INTRODUCTION

Obesity is considered a worldwide public health problem. Its incidence has been increasing in men, women and children from developed and in development countries\(^1\).\(^{17,20,26}\). Nowadays, gastrointestinal surgery is accepted as the most effective approach to reach weight loss in morbid obesity patients. Roux-en-Y gastric bypass (RYGB) is the most used surgical technique for the treatment of morbid obesity in the American continent\(^1\) .

Initially, this surgery was accomplished with the use of a ring involving the gastric pouch distal portion, with the aim of decreasing the gastric deflation period and promoting early satiation. Thousands of patients have been operated in this manner. Nowadays, the ring is not frequently used due to complications that may occur, such as ring rupture, sliding with gastric obstruction or erosion, and entrance to gastric lumen interior, requiring sometimes surgical intervention or endoscopy for resolution\(^9,13\).

\(^{1}\) Declared conflict of interest of all authors: none

Disclosure of funding: Coordination for the Improvement of Higher Education Personnel (CAPES)
Departamento de Gastroenterologia Cirúrgica, Universidade Federal de São Paulo, São Paulo, SP, Brasil.
Correspondence: Thiago De Bortoli Nogueira. Av. Rei Alberto I, 341, ap. 122 - CEP 11030-380 - Santos, SP, Brasil. E-mail: thiagobnogueira@hotmail.com
Furthermore, the RYGB technique may create conditions that contribute to the development of potentially malignant alterations, such as: ulcers in gastrojejunal anastomosis \cite{4,8}; gastric acidity, which may provoke lesions when the gastric pouch is larger or when gastrogastric fistula is developed \cite{3}; and *H. pylori* infection, which appears to be more frequent in the group submitted to RYGB \cite{2,21,31}. Promoting inflammatory alterations in the gastric mucosa with glandular loss, atrophy and gastritis and ulcer increase \cite{30}.

RYGB with a ring was performed in Brazil in large scale. However, there is scarcity of long-term studies evaluating macroscopic and histopathological alterations with a long period of patient follow-up. The objective of this study was to analyze the endoscopic and histological alterations in the gastric mucosa of patients submitted to RYGB with a contention ring. The hypothesis was that physiopathological alterations in the gastric mucosa after gastropasty could predispose chronic and proliferative inflammatory alterations.

**METHODS**

This is an observational, cross-sectional study, performed in a public university hospital and approved by the Institutional Research Ethics Committee. Patient informed consent forms were waived since this is a retrospective study based in archived material.

We searched records of all patients consecutively admitted to undergo open surgical treatment for morbid obesity with Roux-en-Y gastric bypass using a contention ring from 1997 to 2009. Therefore we selected the sample in the period before 2009 to allow a homogenous sample of patients operated with the same technique and long-term follow-up.

We included all consecutive adult patients submitted to ring Roux-en-Y gastric bypass who met the following criteria:

- submitted to preoperative upper digestive endoscopy;
- submitted to preoperative gastric antrum and body biopsy and postoperative gastric pouch biopsy;
- submitted endoscopy again in the postoperative period and after a minimum period of 60 months.

The exclusion criterion was the presence of neoplastic or pre-neoplastic gastric lesions, observed in the upper digestive endoscopy performed in the preoperative period.

We gathered patients' charts, pre and postoperative endoscopic reports and histopathological exams from preoperative gastric antrum and body biopsy and postoperative gastric pouch biopsy, in slides stained with haematoxylin and eosin (HE). We evaluated *H. pylori* presence in the gastric pouch archived slides and performed an immunohistochemical study to assess cell proliferation, as described next.

**Histopathological study**

The archived slides contained tissues from gastric biopsies, fixed in 10% formalin and processed for histological analysis as the hospital usual routine, through paraffin embedding, with 4 mm histological sections and HE staining. Modified Giemsa staining was used to detect *H. pylori* presence or absence in the gastric tissue.

