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CLINICAL RESEARCH

Early Identification of Infectious Complications in Bariatric Surgery by the Determination of Peritoneal and Systemic Cytokines

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Abstract
Background Obesity has become a global epidemic and bariatric surgery is one of the therapeutic tools to deal with it. Postoperative complications can occur, such as staple line dehiscence and anastomotic leaks, leading to increased patient mortality. The diagnosis of these complications is frequently difficult. The objective of the present study was to determine whether peritoneal and systemic cytokines could early detect those complications.
Methods All patients who underwent open Roux-en-Y gastric bypass from February 2007 to August 2008 were prospectively evaluated. Blood and peritoneal effluent from the drain were collected for the determination of cytokine levels. We also evaluated the clinical signs and the leukograms of the patients.
Results A total of 107 obese patients were studied. Ninety patients had no complications; 17 had at least one infectious complication which include five cases of staple line dehiscence. Until the third postoperative day, the vital signs and the leukogram did not predict the onset of infectious complications, but the cytokines (interleukin-1β and interleukin-6) were early markers of these complications.
Conclusion Cytokines are good predictors of poor postoperative evolution in bariatric surgery since peritoneal cytokines diagnose better these infectious complications even before changes in blood count and before the occurrence of clinical manifestations.

Keywords Cytokines · Bariatric surgery · Complications · Staple line dehiscence · Inflammatory response

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Introduction

Obesity has become a global epidemic that currently affects 185 million adults in the developed countries [1, 2]. In the United States, 27% of the population are obese [2]. The surgical treatment of morbid obesity appeared in the 1950 decade and started to be seen as a magical solution for all the problems of obese persons, a fact that, unfortunately, is not true. There is no ideal operation without inconveniences or risks. The frequency of postoperative complications is varied in the literature [3–5]. Amongst all the possible surgical complications, staple line dehiscence and anastomotic leaks are major concerns because of their extreme severity leading to increased patient mortality. The diagnosis of these complications is frequently difficult and sometimes late [6].

There is a continuous search for a tool to aid the diagnosis, i.e., some exam that can foresee the onset of complications, or detect them in an initial phase before the establishment of severe sepsis. In addition, there is a search for reliable parameters that guarantee a safe early withdrawal of abdominal drains and earlier hospital discharge.

Surgery, infection and trauma induce the production of cytokines that are polypeptides produced by the cells of the immune system and by a wide variety of other tissues. They act as mediators of the immunologic response and the acute phase of inflammation [7–9]. The most important pro-inflammatory cytokines are: interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). Some studies have demonstrated a significant systemic inflammatory response in surgical patients with secondary peritonitis, identified on the basis of the systemic release of these pro-inflammatory cytokines [7–9].

The levels of some cytokines have been reported to increase very early in blood, preceding the onset of the clinical signs of complications by 12 to 48 h [10]. Little it is known, however, about the local cytokine response inside the peritoneal compartment, an important and very early event in the physiopathology of inflammation [11].

The behavior of systemic and peritoneal cytokines in obese patients submitted to bariatric surgery who presented complications has not been previously described. Thus, the objective of the present study was to determine whether pro-inflammatory cytokines (IL-1, IL-6, TNF-α) measured in the peripheral blood and peritoneal fluid of morbidly obese patients during the postoperative period will permit the early identification of infectious complications of bariatric surgery before the occurrence evidence of the first clinical and laboratory manifestations.

Methods

All patients who underwent open RYGBP from February 2007 to August 2008 at our institution were prospectively evaluated. The selection criteria for bariatric surgery followed the indication of the National Institutes of Health Consensus Development Panel. Institutional Ethics Committee approval was obtained and all patients signed a term of informed consent.

Routinely, we created a small (20 ml) gastric pouch with 80 mm using a 3.5 mm linear stapler. The staple line was always oversown with an unabsorbable suture. Both the alimentary and biliopancreatic limbs measured 100 cm. Patency and tightness of the staple line at the gastric pouch were routinely checked by injection of methylene blue dye. At the end of the procedure, a 24-Fr Blake drain (Johnson & Johnson) was inserted into the abdomen proximal to the gastrojejunostomy and exteriorized through the right hypochondrium. The drains were removed on postoperative day 7 after another negative methylene blue test. Two surgeons were responsible for all operative procedures. During the postoperative period the vital signs of each patient (heart rate and axillary temperature) were checked at 4, 24, 48, and 72 h.

One day before the surgical procedure, control blood samples, 3 ml for cytokine determinations (IL-1, IL-6 and TNF-α) and 5 ml for the blood count, were collected. The same amount of blood was again obtained from each patient for cytokine determination and blood count 24 and 48 h after the end of the surgery and 48 h later. A small amount of peritoneal fluid from the abdominal drain was similarly tested for cytokine levels at the same time intervals as the blood cytokines and also on the seventh postoperative day.

