RISK FACTORS IN THE PATHOGENESIS AND TREATMENT OF GASTROESOPHAGEAL REFLUX DISEASE

PhD thesis

István Pregun
Clinical Medicine Doctoral School
Semmelweis University

Consultant: László Herszényi DSc.

Official reviewers:
Ilma Korponay-Szabó DSc.
Antal Dezsőfi PhD.

Head of the Final Examination Committee:
Ferenc Szalay DSc.

Members of the Final Examination Committee:
György Székely PhD.
Margit Abonyi PhD.

Budapest
2015

INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the most common disorders in the general population. In recent years, a marked increase in the occurrence of the disease worldwide has been noted. The approximate prevalence of GERD is 10–20% in Western populations. The possible underlying pathophysiology of gastroesophageal reflux is complex and is not completely understood. In the recent years several risk factors were examined which can be involved in the pathogenesis of GERD (genetic, anatomic and environmental factors, obesity, dietary factors, smoking and alcohol consumption, medicines or physical activity).

GERD is commonly diagnosed based on typical symptoms (heartburn and regurgitation). However, a range of other symptoms can be associated with GERD, including noncardiac chest pain and dysphagia. Furthermore, a variety of pulmonary and laryngopharyngeal signs and symptoms can be associated with GERD, including asthma, chronic cough and hoarseness, as known from its extraesophageal manifestations. Pathological findings include mucosal breaks (erosive esophagitis), Barrett's esophagus, peptic esophageal strictures or esophageal adenocarcinoma.

The symptoms of GERD can have a profound impact on patients' quality of life. As a consequence, sleep disturbances, problems with eating and drinking, increased general worry or even impaired social activity and work productivity may occur. GERD symptoms were found

to have an impact on quality of life that is similar to that of other chronic diseases.

Recently a specific, occupation-related susceptibility of professional singers and opera choristers to experience GERD has been hypothesized. On the other hand, it has also been suggested that intense exercise in healthy people can induce esophageal reflux or can exacerbate symptoms of GERD. Episodes of reflux seem to be associated with the length and intensity of the physical activity undertaken. Transient increase in intra-abdominal pressure that overcomes the resistance of antireflux barrier can be a potential factor contributing to GERD symptoms. The prevalence of reflux symptoms has not been evaluated yet in professional wind players, glassblowers or water polo players from this aspect.

The profound, effective gastric acid inhibition with proton pump inhibitors (PPIs) is the mainstay of GERD therapy. Gastrin, released from antral G cells, is a main regulator of gastric acid secretion; furthermore, it has not only stimulatory but also trophic effect on gastric mucosa, especially on neuroendocrine cells, the enterochromaffin-like cells (ECL) of the stomach. Potent acid-suppressive treatment with PPIs, particularly for longer period, may increase serum gastrin levels due to reduced gastric acidity. Chromogranin A (CgA), member of the granin family is widely used as a sensitive marker in the diagnosis of neuroendocrine tumors (NETs). In patients with atrophic gastritis, high gastrin and CgA levels were observed. A similar condition occurs in patients treated with acid-suppressive drugs: some clinical data suggested

that effective antisecretory therapy significantly increases serum gastrin and CgA levels, which can reflect ECL cell hyperfunction or proliferative changes.

The majority of data derive from medium- or longterm treatment, little is known about the shortterm treatment and no data are available about the ultrashort-term (few days) period. The effect of cessation of PPI therapy has been scarcely evaluated.

There are data demonstrating that in *Helicobacter pylori (H.pylori)* positive patients, both gastrin and CgA levels are higher than in *H. pylori* -negative subjects.

Risk factors in the pathogenesis and treatment of GERD were evaluated in our studies.

OBJECTIVES

It has been proposed that professional singers and opera choristers regularly activate the diaphragm when there is a need for a rapid decrease in subglottal pressure, causing an abrupt and prolonged increase in intra-abdominal pressure and straining which can lead to reflux or worsening of GERD symptoms. It has also been suggested that intense physical activity can induce gastroesophageal reflux or can exacerbate GERD symptoms.

