ISSN 0100-6991
ISSN ONLINE: 1809-4546

CBC

Revista do Colégio Brasileiro de Cirurgiões

Journal of the Brazilian College of Surgeons

QUALIS B1 CAPES

Orgão Oficial

COLÉGIO BRASILEIRO DE CIRURGIÕES

Sociedade Brasileira de Hérnia e Parede Abdominal

INGLÊS

Volume 43 • Nº 2
Março / Abril de 2016

www.cbc.org.br

Indexado
DOAJ LILACS PubMed SciELO SJR SCImago Journal & Country Rank SCOPUS

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Revista do Colégio Brasileiro de
Cirurgiões

Órgão Oficial do Colégio Brasileiro de Cirurgiões

A REVISTA DO COLÉGIO BRASILEIRO DE CIRURGIÕES é indexada no Latindex, Lilacs e Scielo, Scopus, Medline/PubMed, DOAJ, Free Medical Journals e enviada bimestralmente a todos os membros do CBC, aos seus assinantes, a entidades médicas, bibliotecas, hospitais, e centros de estudos, publicações com as quais mantém permuta, e aos seus anunciantes.

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Tel.: + 55 21 2138-0659; Fax: + 55 21 2286-2595; E-mail: revistacbc@cbc.org.br
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Preço da assinatura anual: a vista, R$ 150,00
ou três parcelas de R$ 60,00
Números avulsos e/ou atrasados: R$ 40,00
Preço da assinatura para o exterior: US$ 248,00
Tiragem: 5.000 exemplares

International Standard Serial Number
ISSN 0100-6991

PUBLICIDADE

Tel.: (21) 3116-8300
E-mail: medline@medlineeditora.com.br

IMPRESSÃO e ACABAMENTO
Gráfica e Editora Prensa Ltda
Rua João Alvares, 27
Saúde - Rio de Janeiro - RJ
Tel.: (21) 2253-8343

PROJETO GRÁFICO
Artur Farias

PROJETO GRÁFICO - CAPA
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REVISTA DO COLÉGIO BRASILEIRO DE CIRURGIÕES

Indexada no Latindex, LILACS e SciELO, Medline/PubMed, Scopus, DOAJ e Free Medical Journals

DOI: 10.1590/0100-69912016002001

"I would like to be a surgeon, but ...." Will two years be enough?

"I would like to be a surgeon, but ...." Serão dois anos suficientes?

Elizabeth Gomes dos Santos, TCBC-RJ[1]

When Felix Harder presented his Presidential Address in the European Surgical Association Meeting in 2002 about this theme, the setting for general surgeons in the US was worrisome. There was a dwindling demand for the specialty and it was expected that in the near future there would be a lack of general surgeons as a labor force. The reasons for the decrease in demand for General Surgery as a specialty were many at that time: lifestyle, many hours of work for training, low pay, among some others. This is a phenomenon that also affects Brazil, perhaps with even greater intensity. And, at present, the situation is even more disturbing, due to the worsening of the precarious conditions in several training sites in our country, the lack of communication between capitals and country sides, the lack of preceptors with good training, and the the country's health precariousness as a whole.

When established in Brazil in 1977, the Medical Residency was defined by the educators of the time as "the best training method after graduation". One reason for its creation was the difficulty that medical schools had to offer the practical learning. At that time training followed Halsted's model, the apprenticeship. There was always a great Master in a Surgery service and future surgeons gravitated around him repeating what he did.

Times have changed, Surgery has evolved as science, the art has become even more refined. New technologies have been developed and have demanded even more time for skills development.

The process of forming a general surgeon and his practice seems even more difficult, requires even more attention. How much training time a surgeon needs to be considered an expert? How many operations of the same type does he or she need to repeat? How will be the ideal program? In the US some surgeons involved with medical education think that the program should encourage practical training. Others argue that the workload in research should be higher. The point common to all is that the program must have sufficient duration to provide adequate training, which, in surgery, means a chance to relentlessly repeat the moves until they becomes automatic. With these concepts in mind, medical residencies in the US and Canada have a duration of five years and in England and Australasia they range from six to eight years.

Brazil seems to go against the world, maintaining a two-year program, of which only 11 months are completed in general surgery itself due to the numerous required rotations.

Surgery is a specialty mainly dependent on practice. The development of technical skill is sedimented with the hands on, that is, practicing. By completing the training program, the resident should be competent to autonomously establish, but even in the US, only 38% of last-year residents (chief resident) think themselves competent to operate without supervision.

