TITLE:

Vigor of peristalsis during multiple rapid swallows is inversely correlated with acid exposure time in patients with NERD.

RUNNING TITLE: Correlation between multiple rapid swallow and acid exposure time

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All authors approved the final version of the manuscript.

ABSTRACT (250)

Background and aim: Multiple rapid swallowing (MRS) during high-resolution manometry (HRM) is increasingly utilized as provocative test to assess esophageal peristaltic reserve. The aim of the present study was to evaluate the correlation between MRS response and impedance and pH (MII-pH) parameters in endoscopy negative heartburn (ENH) patients.

Methods: We enrolled consecutive ENH patients, who underwent HRM and MII-pH study, with a selected MII-pH profile: abnormal MII-pH (pH+/MII+); normal MII-pH (pH-/MII-). HRM was performed with 10 wet swallows (WS) and one MRS. Mean distal contractile integral (DCI) during WS and MRS were calculated. MII-pH parameters including acid exposure time (AET), reflux events, baseline impedance levels (BI) and the efficacy of chemical clearance evaluated with the post-reflux swallow-induced peristaltic wave (PSPW) index were measured.

Results: We analyzed 103 patients: 49 MII+/pH+ (27 male), and 54 MII-/pH- (19 male). Mean age was similar between the two groups. As expected, mean AET and number of refluxes were higher in pH+/MII+ ($p<0.05$). HRM was normal in all selected patients. Mean DCI-WS was similar between two groups (p=n.s.). Mean DCI-MRS- was higher in MII-/pH- vs. MII+/pH+ ($p<0.05$). The increase of DCI-MRS was inversely correlated with AET (-0.699; $p<0.001$) and directly correlated with BI values (0.631; $p<0.001$) and PSPW index (0.626; $p<0.001$).

Conclusion: Following MRS, patients with abnormal impedance-pH test showed suboptimal contraction response as compared with those with normal impedance-pH test. Moreover, MRS response was inversely correlated with AET and directly correlated with BI values and PSPW index.

Keywords: High resolution manometry, Multiple rapid swallow, Gastroesophageal reflux disease, Acid exposure time

Key Messages:

- We evaluated two different groups of endoscopy negative heartburn patients, classified with impedance and pH test in: pH+/MII+ and pH-/MII- They underwent high resolution manometry with low volume multiple rapid swallow (MRS).

  The main results of our study were the following:
- post-MRS contractile DCI and MRS/WS ratio were lower in MII+/pH+ patients as compared with MII-/pH- (considered as control group);
- esophageal motor response after DCI-MRS and MRS/WS ratio were inversely correlated with AET;
- DCI-MRS and MRS/WS ratio were directly correlated with BI values and PSPW index.

INTRODUCTION

Gastro-esophageal reflux disease (GERD) is present when the passage of gastric contents into the esophagus leads to troublesome symptoms and/or complications (1). Endoscopy may reveal erosive esophagitis or Barrett's columnar lined esophagus; however, most of the patients have no evidence of esophageal injury (non-erosive reflux disease; NERD)(2).

The pathogenesis of GERD is multifactorial in nature, mainly involving transient lower esophageal sphincter (LES) relaxations (TLESRs), as well as LES pressure abnormalities (i.e., hypotensive LES),(3) impairment of the esophagogastric junction (EGJ) (i.e., hiatal hernia), ineffective esophageal acid and bolus clearance, and impaired mucosal defensive factors (3-6).

The recent introduction in clinical and research practice of high-resolution manometry (HRM) and impedance-manometry has represented a major advance in defining and characterizing esophageal motor abnormalities in GERD patients (7-9). Several studies have shown that esophageal dysmotility prevalence parallels the increasing severity of GERD presentation (10-12), and of particular relevance those patients had failed and hypotensive peristaltic contractions, which result in incomplete esophageal emptying (11, 13). Moreover, intermittent and non-specific alterations of esophageal motility are frequently encountered in patients with GERD. However, the true impact and frequency of these abnormalities are not clear, even because standard manometric protocols based on single wet swallows are affected by intrinsic limitations, considering that active esophageal contractions may not be necessary to allow liquid transport, especially if it happens in the upright-seated position (14). On that ground, recent studies highlighted the importance of including provocative tests, aimed at increasing esophageal workload, during HRM studies, in order to enhance the description and interpretation of esophageal motility (15, 16).