Consequently, based on this hypothesis, susceptibility to gastroesophageal reflux in special groups was examined, where straining

and prolonged increase in intra-abdominal pressure occur frequently. The study was designed to evaluate:

- the prevalence of reflux symptoms (heartburn, regurgitation, hoarseness, epigastric pain, chest pain, cough, nausea, vomiting) in a series of professional opera choristers wind players, glassblowers and water polo players in comparison with a sample of general population (controls)
- to determine any correlation between reflux symptoms and the cumulative lifetime duration of professional singing, playing and/or working activity.

To evaluate the effect of potent gastric acid inhibition on neuroendocrine cells, the effect of diverse type and dose of PPI therapy on serum CgA and gastrin levels was analyzed. On the other hand, the effect of PPI treatment cessation was also examined. This study was designed to evaluate:

- the basal CgA and gastrin levels in both *H. pylori*-positive and negative patients
- the trends of changes of serum CgA and gastrin levels in patients with newly diagnosed GERD after starting PPI therapy
- the effect of different PPI dose regimen
- does CgA changes reflect the efficacy of PPI test?
- the CgA and gastrin levels in patients who were at least 6 months on PPI therapy
- the effect of cessation of PPI treatment on CgA and gastrin levels

METHODS

Participants

To evaluate some risk factors of GERD, a total of 485 subjects were involved. Of the 216 opera choristers 14 (6.5%) refused to participate in the study. The refusal rates were similar in the group of wind players, glassblowers and water polo players. Thus, a total of 202 opera choristers, 71 wind players, 43 glassblowers and 54 water polo players were recruited.

A total of 115 subjects were recruited and formed the control group (medical students: n = 45; teachers: n = 19; lawyers: n = 37; engineers: n = 14).

Overall, the prevalence of overweight and obesity was significantly higher among professional wind players and glassblowers in comparison with controls. In addition, the proportion of subjects who reported consuming dinner late in the evening was significantly higher among opera choristers and wind players. The prevalence of never smokers was significantly higher among opera choristers and significantly lower in glassblowers in comparison with the control sample. The consumption of alcoholic beverages was similar between groups. With exception of water polo players, the physical activity in leisure time was also similar between groups.

The effect of PPI therapy on serum CgA and gastrin levels in diverse groups of patients were analyzed. Patients with dyspeptic symptoms referred to our endoscopic unit were enrolled into *Group Hp* (n

= 39, 22 females, 17 males; 17 *H. pylori*-positive and 22 *H. pylori*-negative). Patients under 18 years of age, with malignancy or pathologic conditions associated with elevated serum gastrin and CgA levels or those previously treated with acid suppressive drugs were not enrolled.

Group A (n=54) included patients with clinically and endoscopically newly diagnosed GERD, where PPI therapy was indicated. In *group B* (n=42) H. pylori-negative patients treated continuously with PPIs for at least for 6 months were involved. A subgroup of these patients (g*roup B/1*, n=11) with GERD, where PPI treatment could be stopped due to symptomless or endoscopic healing was further analyzed. All subjects gave informed consent prior to inclusion. The study was approved by the local Ethical Committee of Semmelweis University.

Questionnaire

In the study evaluating some GERD-risk factors, an anonymous questionnaire was delivered to each participant. The questionnaire contained specific questions regarding the presence of reflux symptoms and general questions about individual characteristics: including gender, age, weight, height, lifestyle habits (alcohol and tobacco consumption), eating habits (consume dinner late in the evening), and physical activity in leisure time were also collected. The frequency of reflux symptoms was evaluated on a scale (1-6). The study participants were also asked to classify the intensity of their symptoms. Opera choristers and wind players were requested to record the number of years of singing or

playing activity, the number of hours per week of singing/playing exercises. Glassblowers and water polo players were also requested to record the number of years of working activity, and the number of hours per week of their exercises.

