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Anatomical and functional deficiencies of the crural diaphragm in patients with esophagitis

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MANS, RAN, & PCB performed the research; MANS, DS, & AAS analyzed the data; AAS contributed essential tools; and, MANS & DS designed the research study and wrote the paper.

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Running title: Deficient crural diaphragm in GERD.

Key Points

- The crural diaphragm (CD) is an essential component of the EGJ antireflux barrier. Inspiratory esophagogastric junction (EGJ) pressure is lower in patients with GERD.
- Our study showed that patients with esophagitis may have a thinner crural diaphragm and a deficient EGJ activity during forced inhalation.
- The anatomical changes and functional failure of the CD in esophagitis patients support the possibility of a skeletal muscle deficiency in GERD that may be targeted for exercise-based treatment.

Abstract

Inspiratory esophagogastric junction (EGJ) pressure is lower in GERD and patients fail to increase EGJ pressure during the inspiratory effort. **Aim:** to assess the EGJ activity during inspiratory maneuvers (high-resolution manometry-HRM) and the crural diaphragm (CD) thickness (endoscopic ultrasound-EUS) in GERD. **Methods:** Twenty esophagitis patients (average age 45y, 7 grade A, 13 grade B) had HRM and EUS. Forty-three controls were recruited; thirty had HRM (average age 33y), and 13 had EUS (average age 40y). The EGJ contractility index (EGJ-CI) (mmHg×cm) was measured during normal respiration and two inspiratory maneuvers: without and with inspiratory loads of 12, 24, and 48 cmH_2O (TH-maneuvers). A composite metric for TH-maneuvers ("EGJ total activity") was defined as the product of the maximal EGJ pressure and the length of its aboral excursion during the maneuver (mmHg×cm). The CD thickness (cm) was measured during expiration (12 MHz). **Results:** Expiratory LES pressure and IRP were lower in GERD. The EGJ-CI and the "EGJ total activity" were lower in GERD during TH-maneuvers (48-cmH_2O load: 168.4±13.8 v 114.8±9.6, p = 0.006). Patients failed to sustain the inspiratory CD activity across the 12 and 48-cmH_2O efforts. The CD was thinner in GERD patients (0.37±0.03 v 0.49±0.04, p = 0.02). The CD thickness correlated with the increment in the "EGJ total activity" in GERD without a hiatal hernia (r = 0.702, p = 0.016, n = 11). **Conclusion:** There are anatomical changes and functional failure of the CD in esophagitis patients supporting the possibility of a skeletal muscle deficiency in GERD.

Key words: Gastroesophageal reflux, esophagitis, diaphragm, skeletal muscle, atrophy, endosonography.

GASTROESOPHAGEAL REFLUX DISEASE (GERD) symptoms affect 5% of the population in Asia and 15% in the Western world (1). They develop as a consequence of the reflux of gastric contents (2). The esophagogastric junction (EGJ) provides an efficient antireflux barrier in healthy subjects, and the crural diaphragm (CD) is an essential component of the EGJ antireflux barrier (3). In humans, the diaphragmatic hiatus is the site of minimum EGJ opening aperture, indicating that the CD have a crucial barrier role (4). During manometry, the inspiratory pressure of the EGJ is a hallmark of the CD function. Although both a low LES pressure and a hiatal hernia (LES-CD separation) are associated with GERD, the only independent predictor of GERD described in the analysis of EGJ function during high-resolution manometry was the impaired CD function (5). Recently, several reports suggested a relationship between CD function and GERD features. For example, EGJ inspiratory pressure may increase in systemic sclerosis patients with GERD (6). Also, in children with GERD and sleep apnea syndrome, esophageal acid exposure is lower with more severe airway obstruction (7). Moreover, some GERD patients fail to increase the EGJ pressure during respiratory maneuvers with increasing inspiratory loads (8), and respiratory training improves EGJ pressure in GERD patients (8, 9).

We hypothesized that patients with GERD might have abnormal CD anatomy/function that can underlie antireflux barrier failure. Thus, the primary goal of this study was to assess evidence of CD anatomical and functional disorder in patients with GERD.