For this study, a single pathologist reviewed the slides and evaluated atrophy, inflammation, intestinal metaplasia and dysplasia occurrence. The pathologist also reviewed *H. pylori* diagnosis.

**Immunohistochemical study**

We performed an immunohistochemical study in the material obtained from the pre and postoperative gastric biopsies. We added the monoclonal antibody Ki-67 (Dako Cytomation, Carpinteria, CA, USA) at a 1:100 dilution in BSA (1% bovine serum albumin) to slides previously silanised with 4 mm histological sections (3-aminopropyltrietoxysilane, Sigma Chemical Co., Saint Louis, MO, USA.) and maintained them in a stove at 60°C for 24 hours.

We calculated Ki-67 expression index. Using a microscope with a 400x magnification, we evaluated the percentage of glandular epithelial cells with marked nuclei in four 100,000 μm² areas. We considered as positive the cells marked by the antibody, even if weakly stained. We classified the Ki-67 marker expression as high cell proliferation level when the positive cells count was ≥25%, and low when <25%, based on the Ki-67 immunoreactivity in the stomach of normal individuals \cite{18,25}.

**Endoscopic findings**

We analyzed the endoscopic reports and evaluated the preoperative findings in the esophagus, gastric chamber and duodenum, and the postoperative findings in the esophagus, gastric pouch, gastrojejunal anastomosis, and jejunal afferent and efferent loop.

We also evaluated the presence, absence or internal migration of the contention ring in the gastric pouch. We used the Sydney classification \cite{27} for inflammatory alterations found in the preoperative gastric chamber mucosa and in the postoperative gastric pouch. We evaluated inflammatory findings from the esophagus according to the Los Angeles classification \cite{10}. We also evaluated the jejunal afferent and efferent loop endoscopically.

**Statistical analysis**

We measured the results though arithmetic mean and standard deviation (SD), and analyzed them through the paired t test (Student test), chi-square test and Fisher's exact test. We considered *P* values <0.05 significant. We used the statistical program PASW Statistics, version 18.0 (IBM Corp. New York, NY, USA).

**RESULTS**

The hospital staff operated 33 patients with the ring technique in the selected period. Thus, we included 33 patients in this study. The mean age was 42±9 years (22-55 years) and 22 of them (66.7%) were women. The mean postoperative follow-up was 91±21 months (60-144 months).

We describe the preoperative esophagus and gastric antrum and body endoscopic findings in Table 1 and the postoperative esophagus, gastric pouch, contention ring and gastrojejunal anastomosis endoscopy findings in Table 2.
We describe the pre and postoperative histological findings indicative of gastritis in Table 3. We identified intestinal metaplasia in the preoperative exams in two (6.0%) patients and mucosa atrophy in three (9.1%), two of them presenting both findings. In the postoperative stomach histological study, we identified intestinal metaplasia in three (9.1%) patients, and two (6.0%) of them also presented this alteration in the preoperative study. We found four (12.1%) patients with gastric mucosa atrophy.

We found no cases of gastric mucosa dysplasia in the pre and postoperative period. We found no statistically significant concordance ($P=0.1$) between the endoscopic and histological findings in the pre and postoperative period. The longer postoperative follow-up did not significantly influence the findings of endoscopic ($P=0.5$) or histological ($P=0.3$) abnormalities.

Among the 33 patients, 12 (36.3%) were *H. pylori* negative before and after the surgery; 4 (12.1%) were positive before and after. However, 15 (45.4%) were negative before the surgery and became positive after. Only two (6.0%) were positive before and became negative after the surgery. All patients with a positive preoperative *H. pylori* exam received specific antibiotic therapy, and endoscopic biopsies confirmed the bacteria eradication. The histological gastritis intensity in the gastric pouch was associated to *H. pylori* presence ($P=0.02$) (Table 4). The gastric pouch higher cell proliferation index were associated to *Helicobacter pylori* presence (Table 5), whose infection was significantly higher in the gastric pouch of patients with greater postoperative follow-up period (Table 6).