All the blood and the peritoneal fluid were immediately centrifuged after been collected and stored at −70°C until the cytokines determinations. The TNF-α, IL-6, and IL-1β levels were determined by enzyme-linked immunosorbent assay. Briefly, microtiter plates (Nunc-Maxisorb) were coated overnight at 4°C with sheep anti-human TNF-α, IL-6, or IL-1β. After blocking the plates, human TNF-α, IL-6, or IL-1β standards at various dilutions in medium and 50 μl of samples (plasma) were added in triplicate and maintained at room temperature for 2 h. Sheep TNF-α, IL-6, or IL-1β biotinylated polyclonal antibodies (R&D Systems Inc. Minneapolis, MN 55413) were added at a 1:500 dilution, followed by incubation at room temperature for 1 h. Finally, 100 μL of avidin-HRP (1:5,000 dilution) was added to each well and, after 30 min, the plates were washed and the color reagent OPD (40 μg, 50 μl well^{-1}) was added. After 15 min, the reaction was terminated with H_2SO_4 (1 M, 50 μL well^{-1}) and the absorbance read at 490 nm with the Molecular Devices/Spectramax 250 apparatus. The results were obtained by comparing the absorbance with standard curves. The variability inter-assay was less than 5%. The sensitivity of the assay was up to 0.02 ± 0.003 pg/ml for all cytokines.

Other parameters included were patient demographics and major and minor complications.

The INSTAT program was used for statistical analysis. The variables with normal distribution were compared between groups by the *t*-test for unpaired samples and the variables with non-normal distribution were compared using the Mann–Whitney non-parametric test. For intergroup analysis, Friedman's test with Dunn's post-test was used.

Results

A total of 107 obese patients were included in this study (90 women and 17 men). The mean (±standard deviation) weight, body mass index and age, 1 day before the operation, were 134.57±23.20 kg, 50.59±7.80 kg/m^2 and 38.30± 9.39 years, respectively. The number of co-morbidities per patient was 1.77.

Morbidly obese patients are encouraged to lose weight in order to be operated under better clinical conditions, with a

reduced risk of complications. The maximum weight reached by these patients, before entering the program to lose weight to the surgery, was 147.95±27.48 kg, and the average preoperative weight loss was 10.36%. The abdominal circumference (AC) is an important measure, generally related to a high incidence of associated co-morbidities such as hypertension and diabetes, and is also an indicator of the degree of surgical difficulty. The mean AC of the present group was 141.7±17.82 cm.

Ninety patients had no complications, 17 (15.8%) had at least one infectious complication: eight cases (7.4%) of superficial incision site infection, three cases of urinary tract infection (2.8%), one case of pneumonia (0.9%), two cases of cellulitis (1.8%), and five cases of staple line dehiscence (4.6%), one of them with a lethal outcome (0.9%). The leaks were detected between the fourth and the eighth postoperative days. An important observation is that only one of the patients with staple line dehiscence had clinical manifestation of sepsis and needed to be reoperated on postoperative day 7. The other four patients only had the diagnosis of dehiscence because of a positive methylene blue tests.

Preoperative Characteristics

In order to assess the physiological and laboratory characteristics of the patients before surgery that could indicate a higher risk of development of infectious complications, the groups with (n=90) and without infectious complications (n=17) were compared. As shown in Table 1, the only difference between groups was a higher preoperative IL-6 value in the group without complications.

However, when the group without complications was compared to the group with staple line dehiscence (n=5), the latter group was found to consist of older patients who had lost little weight before surgery (Table 1**)**

Clinical Signs of Postoperative Infection

The infectious complications were diagnosed and confirmed mainly after the fourth postoperative day. A significant difference in vital signs such as temperature and heart rate was not observed between the groups with and without complications (Table 2).

However, in the group with dehiscence, the axillary temperature on the third postoperative day was higher than in the group without complications (Table 2).

Inflammatory Response

Comparison of the groups with and without complications at 48 h showed higher white cell and rod neutrophil counts in the group with complications. Despite this difference, these cell counts were still within normal limits and probably would not alert to the presence of a possible complication. Significant alterations of interleukin-1β (IL-1β) in peritoneal fluid preceded by one day the changes in the white cell count and persisted along the postoperative days, with higher values in the group with complications. Other cytokines did not differ between groups (Table 3).