Methods

Subjects

Control volunteers and patients with gastro-esophageal reflux disease (GERD) participated in this study. A total of 43 control subjects were recruited by advertisement or word of mouth. All controls were interviewed with a checklist of typical/atypical GERD and dyspepsia symptoms and were asymptomatic. Thirty of them had high-resolution manometry (15 males, average age 32.7 years, range from 19 to 69 years; average BMI 26.2, range from 17.7 to 35.7 Kg/m^2; no comorbidity), and other 13 subjects had endoscopic ultrasound (10 males, average age 39.9 years, range from 21 to 65 years; average BMI 25.7, range from 18.1 to 37.2 Kg/m^2; 1 with mild hypertension, and 1 with mild diabetes and hypertension). The controls that underwent EUS had neither esophagitis nor hiatal hernia. The controls that had only HRM did not have double pressure pattern at the EGJ. Twenty GERD patients (7 males, average age 45.5 years, range from 21 to 72 years; average BMI 27.6, range from 20.8 to 32.7 Kg/m^2, 2 with mild diabetes, and 3 with mild hypertension) with heartburn and reflux esophagitis (7 grade A, 13 grade B, Los Angeles Classification) were recruited from the Endoscopy Outpatient Facility at Walter Cantídio University Hospital (Federal University of Ceará, Brazil). All GERD patients had high-resolution manometry and endoscopic ultrasound. The Research Ethics Committee of the Walter Cantídio University Hospital approved the study (nº 022.04.10), and all participants provided written informed consent before entering the study protocol.

Questionnaires

All esophagitis subjects completed the Heartburn Specific Quality of Life (HBQOL) and the Gastroesophageal Reflux Disease Health-Related Quality of Life

(GERD-HRQL) questionnaires (10). These questionnaires were translated and validated to Brazilian Portuguese (11, 12).

High-resolution manometry (HRM)

Manometric studies were done in the supine position after 8-h fast. We used a solid-state HRM system (Given Imaging, Yoqneam, Israel) with a catheter including 36 circumferential sensors at 1-cm intervals. Transducers were calibrated at 0 and 300 mmHg. The manometric catheter was placed transnasally and positioned to record pressures from the upper to lower esophageal sphincters. The manometric protocol included a 5-min baseline recording and six 5-mL liquid swallows in the supine position. After that, the catheter was repositioned with at least five intragastric pressure sensors and the subjects underwent a protocol with two standardized inspiratory maneuvers. First, without airflow resistance, the volunteers carried out six cycles of 5-s deep inhalation and 5-s exhalation [sinus arrhythmia maneuver (SAM)]. Second, with airflow resistance, they did a fast and forced inhalation through a device that incorporated a flow-independent one-way spring-loaded valve that provided an adjustable airflow resistance (Threshold IMT, Philips Respironics, Andover, MA, USA) (8). Each subject trained the maneuvers during few minutes before the study and accomplished inhalations under 12-, 24-, and 48-cmH_2O resistance loads [threshold maneuver (TH)].

Analysis of HRM

The manometric analysis was performed using Manoview Software (Given Imaging, Yoqneam, Israel). Esophageal peristalsis and swallow-induced EGJ

relaxation were analyzed using the standard parameters provided by the Chicago Classification V.3 (13). EGJ pressures were measured at baseline during normal respiration and the two standardized respiratory maneuvers (SAM and TH). The EGJ pressure changes were determined by the newly described EGJ contractile index (EGJ-CI) using the software DCI tool box (14). The upper and lower margins of the EGJ were enclosed in the box (Figure 1). The isobaric contour set at 2 mmHg above the gastric pressure defined the EGJ margins. The duration of the DCI box was different during baseline, SAM and TH maneuvers. During normal baseline respiration, the DCI box included three consecutive respiratory cycles; during SAM, the DCI box extended for 30 s. The value computed by the DCI tool in mmHg × s × cm was then divided by the duration of the respective box (in seconds) yielding EGJ-CI in mmHg × cm for baseline normal respiration and SAM. During the TH maneuvers, we observed a significant aboral inspiratory excursion of the EGJ. The DCI box during the TH maneuver extended from the beginning of the inhalation until maximal EGJ aboral excursion + 1 second (Figure 1). A composite metric for the TH maneuver including the increase in intraluminal pressure and the longitudinal axial excursion ("EGJ total activity") was defined as the product of the maximal EGJ pressure and the length of aboral excursion (mmHg × cm). In the cases of hiatal hernia, we took into account both LES and CD high pressure zones for the EGJ-CI and "EGJ total activity" measurements. The EGJ pressures during the respiratory maneuvers were referenced to atmospheric pressure.

