Vertical gastrectomy in overweight and non-overweight rats

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PURPOSE: To compare the two lines suture (total and seromuscular) after partial gastrectomy in normal and overweight rats.

METHODS: Forty Wistar rats were distributed in two groups. Group A received normal diet; group B, normal diet and supplementation with saccharose in the water. When group B progressed to a statistically greater weight than the animals of group A, the experiment (sleeve-like gastrectomy) was conducted with gastrorraphy in two sutures lines (total and seromuscular). The animals were distributed into two subgroups of 10. A1 and A2 subgroups were sacrificed at 7 and 14 days postoperatively as well as B1 and B2. Mortality, morbidity, complications attributed to the gastric suture, biochemical dosages, Lee index, macroscopy, weight of retroperitoneal and gonadal fat, optical microscopy with hematoxylin-eosin and picrosirius-red, were the evaluation parameters.

RESULTS: The overweight group achieved statistically greater weight after 16 weeks in induced obesity; there was no mortality or complications with clinical consequences attributable to morbidity. The overweight group had statistically greater weight of gonadal and retroperitoneal fat. The difference was observed in urea, albumin, total cholesterol and indirect bilirubin.

CONCLUSION: There was no outcome difference between the overweight and non-overweight group in two suture lines in gastrorrhaphy after sleeve-like gastrectomy.

Key words: Stomach. Sutures. Anastomosis, Surgical. Rats.
Introduction

Obesity reaches alarming levels in Western society. The medical literature has shown that overweight people have higher surgical complications, among them dehiscence, digestive fistulas and higher incidence of abdominal incisional hernias\textsuperscript{1,2}.

Bariatric surgery is well established as a durable option to reduce and maintain the loss of weight excess in patients submitted to it\textsuperscript{3-5}. Most techniques include sutures and/or gastrointestinal anastomoses performed manually or with mechanical staplers\textsuperscript{6-10}.

The manual suture can be made in one\textsuperscript{11-13} or two suture lines\textsuperscript{14}, with good results. Gastrointestinal anastomoses had a long evolution during the history of medicine\textsuperscript{15,16}. The manual suture of the edges of intestinal wounds usually caused by trauma, was probably one of the first attempts to make the synthesis of these intestinal lesions\textsuperscript{15,17-19}.

Even today, gastrointestinal anastomoses are still object of study, and when carried out in overweight can have complications in higher levels, especially those related to dehiscence\textsuperscript{2,20}.

The goal of this study was to compare the healing process and complications of suture lines in overweight and non-overweight animals after sleeve-like gastrectomy.

Methods

Forty EPM-1 Wistar rats, males, with average weight of 180 g were distributed in two groups of 20, being group A with normal weight and B overweight. The animals in both groups received regular diet for the species. The B group received sucrose supplementation (300 g/L) rather than water. They were all weighed weekly.

After 16 weeks B group reached statistically higher weight than group A. At this time was initiated the experiment consisting of general anesthesia with isoflurane (3 ml), xylazine (10 mg/kg) and ketamine (100 mg/kg). Antibiotic prophylaxis was performed with ceftriaxone (50 mg/kg). After being anesthetized, the naso-anal length of the rat was measured in centimeters and the correlation weight/length gave the body mass index, the Lee index, which is the ratio of the cube root of body weight (in grams) of the naso-anal length (in centimeters) multiplied by one thousand (Figure 1).

\[
\text{ÍNDICE DE LEE } = \frac{\sqrt[3]{\text{peso}}}{\text{CNA}} \times 1000
\]

FIGURE 1 - Lee index formula.

![FIGURE 2 - Surgical procedure: A) Median laparotomy; B) Schematic drawing of the sleeve-like gastrectomy; C) Vertical gastrectomy; D) Gastrorrhaphy.](image)

The surgical procedure was median laparotomy (Figure 2A), vertical gastrectomy (Figure 2B and 2C) and gastrorrhaphy in two suture lines with 6-0 polypropylene, one full invaginating continuous suture and the second seromuscular with separated stitches (Figure 2D).