The cell proliferation index in the gastric pouch (Ki67) was significantly higher ($P=0.001$) than in the preoperative gastric chamber (Table 7 and Figures 1 and 2).
TABLE 6. Helicobacter pylori distribution gastric pouch and postoperative follow-up period of patients submitted to Roux-en-Y gastric bypass with a restraining ring

<table>
<thead>
<tr>
<th>Postoperative follow-up (months)</th>
<th>H. pylori</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Variation (minimum-maximum)</td>
<td>60-108</td>
<td>84-144</td>
</tr>
<tr>
<td>Median</td>
<td>72</td>
<td>96</td>
</tr>
<tr>
<td>Average (standard deviation)</td>
<td>73.7 (13.2)</td>
<td>104.2 (14.4)</td>
</tr>
</tbody>
</table>

* Significant; Student’s test.

TABLE 7. Ki-67 expressions preoperatively and postoperatively in patients submitted to Roux-en-Y gastric bypass with a restraining ring

<table>
<thead>
<tr>
<th></th>
<th>Preoperative</th>
<th>Postoperative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki-67</td>
<td>N% SD</td>
<td>N% SD</td>
<td></td>
</tr>
<tr>
<td>Gastric antrum</td>
<td>18.1 8.8</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>Gastric body</td>
<td>16.2 10.2</td>
<td>0.02*</td>
<td></td>
</tr>
<tr>
<td>Gastric pouch</td>
<td>23.8 16.2</td>
<td>0.001*</td>
<td></td>
</tr>
</tbody>
</table>

SD: standard deviation, *Significant; Student’s test.

DISCUSSION

Nowadays the most used bariatric surgery technique for morbid obesity treatment is the Roux-en-Y gastric bypass (RYGB), in which a contention ring may be used. There are no prevalence reports of gastric neoplasia after RYGB. However, symptoms such as abdominal pain, bleeding, uncontrollable vomiting and weight loss, which are common in gastric cancer, can also occur after RYGB. Therefore this procedure may theoretically contribute to late diagnosis of gastric cancer. Malignant gastric tumours after 5 to 22 years of the postoperative period have been described in 21 obese patients submitted to RYGB, and 2 of them were located in the gastric pouch. Due to reports of late neoplasia after RYGB, there is a concern regarding gastric pouch evaluation.

A retrospective study with 161 patients submitted to preoperative gastric biopsy demonstrated alterations in 109 (68%) of them, mainly chronic gastritis. The alterations were significantly more frequent (94%) in the H. pylori positive cases when compared to the negative (51%). The present study identified chronic gastritis in similar proportions and significantly more related to H. pylori infection cases.

Csendes et al. evaluated 227 patients submitted to gastric bypass, with a mean follow-up of 27 months after operation. The endoscopic exam showed no alterations in 225 (99%) patients. One patient (0.4%) presented ulcer in the gastrojejunal anastomosis and in 96 (56%) cases the gastric pouch mucosa...
was histologically normal. While 48 (28%) patients presented active chronic gastritis, it was associated to *H. pylori* presence in 43 (89.6%) cases. After 2 years of evaluation, 53 (31%) cases presented *H. pylori* positivity and 9 (5.5%) cases presented inactive chronic gastritis. These authors reported late marginal ulcer in 0.6% to 25% of operated patients. Our study found one (3%) patient with gastrojejunal anastomotic ulcer only.

Flickinger et al. performed endoscopies 13 to 20 months after the gastric bypass in 53 patients. These authors found the gastric pouch without detectable macroscopic alterations in 45 (85%) patients. On the other hand, 11% of patients had biliary stasis, probably due to the construction of an afferent loop shorter than usual in the Roux-en-Y. The gastric pouch histological analysis revealed the mucosa was normal in 45% of patients, with acute gastritis in 23%, chronic gastritis in 30% and with intestinal metaplasia in 13%. These results indicate no correlation between endoscopic appearance and histological findings, which may justify the necessity of histological evaluation even when the gastric mucosa macroscopic appears to be normal. In the present study we observed a similar result: normal postoperative gastric pouch macroscopic aspect in 24 (72.7%) cases and mucosa inflammatory process with histological evidence in 28 (84.8%) patients.