Multiple rapid swallows (MRS) that consists in the administration of five swallows in rapid sequence, represents the simplest provocative maneuver. Indeed, when multiple swallows are rapidly administered, esophageal peristalsis is deeply inhibited, and pronounced LES relaxation ensues. After the last swallow of the series, a robust esophageal contraction is expected (17).

Abnormal responses consist of incomplete inhibition (when contraction fragments are seen during the period of expected inhibition), or suboptimal contraction (when the post-MRS sequence fails to demonstrate augmentation of smooth muscle contraction) (17, 18). In particular, Shaker et al.(19) showed that the strength of smooth muscle contraction augments almost twofold with MRS in normal controls and that lack of augmentation of contraction is significantly more prevalent in GERD patients who develop late postoperative dysphagia (18, 19). Therefore, this alteration is considered to represent an inadequate peristaltic reserve of the esophageal smooth muscle (18).

The aim of the present study was to evaluate the correlation between MRS response and esophageal impedance and pH parameters in patients with heartburn and negative endoscopy.

MATERIALS AND METHODS

Patients

Between September 2014 and March 2015, we prospectively enrolled 103 consecutive endoscopic negative heartburn (ENH) patients with a selected MII-pH profile (detailed below), referred to the outpatients motility laboratories at the University of Pisa, Padua, Bern and Genoa. The inclusion criteria were: age higher than 18 years; complaining of heartburn with/without regurgitation, at least three times in a week, for 6 months in the previous year and normal esophageal peristalsis. The exclusion criteria were: pregnancy (excluded by urine analysis) and/or breast feeding; eating disorders; history of thoracic, esophageal or gastric surgery; neoplasia; achalasia, outflow obstruction, scleroderma, major and minor disorders of peristalsis (20); hiatal hernia larger than 3 cm (defined as separation larger than 3 cm between the two high pressure zones in the distal esophagus: EGJ and crural diaphragm); underlying psychiatric illness or psychiatric therapies; use of non-steroidal anti-inflammatory drugs and aspirin; peptic ulcer or erosive esophagitis at a previous endoscopy. All patients signed the informed consent. The study was designed and carried out in accordance with the Helsinki Declaration (Sixth revision, Seoul 2008) was approved by the local Institutional Review Boards.

The presence of erosive esophagitis and other mucosal abnormalities were excluded by means of an upper endoscopy performed off-therapy (proton pump inhibitors, PPIs or H2-receptor antagonists were discontinued at least 20 days prior to endoscopy) in each of the above mentioned centers, within 6 months prior to the initial visit. A distinct investigator completed a detailed interview to the patient, including a careful medical history (with recording of height and weight), current medications, tobacco and alcohol consumption. All patients completed a detailed questionnaire for GERD diagnosis (GERDQ) (21). The response to PPI therapy was assessed with a visual analogue scale (VAS) and recorded as positive if symptom relief was higher than 50% (22).

Then, all the subjects underwent solid state HRM and 24-h multichannel intraluminal impedance and pH monitoring (MII-pH) off-therapy (14-day wash-out). Patients were only allowed to take alginates, on as-needed basis, as rescue therapy for controlling heartburn (23). The HRM and MII-pH were performed after an overnight fast.

High resolution manometry and provocative test

All the patients underwent esophageal HRM using a 36-sensor solid state system (Sierra Scientific/Given Imaging, Los Angeles, CA, USA) capable of generating spatiotemporal Clouse plots of esophageal peristalsis.(24, 25)

The basal EGJ pressure and peristaltic performance were assessed. The distal contractile integral (DCI), distal latency (DL) and the EGJ relaxation with integrated relaxation pressure (IRP) were calculated for each swallow (26-28). All manometric findings, including EGJ morphology, were classified according to the current Chicago Classification (Version 3.0) (20).

The MRS was performed for each patient, after the standard HRM protocol, through administration of 4 ml of waters rapidly swallowed for 5 consecutive times, in less than 10 s. MRS-induced esophageal body inhibition was considered incomplete if contractions, measuring >3 cm, were identified using the 30 mmHg isobaric contour tool (29). Further analysis of the esophageal body contraction response was performed using the DCI metric. Averaged DCI was first recorded for the

single wet swallows (WS) in all peristaltic patterns. Moreover, DCI was determined for each MRS contractile rebound sequence and compared to the averaged DCI-WS.(19) The number of failed post-MRS contractions (DCI <100 mmHg*s*cm), and of MRS with lack of inhibition of esophageal body were recorded.