Endoscopic Ultrasound (EUS)

Control subjects and GERD patients underwent EUS after 8-h fasting, under

conscious sedation. The same investigator (MANS) performed all the procedures. We used an 11mm-diameter radial echoendoscope (12 MHz) (SU-8000, Fujifilm Corporation, Tokyo, Japan). The system configuration was kept constant across all studies [power 100%, gain 50 (0 - 100), dynamic range DR65 (DR40 – DR100), gray map M3 (M1 – M5), contour enhancement E2 (E0 – E3)]. After esophageal intubation, the ultrasound transducer was maintained across the EGJ at a position so that the right crus of the diaphragm could be seen between the esophageal wall and the aorta. Mild balloon inflation was done for sonographic coupling whenever necessary. The CD right crus is a conventional endosonographic landmark nearby the celiac trunk and pancreas (15). Two DICOM images were obtained (during exhalation) in each subject.

Analysis of endosonography

Endosonographic images were analyzed by another investigator (DS), blinded to the subject group (control vs esophagitis) and the HRM results. The CD was identified in front of the aorta, and cross-sections at three different positions along the right crus length (Figure 2) were measured using the "length tool" of a DICOM analysis software (OsiriX, Pixmeo SARL, Bernex, Switzerland). The mean of 3 cross-sections measurements defined the CD "thickness" in each subject.

Statistical analysis

Continuous data were presented as means ± SEM. Student's unpaired *t* test was employed to compare healthy subjects and GERD patients' variables. The difference between the "EGJ total activities" (delta "EGJ total activities") during the 48 and 12 cmH_2O inspiratory loads and the CD thickness were tested for correlation

with the Spearman's rho. The level of statistical significance was set at 0.05 for differences in mean values and distributions [JMP Statistical Discovery Software, version 7.0.1, SAS Institute (Cary, NC); and, GraphPad Prism, GraphPad Software (La Jolla, CA)].

Results

All the 20 GERD patients recruited had typical reflux symptoms (heartburn in 18, regurgitation in 17). Eight reported mild dysphagia. The median/range of HBQOL score was 25 (15-45), and the GERD-QOL was 14.5 (1-27). Endoscopy showed esophagitis grade A in 7 patients and grade B in the 13 remaining patients. Nine out of 20 GERD patients had a high-resolution manometry pattern suggestive of a hiatal hernia (separation of the LES and CD). There were three type II, five type IIIa, and one type IIIb EGJ according to the Chicago Classification v3.0. The LES-CD separation ranged from 1.2 to 5.8 cm. Demographic data of the control groups are presented in table 1.

HRM standard parameters (esophageal body and EGJ relaxation)

Esophageal body motility was normal in 27 (out of 30) healthy subjects. Three subjects had esophageal distal contractility integral (DCI) lower than 450 mmHg × s × cm (average of all swallows), and none had failed esophageal contractions. Eighteen (out of 20) GERD patients had normal esophageal body motility. Two patients had DCI lower than 450 mmHg × s × cm (average of all swallows), and none had failed esophageal contractions. Six (30%) esophagitis patients had ineffective individual swallows (DCI < 450 mmHg × s × cm) for more than 30% of the liquid

swallows. The distribution of ineffective swallows was as follows: 0% for 12 patients, 17% for 2 patients, 33% for 4 patients, 67% for 1 patient, and 100% for 1 patient. Standard EGJ pressures in the GERD patients were lower than in the healthy controls. The values of LES pressure and relaxation, as well as DCI, are presented in table 2.