A factor that could explain the high histological gastritis index we found is *H. pylori* presence. *H. pylori* infection is more prevalent (61.3%) in the obese population in general, while in the obese patients submitted to bariatric surgery, *H. pylori* presence varied from 24% to 70%.

Csendes et al. found *H. pylori* in the preoperative period in 47% of obese patients submitted to bariatric surgery. None of them received eradication treatment, since all had the distal gastric segment totally dissected. *H. pylori* was present in the antrum in 20% of patients and in the gastric fundus in 5%. Two years after operation, *H. pylori* in the gastric pouch was 31%. In only 50% of patients with *H. pylori* in the gastric pouch the bacterium was present in the preoperative period. This finding suggests *H. pylori* quickly colonizes this little gastric pouch with a minimum amount of parietal cells, probably since in this portion there is no acid secretion in great quantity, which contributes to the rapid *H. pylori* infection of the mucosa.

In the present study, *H. pylori* was present in the preoperative gastric antrum and body of six (18.2%) patients and in the postoperative gastric pouch of 19 (57.5%). All patients with preoperative *H. pylori* received specific antibiotic therapy and bacteria eradication confirmed by endoscopic biopsy. This result suggests that the *H. pylori* infection persistence in the postoperative period was probably due to reinfection episodes. As in the study from Csendes et al., in the present study there was a higher *H. pylori* prevalence in the postoperative gastric pouch. The bacteria greater permanence in the gastric pouch after gastric derivation could favor the appearance of long-term histological alterations in the gastric mucosa.

Kuga et al., in a study with 40 patients submitted to gastrojejunal derivation in Roux-en-Y and a 77.3-month follow-up, observed *H. pylori* gastric pouch presence in 34.3% of cases. The histological gastritis intensity of the gastric pouch was associated with *H. pylori* presence. In the present study, 84.8% of patients presented light or moderate histological gastritis in the gastric pouch. And, as in the study from Kuga et al., the gastritis level in the gastric pouch was associated to *H. pylori* presence. 

Usually an inflammatory response in the underlying mucosa accompanies *H. pylori* gastric mucosa colonization. This induces lymphocytes, plasma cells, neutrophils and monocytes inflammatory infiltrates and proinflammatory cytokines such as interleukins, interferon and tumor necrosis factor. *H. pylori*, besides being an important peptic ulcer and gastritis etiological agent, is related to chronicity of these lesions and progression to premalignant conditions.

The Ki-67 used to evaluate cell proliferation allows a very approximate identification of a cell population growth fraction. For that reason, we pioneered used Ki-67 in this study to determine eventual histological alterations in the gastric mucosa of patients submitted to RYG. Gerdes et al. reported increased expression of this antigen with progression of the cell cycle in normal and neoplastic tissues.

Safatle-Ribeiro et al. evaluated 35 patients submitted to RYG with a postoperative follow-up higher than 36 months and observed Ki-67 antigen expression in the gastric pouch and in the excluded stomach mucosa. They compared these results to the expression of this antigen in the gastric antrum and body of not operated obese patients. In operated patients, the cell proliferation index evaluated through Ki-67 in the gastric antrum was of 24.9%, in the body 24.7% and in the pouch 18.3%. In the control group, Ki-67 proliferation index in the gastric antrum was of 17.7%, and in the body 15%.