Study design

In the study evaluating the effect of GERD-treatment, blood samples were collected from resting patients from a cubital vein after 10 h fasting for CgA and gastrin determination *(group Hp)*. In *group A*, blood samples were collected for CgA and gastrin determination before starting PPI therapy (day 0). Diverse PPIs and different doses were administered according to the severity of GERD; thus, *group A* was subdivided into the following subgroups: low-dose PPI (esomeprazole 20 mg o.d. n = 11), standard-dose PPI (lansoprazole 30 mg o.d. n = 5, pantoprazole 40 mg o.d. n = 5, rabeprazole 20 mg o.d. n = 5, esomeprazole 40 mg o.d. n = 6), and high-dose PPI (esomeprazole 40 mg b.d. n = 14, pantoprazole 40 mg b.d. n = 5, rabeprazole 20 mg b.d. n = 3) subgroups. Further blood samples were taken for CgA and gastrin determination after 5 days (day 5), 10 days (day 10) and 28 days (day 28) of initiation of PPI treatment.

Patients involved in *group B* were treated continuously with a standard dose of PPI for at least 6 months (rabeprazole 20 mg o.d. n = 6, pantoprazole 40 mg o.d. n = 16, lansoprazole 30 mg o.d. n = 3, esomeprazole 40 mg o.d. n = 17). Blood samples for CgA and gastrin

determination were taken from all patients ('on-therapy') as described in *group A*. Thereafter, in a subgroup of patients (*group B/1*, n = 11), where PPI treatment could be stopped, blood sampling was repeated with a mean of 5 days (range 3–8) after cessation of PPI therapy ('off-therapy').

Laboratory analysis

CGA-RIA CT was used to measure serum CgA levels (CIS Bio International, Gif-sur-Yvette, France). 125 I anti-CgA monoclonal antibody was used as a tracer, and recombinant human CgA as standard (normal: 19.4–98.1 ng/ml). Gastrin was determined by a competitive radioimmunoassay method (BioSource Europe SA, Belgium), using a rabbit antiserum raised against a gastrin 17 albumin conjugate (normal 11–54 pmol/l). Samples were frozen after sampling till the analysis.

Statistics

The distribution of a series of individual characteristics among professional wind players, professional opera choristers, glassblowers, water polo players and the general population (controls) sample were compared. Differences between groups were statistically tested using *Student's t-test* and *one-way ANOVA*. Continuous variables were expressed as mean ± standard deviation. The hypothesis of independence in cross-tabulated data was examined with the χ^2*statistics* or *Fisher's exact test*. Differences were considered significant with $p<0.05$.

The prevalence of each reflux symptom was calculated based on the presence of the symptom at least once in the year before survey. The crude prevalence rate ratios (PRRs) of each symptom according to the presence or absence in the year before the survey among groups were analyzed. To consider differences between two study groups in demographic and lifestyle habits characteristics, we also evaluated the PRR adjusted for age, gender, BMI, smoking habits, alcohol consumption, physical activity in leisure time, consume dinner late in the evening (expressed as PRR and 95% confidence interval, CI). The SAS PROC GENMOD software package (SAS Institute, Cary, N.C., USA) was used to perform statistical analyses.

In the study, where PPIs effect on CgA and gastrin levels were analyzed, due to the high standard deviations of some of the series, values were expressed as medians. Differences between groups were tested by using the *Kruskal-Wallis analysis of variance* (followed by *Dunn's multiple comparisons test*) and *Mann-Whitney test*. *Spearman rank correlation test* was performed to evaluate the correlation between variables. All data were analyzed by GraphPad InStat version 3.00, GraphPad Software, San Diego, Calif., USA. Significance was assumed at $p<0,05$.

RESULTS

Heartburn, regurgitation, and hoarseness were significantly more frequent in opera choristers in comparison with controls ($p<0.001$).

Heartburn and regurgitation were also different between wind players and controls ($p<0.05$ and $p<0.01$, respectively). Finally, regurgitation was significantly more frequent in glassblowers compared with controls ($p<0.01$). A trend toward higher frequency of heartburn, regurgitation, epigastric pain, and hoarse voice was reported by water polo players compared with control subjects, but the differences were not statistically significant.