The musculoaponeurotic layer was closed with continuous suture using polydioxanone 3-0 and the skin with polyamide 4-0 in separate stitches.

The animals received water (group A) or sucrose solution (group B) once recovered from anesthesia and normal diet for the species after 12 hours for both; then they were divided into two subgroups of 10 to be euthanized on 7 and 14 days of the postoperative period. The same anesthetic technique was used with intracardiac puncture for blood collection for biochemical analysis until death.

Postoperative analysis of kidney function, lipid profile, blood glucose and insulin were asked. The preoperative clinical analysis parameters were those described in the literature as normal for mice. The operative evolution and surgical complications of all animals were recorded as well.

Necropsy evaluated presence of adhesions and fistula. The stomach with gastrorrhaphy was removed for optical microscopy with H&E and picrosirius-red collagen dosage of the corpus as well of the rumen (Figure 3A). After removal of the stomach, extraction of retroperitoneal (Figure 3B) and gonadal fat (Figure 3C) for weighing was realized.
Vertical gastrectomy in overweight and non-overweight rats

Statistical analysis

The statistical analysis were considered quantitative variables were described as mean, median, minimum, maximum and standard deviation and qualitative variables as frequencies and percentages. To compare the weights evaluated in three stages (initial weight, weight at surgery and weight sacrifice), it was considered the model of analysis of variance with repeated measures. Multiple comparisons were made using the LSD test (least significant difference). To compare two groups regarding the quantitative variables was considered the Student t test for independent samples or assessed by the Kolmogorov-Smirnov test. To evaluate the association between two dichotomous qualitative variables was used Fisher’s exact test.

P0.05 values indicated statistical significance. Data were analyzed with the software Statistica v.8.0.

Results

The overweight group evolved after 16 weeks for an average weight of 519.83 ± 67.66, statistically higher than the non-overweight group (average weight of 402.41 ± 46.51).

Most of the animals showed loose adhesions to neighboring structures. One rat had a small subcutaneous seroma. Two rats (one from Group A and one from group B) showed fistulas of the gastric suture blocked by the adjacent organs, but without changing the clinical behavior or feeding. These complications were not identified during the autopsy.

Regarding the Lee index there was no statistical difference when compared non-overweight with overweight group.

When comparing the weight of gonadal and retroperitoneal fat of the overweight and non-overweight group, it was noted that the overweight showed statistically greater weight in both gonadal and retroperitoneal fat when compared to non-overweight group.

In hematological study of leukocytes, platelets, biochemical analysis of glucose, evidence of hepatic and renal function, and insulin, changes were observed only in urea which was statistically lower in the overweight group on the 7th postoperative and lower in the non-overweight group on the 14th day when compared to the normal standard for mice. The indirect bilirubin was statistically lower in the overweight group on the 14th day. Albumin was higher in the overweight group on the 7th day and total cholesterol higher in 7th and 14th days in overweight group (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Standard deviation</th>
<th>p value</th>
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<td>Urea</td>
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<td>10</td>
<td>53,10</td>
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<td>104,00</td>
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<td>0,22</td>
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<td>0,00</td>
<td>0,31</td>
<td>0,09</td>
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<td>70,00</td>
<td>49,00</td>
<td>90,00</td>
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P.O.: Post operative; * Student’s t test for independent samples, p<0.05; * Nonparametric Mann-Whitney, p<0.05.

FIGURE 3- Stomach was removed with the scar: A) Identification of the corpus and rumen in the scar; B) Removal of the retroperitoneal fat; C) Removal of gonadal fat.
The analysis of optical microscopy with H&E staining showed no statistical difference in re-epithelialization of the mucosa, coaptation of layers (serous and muscle, mucosa and submucosa) and inflammation of the serous and muscular layers, mucosa and submucosa between non-overweight and overweight subgroups at 7 and 14 days, both rumen and corpus. Good cicatrization was observed in most animals; they had re-epithelialization of the mucosa, mucosa/submucosa and serous/muscle coaptation, consistent with inflammatory process in respective days.