Esophageal multichannel intraluminal impedance and pH monitoring

MII-pH was performed using a polyvinyl catheter (diameter: 2.3 mm), equipped with an antimony pH electrode (Sandhill Scientific Inc. Highland Ranch, CO). During 24-h MII-pH test all patients consumed foods and beverages exclusively during three standard meals (lunch at 1.00 p.m., dinner at 8.00 p.m. and breakfast at 8.00 a.m. of the next day) on the basis of a Mediterranean diet,(30) without alcohol and coffee, to reduce the variability due to alimentary habits. All patients received detailed instruction for the recording period as described in a previous paper.(31)

At the end of the recording period, data were edited with a dedicated software program (Bioview Analysis, Sandhill Scientific, Highlands Ranch, CO; USA) and analyzed using Microsoft Excel 2000 (Microsoft Inc., CA, USA).

MII-pH data analysis

MII-pH tracings were centrally collected and reviewed manually by two investigators (NdB, ES) in order to ensure accurate detection and classification of reflux episodes. Meal periods were excluded from the analysis. Impedance and pH data were used to determine the number and type of reflux episodes as well as acid exposure time (AET, reflux per cent time) in each patient. Then, a per cent time lower than 4.2% with pH <4, over 24-h, was considered as normal.(30, 32) Acid, weakly acidic and weakly alkaline refluxes were defined according to the literature (33) and the number of total refluxes was calculated (normal value <54) (34, 35). Proximal reflux extents were calculated.

For the purpose of this study, we further we selected patients with a MII-pH specific profile as follows: patients with abnormal AET and number of reflux events (MII+/pH+)and those with

normal AET and number of reflux events (MII-/pH -). Patients with either normal AET or normal number of reflux events were excluded from the analysis.

Further, we evaluated two additional impedance parameters whose role was recently emphasized in terms of diagnostic utility: the post-reflux swallow-induced peristaltic wave (PSPW) index,(36) and baseline impedance (BI) values.(35, 37) The PSPW index, previously described by Frazzoni and co-workers,(36) has been manually calculated and it is defined as the number of refluxes followed within 30-s by a swallow-induced peristaltic wave divided by the number of total refluxes. This MII-pH parameter shows the efficacy of chemical clearance and was strongly correlated with the presence of esophageal mucosal damage. BI values (Ohms) were assessed from the same channel (5 cm above the LES) during the overnight rest, at three time points (around 1.00 am, 2.00 am, and 3.00 am). In particular, we selected 10 minutes around each time point avoiding swallows, refluxes and pH drops. Indeed, as previously described, short nocturnal time measurements of BI are reliably representative of long period measurements (35, 37).

The DCI values during single and WS after MRS and the MRS/WS ratio were correlated with above-mentioned MII-pH parameters (AET, number of refluxes, BI levels, and PSPW index) in both groups.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess normality of data. The logarithmic transformation was applied to skewed variables to approximate a normal distribution. Statistical tests used to compare groups of patients included Student t-test for difference in mean values, and Kruskal-Wallis test for skewed variables. Spearman (ρ) correlation coefficient was employed for Gaussian and skewed variables, respectively to detect a correlation between MRS (DCI) and MRS/WS ratio with AET, BI and PSPW index. A p-value less than 0.05 was considered statistically significant. Data are presented as mean ± standard deviation (sd). Analyses were performed using SPSS (version 21, IBM Corp, Armonk, NY)

RESULTS

Demographic and clinical characteristics

One-hundred and three ENH patients (46 M/57F), with a mean age of 46.8±13.8 years, underwent MII-pH testing and were further subdivided into two distinct groups as follows: 49 MII+/pH+ (27M and 22F, mean age 47.7±14.1 yrs); and 54 MII-/pH- (19M and 35F, mean age 44.6±13.6 yrs). Female were significantly more frequent in MII-/pH- group (P=0.0493). No statistically significant differences in terms of age, body mass index (BMI) and discretionary behaviors in the two different subgroups were found. GERDQ was similar in both group (9.2±3.1 in MII+/pH+ and 9.6±3 in MII-/pH-; p=0.54). The prevalence of HRM-detected hiatal hernia was greater in pH+/MII+ group (see **Table 1**). As expected, we observed a different response to PPI treatment and symptom relief: 78.7±9.4% in MII+/pH+, and 24.7±7.1% in MII-/pH-.