HRM esophagogastric junction pressures

During normal baseline respiration, the values of EGJ-CI were similar in the controls and GERD patients (60.9 ± 5.6 mmHg × cm and 60.1 ± 8.2 mmHg × cm, respectively, p = 0.935). During the sinus arrhythmia maneuver (SAM) (without airflow resistance) the values of EGJ-CI were also similar in the controls and GERD patients (96.5 ± 6.4 mmHg × cm and 87.8 ± 8.5 mmHg × cm, respectively, p > 0.5). On the other hand, the EGJ-CI values were lower in the GERD patients than controls during the threshold maneuvers (TH) (with airflow resistance), being statistically significant with the 24- and 48-cmH_2O-load thresholds (Table 3) (Figure 3).

During the TH maneuvers, the GERD patients failed to sustain or increase the inspiratory CD activity along the increasing inspiratory effort (from 12 to 48-cmH_2O-load). The "EGJ total activity" was significantly lower in the GERD patients than the controls during the 48-cmH_2O inspiratory effort. This failure was more evident with the "EGJ total activity" score than the EGJ-CI. "EGJ total activity" and EGJ-CI delta values (48-cmH_2O-load - 12-cmH_2O-load) were -217.2 ± 85.8 mmHg × cm and -17.9 ± 7.3 mmHg × cm, respectively.

Correlation between EGJ-CI and demographic data

In GERD patients, the BMI varied from 20.8 Kg/m^2 to 32.7 Kg/m^2 (average 27.6 ± 0.8 Kg/m^2) and did not differ from controls. Age and BMI did not correlate with the baseline EGJ-CI or the EGJ-CI during the TH maneuvers.

Correlation between severity of esophagitis, hiatal hernia, and EGJ pressures

Baseline or forced inspiratory EGJ pressures did not differ between patients with esophagitis grades A or B, or between patients with or without a manometric hiatal hernia (Tables 4 and 5). HH length, as determined by HRM, did not correlate with the EGJ baseline or forced inspiratory pressures.

Analysis of endosonography images

The CD right crus was thinner in GERD patients (0.37 ± 0.03 cm, n = 20) compared to controls (0.49 ± 0.04 cm, n = 13) ($p < 0.02$) (Figure 4). The CD thickness was similar between esophagitis patients without or with a hiatal hernia (Table 5). Although the thickness of the CD correlated positively with the BMI ($r = 0.513$, $p = 0.021$, $n = 20$), the controls and GERD patients had similar BMI (C: 25.7, n = 13 vs GERD: 27.6, n = 20, $p = 0.23$).

Correlation between CD thickness and EGJ pressures

In the whole group of GERD/esophagitis patients, there was no correlation between the values of CD thickness and the EGJ-CI, the "EGJ total activity", or the maximal EGJ pressures across the three inspiratory loads. However, the values of CD thickness correlated positively and significantly with the increment in the "EGJ total activity" between 12 and 48 cmH_2O, both in the subset of esophagitis patients without

HH as well as in all patients. In contrast, there was no such correlation in the esophagitis patients with HH (Figure 5).

Discussion

This study shows that the CD right crus is thinner in esophagitis patients compared to healthy subjects. Also, esophagitis patients failed to keep the EGJ-CI and the "EGJ total activity" at high levels as the inspiratory load increased. This phenomenon did not occur in healthy subjects. The thickness of the CD right crus did not correlate with the EGJ contractility indexes; however, the increment in the "EGJ total activity" did correlate with the CD thickness in the subset of patients without an anatomical EGJ defect, i.e., hiatal hernia. These findings suggest that there is an anatomical and functional deficiency of the CD in esophagitis patients.

The data presented herein and in previous works support the hypothesis that the CD is deficient in esophagitis patients. Previous studies showed lower inspiratory LES pressure in esophagitis patients (5). We recently showed that esophagitis patients failed to achieve a high EGJ pressure during inspiratory maneuvers by conventional manometry. Furthermore, EGJ pressures and GERD symptoms improved after inspiratory muscle training (8). Two other groups have showed that respiratory exercises may improve GERD (9, 16). This study has taken a step further and demonstrated that esophagitis patients have a thinner CD and their EGJ function cannot compensate during increasing inspiratory load.