The Brazilian College of Surgeons (CBC) has always been committed to surgeons education. Its active participation in the National Medical Residency Comission (CNRM), with some of its registered members as evaluators of residency programs, has a long history. Its has long been fighting a constant struggle to building a program with curriculum matrices based on competences, where every year residents must fill the gaps in their learning according to specific requirements to be achieved at each stage. Also

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in the certification, ie, the granting of the Specialist title, the CBC follows strict standards through a high quality selection process and it requires a longer surgeon practical training.

The evaluation of technical skill gain is a vital component of the training and certification of experts. To certify is to assure, take for granted. In surgery it means that the specialist surgeon has the necessary requirements for practice. To automatically certify without knowing if the resident is properly prepared is a potential danger to patients. A recent CBC study, with national reach among all its Full and Emeritus members, concluded that three years is the minimum acceptable time for training a future surgeon. Based on this principle, an evaluation instrument was constructed, validated and applied to 60 residents of General Surgery programs and General Surgery - Advanced Program in Surgery Services in two major Brazilian cities, leading to a PhD thesis at the Federal University of Rio de Janeiro, which concluded that two years of training are not enough for the development of technical skills for daily and autonomous specialty practice. This conclusion is certainly the same as of any experienced surgeon. The difference is that with this work, we statistically proved that two years are insufficient for the training of a specialist.

The mission of the CBC is to bring this study to the CNRM for a change in the Medical Residency Program in General Surgery Curriculum Matrices, so that it be based on competences, and the certification based on technical skills assessment.

DOI: 10.1590/0100-69912016002002

Expression of Ki-67 and P16^{INK4a} in chemically-induced perioral squamous cell carcinomas in mice.

Expressão KI-67 e P16INK4a em carcinomas espinocelulares periorais quimicamente induzidos em camundongos.

Ângela Valéria Farias Alves[1]; Danielle Rodrigues Ribeiro[1]; Sonia Oliveira Lima[2]; Francisco Prado Reis[2]; Andréa Ferreira Soares[3]; Margarete Zanardo Gomes[4]; Ricardo Luiz Cavalcanti de Albuquerque Júnior[4].

ABSTRACT

Objective: to evaluate the influence of Ki-67 and P16^{INK4a} proteins immunohistochemical expressions on the clinical and morphological parameters of perioral squamous cell carcinoma induced with 9,10-dimethyl-1,2-benzanthracene (DMBA) in mice. **Methods:** we topically induced the lesions in the oral commissure of ten Swiss mice for 20 weeks, determining the time to tumors onset and the average tumor volume up to 26 weeks. In histopathological analysis, the variables studied were histological malignancy grade and the immunohistochemical expression of Ki-67 and P16^{INK4a} proteins. The correlation between variables was determined by application of the Spearman correlation test. **Results:** the mean time to onset of perioral lesions was 21.1 ± 2.13 weeks; mean tumor volume was 555.91 ± 205.52 mm3. Of the induced tumors, 80% were classified as low score and 20% high score. There was diffuse positivity for Ki-67 in 100% of lesions – Proliferation Index (PI) of 50.1 ± 18.0. There was a strong direct correlation between Ki-67 immunoreactivity and tumor volume (R = 0.702) and a low correlation with the malignancy score (R = 0.486). The P16^{INK4a} protein expression was heterogeneous, showing a weak correlation with tumor volume (R = 0.334). There was no correlation between the immunohistochemical expression of the two proteins studied. **Conclusion:** in an experimental model of DMBA-induced perioral carcinogenesis, tumor progression was associated with the tumor proliferative fraction (Ki-67 positive cells) and with tumor histological grading, but not with P16^{INK4a} expression.

Keywords: Carcinogenesis. 9,10-Dimetil-1,2-benzanthracene. Immunohistochemistry. Ki-67 Antigen. Genes, p16.

INTRODUCTION

Squamous cell carcinomas (SCC) represent the most prevalent oral cancer, accounting for about 90% to 95% of cases, being more frequent in the lower lip, tongue and oral floor[1]. Among the etiological factors of these malignancies, there is the action of tobacco combustion products in chronic smokers[2].

One of the most used chemical carcinogens in neoplastic dynamic study is the compound 9,10-dimethyl-1,2-benzanthracene (DMBA), which is an organic pollutant of polycyclic aromatic hydrocarbon type, largely released into the environment, especially due to human activity[3]. DMBA has cytotoxic, mutagenic and immunosuppressant properties[4,5].

The transformation of normal cells into malignant ones is mediated by disorders in several cell cycle regulating agents, whether positive or negative. Cell cycle progression is positively regulated by multiple cyclins and cyclin-dependent kinases, and negatively by a number of cyclin-dependent kinase inhibitors[6].