HRM and Impedance-pH Data

Both HRM, with provocative test, and 24-h MII-pH have been completed in all patients without any complications. There were no differences in mean basal and respiratory EGJ pressure, in mean DCI, IRP and DL between the two subgroups. Detailed results have been reported in **Table 1**.

As expected, AET (8.1±3.9 versus 1.7±1.1), number of reflux events (73.6±30.7 versus 32.7±10.3), proximal extension (30.9±16 versus 11.1±4.4), and number of acid refluxes (52.5±29.3 versus 18.7±6.8) were higher in MII+/pH+ than in MII-/pH- patients ($p<0.05$). On the contrary, PSPW index (30.9±6.6 versus 74.2±6.6) and BI levels (1021.3±279.3 versus 3768.9±717.2) were lower in MII+/pH+ subgroup ($p<0.05$)

Multiple Rapid Swallowing Data

After MRS, only seven patients reported a failed esophageal contraction (all from MII+/pH+ subgroup). No patient had alterations in the esophageal body inhibition during MRS.

DCI-MRS was higher in MII-/pH- patients as compared with MII+/pH+ (2133.7±1217.8 *vs* 1386.2±1120.6; p=0.0017). Similarly, MRS/WS ratio was higher in MII-/pH- (1.6±0.8 *vs* 0.8±0.5; p=0.0001). In **Figure 1** it is reported an example of normal WS and MRS from both group.

Moreover, we found that DCI-MRS was inversely correlated with total number of refluxes and AET (p<0.0001), and directly correlated with BI values and PSPW index (p<0.0001). These same correlations have been confirmed and resulted enforced when considering MRS/WS ratio and AET (inverse correlation, p<0.0001), as well as MRS/WS ratio with BI and PSPW index (direct correlation, p<0.0001). All results have been detailed in **Table 2**.

DISCUSSION

This study was designed to evaluate the esophageal response to the low volume MRS provocative test in ENH patients, and to correlate such MRS response with relevant MII-pH parameters (AET, number of reflux episodes, BI, and PSPW index). The main results of this study were the following: (i) post-MRS contractile DCI and MRS/WS ratio were lower in MII+/pH+ patients as compared with MII-/pH- (considered as control group); (ii) an inverse correlation between esophageal motor response after DCI-MRS and MRS/WS ratio with AET was found; (iii) and a direct correlation between DCI-MRS and MRS/WS ratio with BI values and PSPW index were observed.

During HRM study, the use of provocative tests, such as MRS, have been shown to better detect esophageal motor abnormalities that are not recognized during the standard protocol with 10 WS.(38, 39). Indeed, we found that MII+/pH+ patients present more frequently abnormalities at MRS compared to patients with MII-/pH-. In particular, we observed that DCI-MRS and MRS/WS ratio were lower in patients with impedance-pH evidence of GERD that in those without evidence of GERD. Recently, other studies took into account the use of high volume provocative test (multiple water swallow, with 200 ml of water) during HRM, showing an increase into the diagnostic yield of esophageal motor dysfunction in GERD patients. (40, 41) In our study we used a low volume liquid provocative test (MRS) to investigate the adequate esophageal inhibition during

the repetitive swallows and the presence of strong esophageal contraction after the last swallow. We adopted the MRS test because it is cheap and easy to execute and to interpret, allowing a widespread utilization as a complementary test during HRM.(17) Overall, we consider the current MRS test easier to standardize than approaches with high volume that patients cannot swallow the same way and most will either stop or fail to finish. Moreover, in a recent study performed in healthy controls and symptomatic patients undergoing HRM, Price et al. (29) stated that MRS responses are highly reproducible when repeated. In addition, recent evidences suggest that this test may have strong clinical implications.(18, 19) Finally, a study performed by means of magnetic resonance showed that reflux volume is higher than 5-10 ml of water administered during a standard HRM protocol study.(42) For this reason, the esophageal contraction after a multiple rapid sequence of swallows may better represent the motor contraction elicited after a gastroesophageal reflux event.