Ultrasound has been used to measure the costal diaphragm thickness as a measure of muscle mass (17). However, the CD is not easily seen with superficial ultrasound. This difficulty has been supervened by endoscopic ultrasound (EUS)

where the CD is a typical landmark during the first stage of the pancreas examination (15). Therefore, the right crus thickness may be considered a marker of the CD mass and strength. In the present work, the EUS procedure was performed under sedation. Consequently, the CD thickness measurement was not performed during inspiratory maneuvers. Such technical drawback could explain the absence of a positive correlation between the CD thickness and the raw values of EGJ motility. However, the CD thickness did correlate with the increment in the "EGJ total activity" as the inspiratory load was increased, except in the HH patients, where the gross EGJ derangement might have concealed this effect. Esophagitis patients with a hiatal hernia had the CD muscle as thick as non hiatal hernia subjects, and the EGJ activity could be clearly detected in both groups; therefore, it is plausible to accept similar EGJ activity in both patients without or with a hiatal hernia during inspiratory maneuvers.

The diaphragm function should not be understood based only on its contractility. One can better grasp the significance of the mechanical diaphragm action by considering its anatomy, its attachment to the chest wall (18), and its possible compensatory ability under specific conditions. For example, it has been shown that the diaphragm strength is reduced in patients with symptomatic chronic obstructive pulmonary disease (COPD) (19). Unusually, CD function was reported normal in exceptional cases of stable patients with COPD (20). Indeed, the diaphragm and other respiratory muscles may undergo extensive remodeling in both animal models of emphysema and human COPD (21). Paradoxical findings have also been reported in systemic sclerosis patients with esophageal involvement and GERD. These patients have higher inspiratory EGJ pressure than controls (6). The authors interpreted this phenomenon in the light of diaphragm adaptation. The diaphragm

contraction is complex and over multiple directions so that the EGJ-CI and the inspiratory pressures may not depend (only) on the thickness of the CD right crus. In fact, it can be expected that the downward diaphragm displacement could increase the EGJ pressure without squeezing it. Nonetheless, the easiest way to assess CD thickness (that has to do with muscle mass/strength) is by ultrasound. Our data suggests that this metric is abnormal in some patients with esophagitis.

New insights into the etiology of skeletal muscle wasting/atrophy under diverse clinical settings including denervation, AIDS, cancer, diabetes, and chronic heart failure have been reported in the literature (22). Both respiratory and peripheral skeletal muscles wasting/atrophy, including the diaphragm, can occur as a result of neuromyopathies, aging, inflammation, disuse (23). In fact, the proteasome activity of the CD in a rat model of upper gut inflammation is increased, meaning an increased muscle fiber turnover (due to wasting?) (24). Then, it is reasonable to propose that the CD may be mildly atrophic in some GERD patients. Thus, the right crus thickness and the failure to deal with inspiratory loads may be anatomical and functional markers of this condition.

In summary, some esophagitis patients have a deficient CD, both anatomically and functionally. It is possible that the diaphragm dynamics partially compensates in GERD, as can occur in respiratory diseases. The cellular and molecular mechanisms underlying this phenomenon are yet to be investigated.

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Table 1. Demographic data of the endoscopic ultrasound (EUS) and high resolution manometry (HRM) controls, and the esophagitis volunteers.

	EUS Controls n = 13	HRM Controls n = 30	Esophagitis n = 20	p value (ANOVA)
Age (years ± SEM)	39.9 □□□□□	32.7 ± 2.4	45.5 ± 3.3	0.01*
Males/Females (counts)	10/3	15/15	7/13	
BMI (Kg/m^2)	25.7 ± 1.5	26.2 ± 0.9	27.6 ± 0.8	0.44

* HRM Controls v Esophagitis only; BMI – body mass index.

Table 2. Motility parameters of the lower esophageal sphincter (LES) and distal esophageal body of the controls and esophagitis volunteers.

Parameters	Controls N = 30	Esophagitis N = 20	p value *
Expiratory LES pressure (mmHg)	20.8 ± 1.9	15 ± 1.9	0.045
Ins/expiratory LES average pressure (mmHg)	30.5 ± 2.0	24.5 ± 2.3	0.060
IRP (mmHg)	11.6 ± 1.0	8.2 ± 0.8	0.018
DCI (mmHg x cm x s)	3277 ± 994.6	1588 ± 242.3	0.179

Mean ± SEM; * 2-tail, unpaired t test, controls vs esophagitis; IRP - integrated relaxation pressure, DCI - the distal contractile integral.