Ki-67 is a nuclear protein expressed in all phases of the cell cycle (G1, S, G2 and M), which, however, is absent in the G0 phase ("resting phase"). The precise function of the Ki-67 antigen is still unknown, but it has been suggested that this protein is possibly associated with the nucleolus and fibrillar components, and also seems to play an essential role in ribosome synthesis during cell division. Studies have shown that the Ki-67 immunohistochemical expression correlates with the proliferative potential of oral malignant tumors[7,8].

The p16^{INK4a} (p16) is a oncosuppressor protein encoded by the INK4a gene (also known as

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MTSI, CDK4I or CDKN2) located on chromosome 9p, locus 21, involved in cell cycle progression blocking process. It is inactive in a wide range human malignancies. The loss of p16^{INK4a} immunoexpression has been observed in the early stages of oral carcinogenesis and has been considered a molecular event of significant value in the prognostic analysis of such tumors[9,10].

This study evaluated the influence of Ki-67 and p16 proteins immunohistochemical expression on morphological parameters (mean tumor volume and histological malignancy grade) of DMBA-induced perioral SCC in mice. In addition, it sought to verify the existence of correlation between the immunoreactivity of p16 and Ki-67 proteins.

METHODS

The development of the study had the approval of the Ethics in Research Committee of the Universidade Tiradentes - Aracaju / SE, with protocol number 191208.

Animals and chemical carcinogenesis induction procedure

We used ten Swiss mice without distinction between gender, from the vivarium of the Universidade Tiradentes, with a body mass of about 150 ± 30g (Average age 100 days).

We induced the oral lesions in the mice left oral commissure by the topical application of 9,10-dimethyl-1,2-benzanthracene (DMBA), diluted to 0.5% in acetone, on a weekly basis in three alternate days for 20 weeks[11]. After this period, the animals were kept under observation for six weeks, and we duly registered the time of tumor onset (clinical) of each animal.

Macroscopic analysis of DMBA-induced injuries

To determine tumor volume, we used a digital caliper so that we could verify the average diameter of the induced lesions and apply the following formula[12]: $V = 4/3.\varpi.d$, where: V = volume; $\varpi = 3,14$; d = average diameter.

Specimens collection and histologic processing

After 26 weeks the animals were sacrificed in a CO_2 chamber (Insight, Ribeirão Preto, SP – continuous flow of 100% CO_2 for 50 minutes). Then the tumor area was subjected to post-mortem removal. The tissue specimens were fixed in buffered formalin (10%, pH 7.4) for 24 hours, dehydrated in increasing ethanol solutions and diaphanized in xylene for subsequent impregnation and embedding in paraffin.

For each tumor we obtained 15, 5µm-thick histologic sections, subject to routine staining with hematoxylin and eosin. The lesions were morphologically analyzed by light microscopy (Optical Microscope Olympus CX31). Two previously trained observers examined ten histological fields and classified the tumors according to a histological malignancy grading system[13]. This system aims both at the analysis of the tumor cell population, and at the host response by assessing parameters such as the degree of keratinization, nuclear pleomorphism, number of mitoses, invasion pattern, invasion stage and lymphoplasmacytic infiltrate. It has a established score between 1 and 4, as recommended by the authors. The sum of the scores was divided by six (the number of evaluated parameters) to obtain the average final score for each case. The evaluated cases were divided into two groups, based on the average final score: Group I, low score, with cases whose average value was less than 2.6; and Group II, high score, those with average values equal to or greater than 2.6.

Immunohistochemical analysis

We mounted 3µm-thickness histological sections on previously marked glass slides, which were than subjected to immunohistochemical reaction by the method of the indirect biotin-streptavidin. Sections were deparaffinized in xylene and rinsed in decreasing concentrations of ethanol (100%, 95%, 90%, 80% and 70%). To enhance the reaction, antigen retrieval was performed by immersing the sections into citrate solution and heating for 20 minutes in microwave. We performed the marking of p16^{IN-}

^{K4a} and Ki-67 proteins with rabbit anti-mouse monoclonal antibodies, types Ab-7 (Neomarkers, Fremont, CA, USA, dilution 1:100) and MIB-1 (Dako, Glostrup, Denmark, dilution 1:50), respectively, both for 30 minutes. The reaction was revealed using diaminobenzidine (DAB, Ventana Medical Systems, Tucson, AZ, USA) and counterstained with Meyer's hematoxylin. Both steps were developed in a four minute interval each. The positive control was performed with human tonsil (for Ki-67) and dermal nevocellular nevus (for p16)[14]. For negative control, we substituted primary antibody by phosphate buffered saline in the reaction.