The patients with staple line dehiscence presented higher values of interleukin-6 (IL-6) in the peritoneal fluid

Table 1 Preoperative characteristics of the groups with and without infectious complications and with staple line dehiscence (mean±SD)

Characteristic	Group without complications (n=90)	Group with complications (n=17)	Group with dehiscense (n=5)	p value
Age	**38.38 ± 9.69**	37.82 ± 7.89	**43 ± 2**	**0.003**
Gender	13♂/77♀	4♂/13♀	5♀	NS
Weight (kg)	133.64 ± 22.47	139.48 ± 27.40	124.56 ± 19.9	NS
BMI (kg/m^2)	50.24 ± 7.84	52.48 ± 7.51	49.04 ± 6.05	NS
Co-morbidities/patient	1.8 ± 1.11	1.63 ± 0.07	1.6 ± 0.89	NS
Abdominal circumference(cm)	140.87 ± 18.15	145.29 ± 16.57	144.66 ± 7.37	NS
Maximum weight (kg)	146.87 ± 26.26	159.21 ± 35.2	145.32 ± 27.32	NS
% of preoperative weight loss	**10.63 ± 6.29**	8.24 ± 3.98	**4.86 ± 1.63**	**<0.0001**
Leucocytes	7,847.77 ± 2302.28	8,329 ± 2,003.98	8,800 ± 1,816.59	NS
Rod neutrophils (%)	0.02 ± 0.15	0.29 ± 0.84	0	NS
Interleukin-1-beta (pg/ml)	14.92 ± 23.47	5.81 ± 15.2	14.35 ± 26.81	NS
Interleukin-6 (pg/ml)	**57.20 ± 74.64**	**6.81 ± 12.84**	9.43 ± 8.35	**0.02**
Tumor necrosis factor-α (pg/ml)	12.04 ± 37.26	26.36 ± 89.39	2.53 ± 5.66	NS

The statistical differences are presented in bold

BMI body mass index, *NS* not significant

Table 2 Clinical signs of the groups with and without complications and with staple line dehiscence along time (mean±SD)

Characteristic	Group without complications (n=90)	Group with complications (n=17)	Group with dehiscense (n=5)	p value
Heart rate (HR)—4 h	80.9±13.70	85.58±13.50	72.6±8.59	NS
HR—24 h	88.75±10.21	88.35±12.69	84.8±14.25	NS
HR—48 h	89.42±10.63	94.35±12.12	98.4±13.37	NS
HR—72 h	88.26±8.91	86.58±6.73	86.4±9.20	NS
Temperature (T)—4 h	36.4±0.49	36.61±0.61	36.28±0.22	NS
T—24 h	36.92±0.44	37.02±0.54	37.06±0.19	NS
T—48 h	37.09±1.12	37.03±0.61	37.14±0.68	NS
T—72 h	**36.93±0.48**	37.14±0.51	**37.2±0.01**	**<0.0001**

The statistical differences are presented in bold

NS not significant

compared to the group without complications as early as 24 h after surgery. Other alterations observed were higher values of rod neutrophils and plasma and peritoneal levels of IL-1 β 48 h after surgery. Similarly, peritoneal IL-6 levels were higher in the group with dehiscence on the seventh postoperative day (Table 3).

Discussion

In obese patients, the diagnosis of gastric dehiscence and leakage is frequently difficult. The clinical symptoms may not be obvious and the abdominal physical examination is not very enlightening. Many times this complication is not

Table 3 Laboratory evaluation of the patients in the groups with and without complications and with staple line dehiscence along time (mean±SD)

Characteristic	Group without complications (n=90)	Group with complications (n=17)	Group with dehiscense (n=5)	p value
White cell counts (24 h)	16,146±3,035.30	17,372.59±3,607.74	15,440±901.66	NS
White cell counts (48 h)	**11,742±2,400.70**	**13,329.41±2,985.54**	12,640±1,158.87	**0.01**
Rods neutrophils—24 h	506.41±722.22	536.23±574.22	289±388.39	NS
Rods neutrophils—48 h	**74.97±147.60**	**300.35±361.14**	**583.8±552.37**	**0.001/0.02***
Plasma interleukin 1 β—24 h	12.11±20.98	10±29.46	26.68±51.71	NS
Plasma interleukin 1 β—48 h	**9.55±15.33**	8.27±16.14	**26.37±21.20**	**0.02**
Peritoneal interleukin 1 β—24 h	**61.61±99.32**	**154.20±151.86**	140.55±157.03	**0.004**
Peritoneal interleukin 1 β—48 h	**39.69±88.68**	**213.63±209.88**	**212.43±180.62**	**<0.0001****
Peritoneal interleukin 1 β—7th day	**297.19±284.43**	**545.54±323.69**	419.74±230.67	**0.03**
Plasma interleukin 6—24 h	70.08±90.44	68.38±80.55	88.99±89.58	NS
Plasma interleukin 6—48 h	76.09±78.33	39.03±27.50	45.97±22.89	NS
Peritoneal interleukin 6—24 h	**635.29±135.72**	697.66±143.47	**791.10±107.92**	**0.01**
Peritoneal interleukin 6—48 h	595.15±154.64	658.71±137.97	707.46±100.58	NS
Peritoneal interleukin 6—7th day	**608.50±136.07**	611.74±273.37	**755.18±92.68**	**0.02**
Plasma TNF—24 h	8.56±24.01	21.26±82.10	1.28±2.86	NS
Plasma TNF—48 h	6.15±17.63	14.82±51.70	1.90±4.26	NS
Peritoneal TNF—24 h	54.33±98.31	90.77±133.90	82.23±77.16	NS
Peritoneal TNF—48 h	45.64±83.69	37.75±56.45	29.85±37.83	NS
Peritoneal TNF—7th day	**295.73±338.56**	**114.49±139.14**	107.02±111.35	**0.04**