In our investigation, the proliferative index of epithelial cells through Ki-67 antigen expression increased in the postoperative period (23.8%) when compared to the preoperative (17.1%), mainly in cases with *H. pylori* presence. Safatle-Ribeiro et al., observed a different result: gastric pouch cell proliferation was of 18.3% (the authors mentioned a treatment for pathogen eradication, but presented no cure confirmation). This result discrepancy could be explained by the higher postoperative *H. pylori* infection rate we found in our study, mainly in patients with greater period of postoperative follow-up.

We conclude that, in patients submitted to surgical treatment for morbid obesity through vertical gastroplasty Roux-en-Y gastric bypass with a contention ring, histological findings indicated high chronic gastritis prevalence in the gastric pouch, unrelated to endoscopical findings. Furthermore, the gastric pouch inflammation intensity and higher cell proliferation index were associated to *Helicobacter pylori* presence, whose infection was significantly higher in the gastric pouch of patients with greater postoperative follow-up period.

**Authors’ contributions**

Nogueira TDB participated in the study design, data collection and interpretation, manuscript writing, and he revised the final version of the manuscript to be published. Artigiani Neto R participated in the study design and in the histological analysis, and he revised the final version of the manuscript to be published. Herani Filho B participated in the study design and data collection and interpretation and he revised the final version of the manuscript to be published. Waisberg J participated in the study design, data interpretation and he revised the final version of the manuscript to be published.

RESUMO - Contexto - O tratamento da obesidade mórbida através da gastrosfisia vertical com derivação gastrojejunal em Y de Roux inicialmente utilizou o anel de contenção. No entanto, essa técnica pode criar condições para o desenvolvimento de alterações potencialmente malignas na mucosa gástrica. Apesar de eficaz e realizada anteriormente em grande escala, essa técnica precisava ser melhor avaliada em estudos de longo prazo em relação às alterações causadas na mucosa gástrica. Objetivo - Analisar os achados endoscópicos, histológicos e da proliferação cellular na mucosa do antro e corpo gástricos no pré-operatório e no pós-operatório de pacientes submetidos à derivação gastrojejunal em Y de Roux com anel de contenção. Métodos - Avaliamos retrospectivamente todos os pacientes submetidos à derivação gastrojejunal em Y de Roux com anel de contenção e 40 meses de seguimento pós-operatório. Comparamos os achados endoscópicos da mucosa gástrica, o índice de proliferação celular e a prevalência do H. pylori no pré-operatório (antro e corpo gástricos) e no pós-operatório (bolsa gástrica). Avaliamos a proliferação celular pela expressão imuno-histoquímica do antígeno Ki67. Resultados - No período do estudo, 33 pacientes foram operados com a derivação gastrojejunal em Y de Roux usando anel de contenção. Encontramos uma taxa de gastrite crónica de 69,7% no período pré-operatório (antro e corpo gástricos) e 84,8% no pós-operatório (bolsa gástrica). O H. pylori estava presente em 18,2% dos pacientes no período pré-operatório (antro e corpo gástrico) e em 57,5% no pós-operatório (bolsa gástrica). O índice de proliferação celular pré-operatório foi de 18,1% no antro gástrico e 16,2% no corpo gástrico, e de 23,8% na bolsa gástrica no pós-operatório. O índice de proliferação celular pós-operatório na bolsa gástrica foi significativamente maior (P=0,001) do que no antro e corpo gástrico no pré-operatório. O maior índice de proliferação celular e a intensidade da gastrite crónica na bolsa gástrica associaram-se significativamente à presença do H. pylori (P=0,01 e P=0,02, respectivamente). Conclusão - Após a derivação gastrojejunal em Y de Roux usando anel de contenção, houve maior incidência de gastrite crónica e maior índice de proliferação celular na bolsa gástrica do que no antro e corpo gástricos no pré-operatório. A intensidade da inflamação da mucosa e o índice de proliferação celular encontrados na bolsa gástrica no pós-operatório associaram-se à presença do H. pylori e foram maiores do que os encontrados na mucosa gástrica do antro e corpo gástricos no pré-operatório.


REFERENCES