Among the 202 opera choristers, 109 (53.9%) reported heartburn in the previous year, whereas among the 115 subjects in general population sample, 45 (39.1%) reported heartburn in the previous year ($p<0.001$). A total of 91 (45%) opera choristers and 36 (31.3%) control subjects recorded regurgitation in the past year ($p<0.001$). Hoarseness was also significantly more frequent in opera choristers (129, 63.8%) compared with controls (41, 35.6%; $p<0.001$). In multivariate analyses, both heartburn and regurgitation were significantly associated with the duration inyears of the singing activity ($p<0.05$) and BMI ($p<0.05$). There were no significant differences between the prevalence of reflux symptoms and the singing specialties of the opera choristers.

Among the 71 wind players, heartburn (n = 36, 50.7%) and regurgitation (n = 37, 52.1%) were significantly more frequent compared with controls ($p<0.05$ and $p< 0.01$, respectively).

In glassblowers (n = 43), regurgitation (n = 24, 55.8%) was significantly more frequent compared with controls ($p<0.01$). The frequency of heartburn, epigastric pain and hoarse voice showed a trend towards increase in glassblowers, but the differences were not statistically

significant. In multivariate analyses, reflux symptoms in both wind players (heartburn, regurgitation) and glassblowers (regurgitation) were significantly associated with the duration in years of the playing/working activity and BMI ($p < 0.05$).

No differences were evident in the prevalence of earlier described reflux symptoms in water polo players. No significant association was found between reflux symptoms and duration of physical exercises. When heartburn and regurgitation were examined according to severity characteristics, professional opera choristers reported more severe symptoms in comparison with controls. No statistically significant differences were seen with respect to wind players or glassblowers.

Evaluating the effect of GERD treatment with PPIs on serum CgA and gastrin levels, our results were as follow:

Group Hp

There was no statistically significant difference between *H. pylori*-positive and *H.pylori*-negative patients neither in CgA [48.21 ng/ml (26.5–79.2) vs. 44.3 ng/ml (30.2–95.0)] nor in gastrin levels [28 pmol/l (8.3–50) vs. 25.3 pmol/l (5–52)]. Despite the above described findings, only *H.pylori*-negative patients were enrolled in *group A*, where the effect of PPI treatment was examined.

Group A

No correlations were found between basal CgA and BMI, age or gender, neither between gastrin and all these parameters.

Serum CgA and gastrin level changes during PPI treatment

In the low-dose PPI subgroup (n = 11), a statistically significant stepwise increase was observed in median level of CgA during the treatment ($p = 0.0017$), even after 5 days of initiation ($p<0.05$). Albeit fasting gastrin levels increased during the treatment, differences were statistically not significant.

Twenty-one patients were treated with a standard dose of PPI. Again, serum CgA increased rapidly (after 5 days) and significantly after initiation of PPI therapy ($p< 0.01$). Comparison test showed significant differences between CgA levels of day 0 and day 5, day 0 and day 10 and between day 0 and day 28, respectively ($p<0.0001$). Serum gastrin levels also increased significantly during the treatment ($p = 0.0022$). There was no significant difference during the observed period neither in CgA nor in gastrin levels with respect to the type of PPI administered.

In the high-dose PPI subgroup (n=22) serum CgA increased rapidly after initiation of PPI therapy ($p< 0.001$). Comparison test showed significant differences between day 0 and day 5, day 0 and day 10, day 0 and day 28 ($p<0.0001$), respectively. Serum gastrin levels also increased significantly during the treatment, with a prominent difference between

day 0 and day 10, day 0 and day 28 ($p<0.001$). Again, there was statistically no significant difference during the observed period neither in CgA nor in gastrin levels with respect to the type of PPI administered.

Differences between groups with respect to PPI dose regimen

Serum CgA and gastrin values were also compared in terms of PPI dosage during the study period. Baseline CgA levels did not differ statistically significantly, then a stepwise increased difference was observed between the groups. There was a significant difference between CgA levels of high-dose and standard/low-dose group on day 5, high-dose and standard/low-dose group on day 10, and the difference became more pronounced on day 28. No significant differences were observed between low-dose and standard-dose groups during the 28-day period. In terms of gastrin level changes, differences among the groups after identical period of treatment were statistically not significant, nevertheless approached borderline significance on day 28 ($p = 0.06$).