In the present study, we analyzed the efficacy of MRS excitatory phase by means of DCI that represents the more realistic measurement of the contraction segment of the smooth muscle of the esophagus. In line with previous data by Shaker et al,(19) who reported that the DCI of MRS was augmented almost twofold in normal controls, our data showed that the vigor of contraction doubled after MRS as compared with WS only in MII-/pH- patients (considered as control group). On the other hand, the MRS/WS ratio was slightly reduced in MII+/pH+ patients. Thus, we can speculate that MII-/pH- patients showed an adequate neural excitatory input/muscle response as healthy volunteers. On the contrary, MII+/pH+ patients showed some defects/inability of the smooth muscle to generate an appropriate response, therefore showing a suboptimal peristaltic reserve.

It is worth noting that all patients had complete inhibition of esophageal body motility during MRS. Similarly, Stoikes et al.(18) observed that the inhibition phase during MRS remained intact both in the esophageal body and in the LES in patients with suboptimal DCI and late postoperative dysphagia, suggesting that the contractile defect is not within the inhibitory pathways.

Data from the present study showed that MRS DCI and MRS/WS ratio were inversely correlated with AET, but directly correlated with BI values and PSPW index. BI values and the PSPW index have been recently found strongly implicated in reflux disease development.(43) In particular, low BI values were related to an impaired esophageal mucosal integrity (43-45) and an increased sensibility to acid stimulations.(35, 37, 46, 47) Instead, the PSPW index, as a measure of impaired chemical clearance, has been shown a primary pathophysiologic mechanism in GERD, as this parameter was not affected by medical or surgical therapy(36). Of note, both the PSPW index and the BI values progressively decrease in parallel with increasing severity of GERD presentation, from healthy controls to hypersensitive patients with normal AET, to NERD and erosive GERD(43). Current results support the hypothesis that an abnormal acid exposure could affect the muscle esophageal contraction, and *vice versa*. In line with these assumptions Rieder et al.(48) showed that the human esophagus produces cytokines (IL-6 and IL-1β) capable of reducing contractility of esophageal muscle cells when exposed to gastric juice. The use of MRS might represent a, easy-to-perform tool to recognize those patients with suboptimal esophageal muscle reserve and reduced clearance efficacy, despite of normal standard HRM protocol study

There are some limitations to mention. First, the lack of a control group with healthy volunteers. However, we selected as controls, a group of consecutive patients without evidence of motility disorder at HRM and with normal impedance-pH findings. Second, we adopted a single MRS as part of our provocative test, although it is unclear which is the good and more reliable number of MRS to adopt in clinical practice.

In conclusion, we foster the inclusion of MRS provocative test in routine HRM studies. Indeed, it is simple, cheap and easy to perform, and, above all, assessing the response to such a provocative test may increase the ability of HRM studies to detect clinically relevant esophageal dysfunction in patients with abnormal esophageal AET. In particular, this “low volume challenge test” could be an easier method to recognize, among ENH patients, those with abnormal AET who may present deficit of peristalsis and thus impairment of esophageal clearance.

We believe that characterizing esophageal subtle motor alterations may represent a further fundamental approach to improve the notions of the pathophysiological mechanisms underlying GERD and, thus, in future, to set up the best therapeutic management for these patients.

Acknowledgments: None.

Funding source: The study was conducted without any financial support.

Conflict of interest disclosure: The authors have no competing interests.

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TABLE PAGES

Table 1: results obtained from high resolutiopn manometry in pH+/MII+ and pH-/MII- patients

	pH+/MII+ (49)	**pH-/MII- (54)**	**p**
Mean EGJ basal pressure (sd)	17.3(±12.9)	19.5 (±13.4)	0.398
Mean EGJ maximal pressure (sd)	31.7 (±15.4)	30.9(±12.8)	0.776
EGJ morphology:			
Type I	16	30	
Type II	17	18	0.013
Type III (<3cm and >2cm)	16	6	
Mean IRP (sd)	6.8 (±4.6)	6.1 (±3.8)	0.404
Mean DL (sd)	6.9 (±1.1)	6.7 (±0.9)	0.317
Mean DCI WS (sd)	1522.8(±1175.4)	1536.2(±1057.7)	0.951