It was found statistical difference in the inflammatory parameters when comparing subgroups within the two groups. Those on the 7th day, both in the non-overweight and overweight group, had higher amount of acute inflammatory process in relation to the subgroups on the 14th day after surgery. These results were expected, since the animals in 14 days would be in later healing time compared to the 7th day.

There was no statistical difference when analyzing the amount of collagen type I and type III in non-overweight and overweight groups at 7 and 14 days.

Laboratorial tests of the rats in the pre surgery were not conducted because the necessary amount of blood for analysis of all the variables is obtained by intracardiac puncturing, making it impossible to continue the study.

Discussion

All animals evolved satisfactorily during the study. Due the fact that rat is animal with resistance to infection, the use of prophylactic antibiotics and antiseptic techniques may have contributed to the good results. These aspects were also observed by other researchers21,22.

In the Lee index analysis there was no statistical difference when comparing the non-overweight with overweight group. Although it has been observed that the overweight group had statistically greater weight than the non-overweight, this finding was not corroborated by the results of Lee index23. This may be due to several reasons: the period after the operation was short (14 days), or as Stephens24 observed in another paper, the correlation length with the weight was not adequate for measuring body mass index.

The vertical gastrectomy afforded weight reduction at the time of euthanasia in both groups. This reduction, after obesity induction in rats, was also observed by other authors with other techniques22,25.

Most of the animals showed loose adhesions to neighboring structures. One had a small subcutaneous seroma and two fistulas of the gastric suture blocked by the adjacent organs. When comparing the weights of gonadal and retroperitoneal fat between the overweight and non-overweight group, it was found that fat had statistical greater weight both gonadal and retroperitoneal compared to the non-overweight group. This fact can contribute to validate the obesity induction model with sucrose used in this study. It was found that in the 14th day the animals exhibited weight reduction of these fats in relation to the 7th day.

Total cholesterol in the overweight group was statistically higher than the values of the non-overweight both on 7th and 14th day. It is believed that there was insufficient time for reduction with sleeve-like gastrectomy surgery. If the animals had had longer postoperative period, 60 or 90 days, the values could be minor, as Bonhomme25 verified. Suzuki26 found decreased triglyceride levels after gastric bypass, but without statistical significance.

After analyzing the optical microscopy with H&E no statistical difference was observed when studying the pathological parameters. It was observed in most animals good cicatrization, with re-epithelialization of the mucous layer and coaptation of mucosa/submucosa and serous/muscle, consistent with inflammatory process in days 7th and 14th days. It was found statistical differences in the inflammatory process comparing the subgroups within groups. On the 7th day both groups had greater acute inflammatory process in relation to the subgroups in the 14th day. These results are as expected, since the animals in day 14 were in later period of cicatrization compared to the 7th. Other authors found similar results, as Santos25 after studying gastorrhaphy with gastric suture with polypropylene in rats sacrificed on days 3 and 7; it was observed in optical microscopy of the animals that most had good coaptation of the edges and discrete acute or moderate reaction. The same was observed by Tabushi11 in healing of rat’s cecum.

There was no statistical difference when analyzing the amount of collagen type I and type III in non-overweight and overweight groups, in both periods. However, it was found that the amount of type I was higher in the non-overweight compared to the overweight, and the amount of type III was higher in the overweight compared to non-overweight group. Given that type I is associated with more mature and well structured wound and type III with initial healing, the tendency of the overweight group, having more collagen type III and less type I, was to have slower cicatrization, like the results found by Xing et al.11, who demonstrated that after the obesity induction in rats, the abdominal cicatrization is delayed in overweight compared to non-overweight.

In this study it was found an albumin dosage statistically higher in the overweight group at 7 P.O. contradicting the results of the literature26. Although not being the objective of this paper, it was not possible to understand why the albumin increased in the overweight group in the 7 P.O. Due the existence of few
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studies dosing serum albumin in rats submitted to gastrectomy in the literature, there is a need to continue this research looking for better explanation for this point.

Conclusion

The healing process in the vertical gastrectomy with two suture lines showed no difference when non-overweight rats were compared with the overweight, although some biochemical variables showed some differences between groups.

References


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