Table 3. The EGJ motility deficit in esophagitis patients is unveiled by respiratory maneuvers under increasing inspiratory loads.

Parameter	Group	Inspiratory load		
		TH12	TH24	TH48
EGJ-CI (mmHg × cm)	Controls	166.9 ± 12.7	172.2 ± 12.2	168.4 ± 13.8
	Esophagitis	132.8 ± 10.3#	135.1 ± 11.4##	114.8 ± 9.6**
EGJ Total Activity (mmHg × cm)	Controls	1270 ± 70.6	1338 ± 80.8	1280 ± 72.5
	Esophagitis	1282 ± 110.4	1241 ± 91.6	1065 ± 73.2*

Mean ± SEM; 2-tail, unpaired t test between controls (n = 30) and esophagitis (n = 20); # p = 0.06, ## p = 0.041; * p = 0.049; ** p = 0.006; EGJ – esophagogastric junction, SAM – sinus arrhythmia maneuver, TH – threshold maneuver under 12, 24, or 48 cmH_2O inspiratory loads.

Table 4. Comparison of crural diaphragm (CD) thickness and high-resolution manometry parameters between GERD patients with grade A or B esophagitis.

Parameters		Esophagitis Grade A N = 7	Esophagitis Grade B N = 13	p value *
CD Thickness (mm)		3.23 ± 0.51	3.99 ± 0.29	0.226
P exp		18.5 ± 4.1	13.0 ± 1.9	0.180
P mean		28.6 ± 4.9	22.2 ± 2.4	0.203
IRP		8.7 ± 1.5	7.9 ± 1.1	0.679
DCI		2262.4 ± 468.0	1224.7 ± 229.5	0.037
EGJ-CI	SAM	58.2 ± 10.8	61.2 ± 11.6	0.867
	12	129.5 ± 19.5	134.5 ± 12.5	0.822
	24	131.9 ± 27.1	136.8 ± 10.9	0.844
	48	109.9 ± 19.4	117.5 ± 11.0	0.714
EGJ Activity	12	1290.2 ± 148.4	1277.8 ± 154.2	0.959
	24	1201.3 ± 137.4	1262.0 ± 123.5	0.761
	48	1008.5 ± 116.2	1095.3 ± 95.8	0.585

Mean ± SEM; * 2-tail, unpaired t test, grade A vs grade B; P exp – LES expiratory pressure, P mean – LES ins/expiratory average pressure, IRP – integrated relaxation pressure, DCI – distal contractility integral, EGJ-CI - EGJ contractility index, SAM – sinus arrhythmia maneuver (without inspiratory load); 12, 24, and 48 - inspiratory loads (cmH_2O).

Table 5. Comparison of the crural diaphragm (CD) thickness and high-resolution manometry parameters between GERD patients without or with a hiatal hernia (HH).

Parameters		Without HH N = 11	With HH N = 9	p value *
CD Thickness (mm)		3.56 ± 0.40	3.92 ± 0.35	0.503
P exp		17.6 ± 2.9	11.7 ± 2.1	0.128
P mean		27.6 ± 3.4	20.6 ± 2.8	0.142
IRP		9.1 ± 1.3	7.0 ± 1.0	0.237
DCI		1799.2 ± 363.8	1329.6 ± 304.2	0.349
EGJ-CI	SAM	63.0 ± 10.5	56.6 ± 13.7	0.710
	12	124.2 ± 15.1	143.3 ± 13.6	0.370
	24	130.9 ± 19.0	140.1 ± 11.4	0.699
	48	115.5 ± 15.6	114.1 ± 10.5	0.945
EGJ Activity	12	1136.7 ± 111.6	1459.8 ± 195.3	0.150
	24	1126.6 ± 90.5	1380.3 ± 165.4	0.175
	48	988.4 ± 84.2	1158.4 ± 124.3	0.258

Mean ± SEM; * 2-tail, unpaired t test, without HH vs with HH; P exp – LES expiratory pressure, P mean – LES ins/expiratory average pressure, IRP – integrated relaxation pressure, DCI – distal contractility integral, EGJ-CI - EGJ contractility index, SAM – sinus arrhythmia maneuver (without inspiratory load); 12, 24, and 48 - inspiratory loads (cmH_2O).