Group B

Forty-two patients were included and treated with a standard dose of PPI continuously for at least 6 months. Serum CgA and gastrin levels were markedly elevated after the treatment [CgA: 222.6 (28.5–1568.6), gastrin: 80.3 (9.7–487.6), respectively]. A subgroup of patients, where PPI therapy could be stopped (n = 11 – *subgroup B/1*), was further

analyzed. After PPI treatment cessation of a mean of 5 days (range 3–8), patients were retested for CgA and gastrin (‘off-therapy’). Both CgA and gastrin levels decreased significantly after PPI discontinuation [CgA ‘on-therapy’: 215.6 (28.5–1080), ‘off-therapy’: 79.6 (35.6–240), p=0.006; gastrin ‘on-therapy’: 79.6 (9.7–236.2), ‘off-therapy’: 30.9 (8.1–101), p=0.047]. Correlation analysis showed a significant positive correlation between serum CgA and gastrin ($r = 0.6421$, $p<0.0001$).

CONCLUSIONS

Potential new risk factors of GERD and the effect of proton pump inhibitors on the neuroendocrine system were analyzed in the present work.

Based on the results of our study, we demonstrate for the first time that not only opera choristers but also professional wind players reported significantly higher frequency of heartburn and regurgitation in comparison with the sample of the general population. We hypothesize that the susceptibility to reflux in professional wind players may also be increased by the disabling of the diaphragmatic sphincter, as a result of prolonged increases in intraabdominal pressure. Our another new finding that the reported prevalence of regurgitation was significantly higher in glassblowers than in the sample of the general population can also support the above-mentioned hypothesis that an abrupt and prolonged increase in intra-abdominal pressure, deep inspiration, and straining,

which are repeated many times over many years, may increase the susceptibility to reflux.

It has been also proposed that strenuous and intense exercise can induce esophageal reflux. In our experience, the prevalence of reflux symptoms in water polo players was similar to controls, although a trend toward higher frequency of heartburn, regurgitation, epigastric pain, and hoarse voice was reported by athletes.

We also demonstrated a significant correlation of symptoms with increasing years of singing, playing or working activity. Gastroesophageal reflux in these professions should be considered as a work-related disorder that may have an impact on quality of life.

The mainstay of GERD treatment is the potent inhibition of gastric acid secretion with PPIs. In our study, not only the dynamics of serum chromogranin A and gastrin change but also its relationship with different PPI type and dose regimen were analyzed within an ultrashort-term period. We also evaluated the effect of cessation of PPI therapy, the time trend of change of serum CgA and gastrin levels. In contrast with previously published data, we did not find any significant differences between *H. pylori* -positive or *H.pylori*-negative patients neither in CgA nor in gastrin levels. Our findings can be partially explained with a supposedly shorter duration of infection not leading to serious mucosal injury.

We demonstrate for the first time that serum CgA increase quickly follows the initiation of PPI treatment (after 5 days) in patients with GERD even using a low dose.

In the standard-dose group, this significant CgA increase occurred similarly to the low-dose group, and fasting gastrin level increase was also significant. Furthermore, in the high-dose group, both gastrin and CgA increased significantly after an ultrashort-term therapy and showed a further increase during the whole period. These findings suggest that the dosage of administered PPI has a notable importance. On the other hand, no differences were observed between the diverse PPI molecules from this aspect.

Our results are consistent with previously reported data evaluating serum CgA changes during medium- and longterm PPI therapy; in addition, based on our findings, CgA increase can occur even within an ultrashort-term period. This finding can indirectly support the efficacy of the ‘PPI test’ used in GERD diagnosis.

In a series of patients treated for at least 6 months with PPIs resulted in a notably elevated CgA and gastrin level. In the subgroup where PPI could be discontinued, after 5 days of cessation of therapy both CgA and gastrin levels decreased significantly. This can indirectly reflect the safety of PPIs.

Since PPIs are used widely worldwide, and CgA is a useful marker of neuroendocrine tumors, PPI use has to be always taken into consideration when screening for neuroendocrine tumors. From the clinical point of view, based on our results a 5-day period of cessation seems to be enough to avoid false-positive results.

PUBLICATIONS

Publications related to the theme of thesis

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