The statistical differences are presented in bold. Cytokines values expressed as pg/ml

TNF tumor necrosis factor, *NS* not significant

*p=0.001 value for difference between groups with and without complications/p=0.02 value for difference between groups without complications and with dehiscence

**p<0.0001 value for both comparisons

recognized until the installation of severe sepsis. In addition, there is no laboratory marker that allows a faster diagnosis of the patients who will develop peritonitis. Radiological examinations are difficult due to the high weight of the patients and provide few reliable results. Thus, the surgeon's decision to reoperate is mainly based on his personal experience and clinical instinct [6].

In our patients, until the third postoperative day, the vital signs did not predict the onset of infectious complications and even in the group with staple line dehiscence the higher body temperature was still within the normal range and did not alert to the presence of this complication.

The alterations of the white cell count in the groups with infectious complications, although early, followed the same pattern as the vital signs. There was no leukocytosis until the second postoperative day.

Baigrie et al. [10] demonstrated that in patients submitted to elective repair of abdominal aortic aneurism, the systemic levels of IL-6 were higher in the sub-group of patients with complications. The increase in this cytokine was very early, only 6 to 8 h after the abdominal incision and preceded the installation of the clinical signs of complication by 12 to 48 h.

Terregrino et al. [12] observed that patients with systemic inflammatory response syndrome (SIRS) associated with infection (sepsis), admitted to an emergency service, and had higher IL-6 and TNF-α levels than patients admitted with SIRS but without infection.

Patel et al. [8] evaluated 41 patients with sepsis of abdominal origin and observed that the systemic levels of IL-6 predict patient mortality patients with 86.4% sensitivity and 78.9% specificity, values that were significantly higher that the APACHE II index (Acute Physiology and Chronic Health Evaluation), which presented 72.7% sensitivity and 57.9%specificity [8].

When we compared the groups with and without infectious complications we observed that there was no difference between them in the levels of systemic cytokines until the second postoperative day. However, when we separately analyzed the group with staple line dehiscence we observed that IL-1β levels were higher 48 h after surgery compared to the group without complications.

Although this is an important topic, little is known about the peritoneal cytokine response during abdominal sepsis.

Henegouwen et al. demonstrated that in patients submitted to pancreatoduodenectomy, the levels of peritoneal cytokines, mainly represented by TNF-α, tended to decrease along time. In the patients who presented complications, there was a second increase of peritoneal TNF-α levels preceding the complication [13]. Similarly, in patients with hepatic cirrhosis, the levels of peritoneal IL-6 were higher in patients with infected ascites compared to patients with sterile ascites [14].

In the present study, the levels of peritoneal cytokines were the most important early signs of infectious complications. In the group with these complications, IL-1 β levels were higher than in the control group as early as 24 h after surgery, a difference observed at all time points evaluated. Similarly, the group with staple line dehiscence showed higher levels of peritoneal cytokines even on the first postoperative day (IL-6–24 h and on postoperative day 7; IL-1 β 48 h after surgery).

This observation suggested that peritoneal cytokines are very early markers of abdominal sepsis and also showed that our cases of dehiscence occurred almost always on the first postoperative day. However, probably due to the fact that leakage was very small, several days passed before the clinical manifestation and identification of dehiscence. Another possibility is that, if leakage occurred so early, it was due to a technical problem. Since there was always a negative intraoperative methylene blue test, we can conclude that even this test has a low sensitivity in diagnosing this complication.

Thus, we can conclude that cytokines are good predictors of poor postoperative evolution in bariatric surgery since peritoneal cytokines diagnose better these infectious complications even before changes in blood count and before the occurrence of clinical manifestations. However, these determinations will need to be implemented in clinical practice.

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