of this study showed that there is cell and tissue injury in the lungs when pH <6.0, with consequently increased levels of BA-LDH in the patients with GERD compared with the control group. The DL_{CO} and KCO values were also significantly lower in the patients with GERD. It is known that DL_{CO} is influenced by various factors including smoking,[31] body position,[32] connective tissue disease,[33,34] heart disease,[35,36] different types of anaemia,[37,38] diabetes,[39,40] inhalation of toxic vapour or gas,[41] as well as breathing technique and measurement method.[42,43]

In our opinion, the reason why DL_{CO} and KCO are reduced in patients with GERD compared with healthy subjects, is that a low pH at the alveolar-capillary membrane most probably causes damage and loss of surfactant with the resultant collapse of alveoli and development of microatelectasis. At the same time, acid affects macrophages and other immunocompetent cells, which then secrete miscellaneous bioactive substances, generating an inflammatory reaction with cellular apoptosis and injury to the pulmonary parenchyma. Indicators of this process are the significantly higher values of BA-LDH, which is a marker for primary or secondary necrosis, as demonstrated in mice,[30] as well as the lower DL_{CO}, KCO and PaO_2 values, all of which are the consequence of injury to the alveolar-capillary membrane. Patients with GERD frequently complain of dyspnoea, which may be the result of reduced DL_{CO} as compared to individuals

without GERD. The long duration of GERD and increased acidity in the lung, as well as processes that occur because of the low pH, may be associated with the inception of miscellaneous respiratory diseases, such as pulmonary fibrosis. This hypothesis has been proposed by a number of investigators,[1,9,11,20–23,44] who demonstrated an increased prevalence of GERD in patients with IPF. Treatment of GERD with proton pump inhibitors was shown to improve pulmonary function after several months.[45,46]

In the early stages of GERD, microatelectasis is not seen on conventional CXR, and probably would not be detected even on high-resolution computed tomography scans; it can only be verified by pulmonary function testing, principally measurement of DL_{CO} and KCO. Only when GER has occurred over a long period of time, do fibrosis and other chronic pathological changes develop in the lung. The duration of pathological acidity in the lung that is necessary for irreversible changes remains an open question, which is an incentive for further research. The results of this study indicate that assessment of pulmonary function, especially DL_{CO}, would be good clinical practice in patients with GERD.

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