Legend: EGJ: esophago-gastric junction (mmHg); IRP: integrated relaxation pressure (mmHg); DL: distal latency (sec); DCI: distal contractile integral (mmHg*cm*s); WS: wet swallow;

Table 2: Spearman correlation between MRS (DCI) and MRS/WS ratio with AET, BI and PSPW index

		AET total	**BI values**	**PSPW index**
MRS (DCI)	Correlation coefficient (ρ)	-0.699	0.631	0.626
	P	**0.0001**	**0.0001**	**0.0001**
MRS/WS ratio	Correlation coefficient (ρ)	-0.733	0.697	0.672
	P	**0.0001**	**0.0001**	**0.0001**

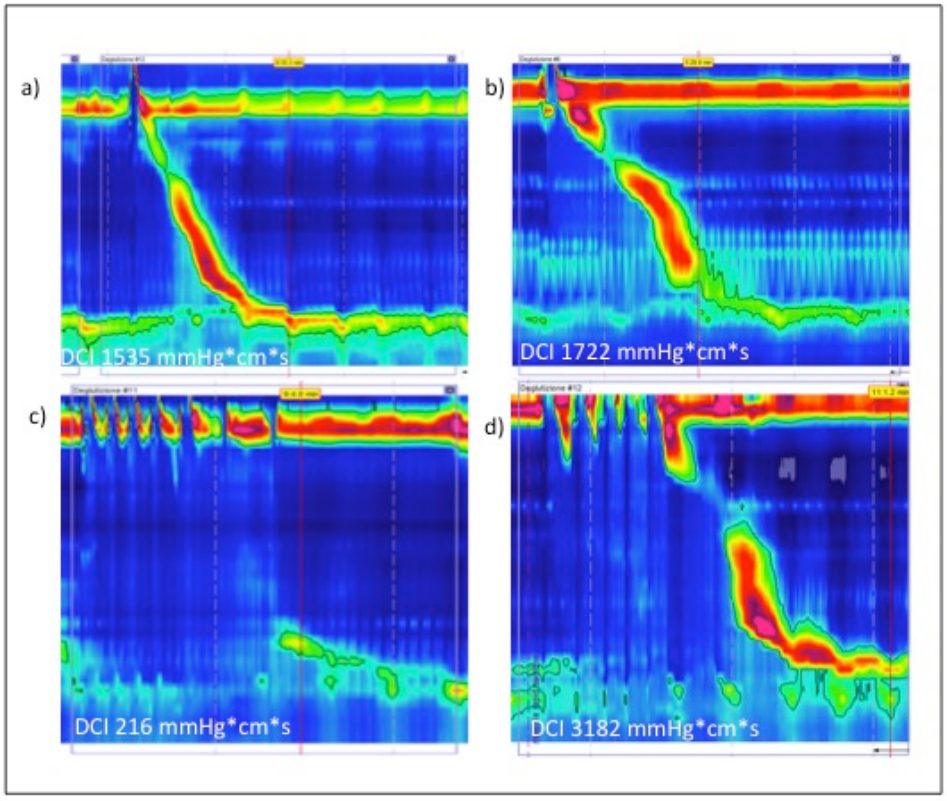
Legend: AET: acid exposure time (%); BI: baseline impedance values (Ω); PSPW: post-reflux swallow-induced peristaltic wave (%)

FIGURE PAGE

FIGURE 1: single example of normal WS and MRS from both group.

Figure Legend: a) wet swallow in patients with pH+/MII+ group (DCI: 1535 mmHg*cm*s); b) wet swallow in patient with pH-/MII- group (DCI 1722 mmHg*cm*s); c) A failed Multiple Rapid Swallow in patients with pH+/MII+ group (DCI: 216 mmHg*cm*s); d) A normal Multiple Rapid Swallow in patients with pH-/MII- group (DCI: 3182 mmHg*cm*s)

FIGURE 1


a)
DCI 1535 mmHg*cm*s
b)
DCI 1722 mmHg*cm*s
c)
DCI 216 mmHg*cm*s
d)
DCI 3182 mmHg*cm*s