Interpretation of the immunohistochemistry results

Cells whose nuclei and / or cytoplasm were stained brown by Ab-7 antibody (anti-p16) were considered positive, regardless of the immunostaining intensity. The grade of immunohistochemical expression was determined by intensity semiquantification (0, negative; 1, weak; 2, moderate; 3, strong) and percentage of positively stained cells (1, less than 30%; 2, between 30 and 60%;. 3, more than 60%) The final score of each tumor was calculated by summing the intensity and percentage scores, as previously described by Prowse et al.[15]. Cells whose nuclei were stained with MIB-1 antibody (anti-Ki-67), regardless of cytoplasmatic staining, were considered positive. The gradw of immunoreactivity was determined by the percentage of positive cells in 1,000 cells.

Statistical analysis

We applied the Spearman linear correlation test to determine the degree of correlation between mean tumor volume, malignancy grade and immunohistochemical expression of Ki-67 and p16^{INK4a} antigens. The correlation was stronger the closer to 1 was the R value.

To compare interobserver means, and determine the average values of the scores, we used the Student's t test, with significance level set to a value of $p < 0.05$.

RESULTS

After 26 weeks, all the animals developed perioral tumor lesions, with mean and standard deviation (SD) of 21.1±2.13 weeks for injuries onset. The mean tumor volume ± SD was 555.91±205.52 mm^3.

With respect to the specimens histological analysis, we observed that all visible tumors were squamous cell carcinomas. These were characterized by proliferation of keratinocytes, well to moderately differentiated, with varying degrees of individual keratinization (dyskeratosis) and in group (keratin pearls), infiltrating the adjacent mucosa and skin. We also found a predominantly lymphocytic inflammatory reaction of intensity ranging between mild, moderate and severe. As shown in table 1, of the ten cases of lip squamous cell carcinoma, eight (80%) were classified as low-grade malignant lesions, while only two (20%) were interpreted as having high degree malignancy. There was a moderate direct correlation between the mean tumor volume and tumor malignancy grade (R=0.659) (Figure 1).

As shown in table 1, all the analyzed tumors showed nuclear staining for the Ki-67 antigen, although at varying grades, with a mean ± SD proliferative index (PI) of 50.1 ± 18.0. In tumors with weak immunostaining (less than 30% reactive cells), we observed the immunohistochemical positivity predominantly in the basal parabasal layers of the nests and neoplastic sheets, whereas tumors with moderate (between 30 and 60% reactive cells) and stronger (more than 60% reactive cells) markings showed a quite diffuse positivity.

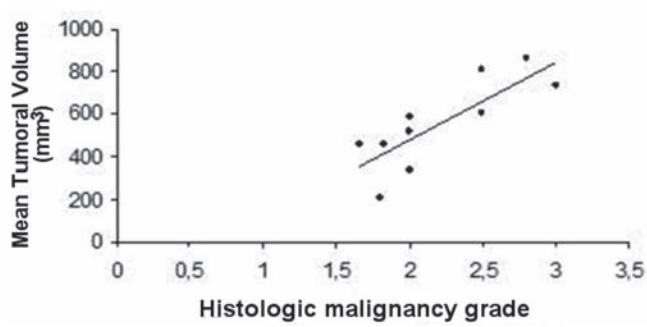

Figure 1 - *Degree of correlation between mean tumoral volume and histological malignancy grade (R=0.659).*

Table 1 - *Histopathological and immunohistochemical evaluation of DMBA-induced perioral squamous cell carcinomas.*

Animals	Score	Mean tumoral volume (mm^3)	Ki-67 (PI index)	P16^{INK4a} (%)
R1	1.8	207.9585	24	3
R2	2.8	858.965	68	1
R3	2	338.4535	39	3
R4	1.83	455.23	34	2
R5	2	517.0158	40	3
R6	2	583.8648	76	2
R7	3	730.5243	61	1
R8	1.66	455.6454	33	1
R9	2.5	600.6244	59	3
R10	2.5	810.85	67	2
Mean	2.209	555.9132	50.1	2.1
SD	0.45797	205.5257	18.05209	0.875595

SD - *Standard Deviation*

This antigen was also well expressed in tumor cells during all phases of mitosis.

We also observed a strong direct correlation between the PI index of Ki-67 positive cells and the mean tumor volume (R=0.702) (Figure 2a), but a weak one between the index and histological malignancy grade (R=0.486) (Figure 2b).

Regarding the p16^{INK4a} antigen immunohistochemical expression, there positivity was mild in 30% of cases, moderate in 30% and intense in 40% of analyzed lesions. The immunoreactivity pattern was quite heterogeneous, with staining sometimes nuclear, sometimes nuclear and cytoplasmic. Eminently nuclear immunostaining was most common in well-differentiated tumor cells located in the surface portion of the tumor. The nuclear / cytoplasmic positivity, on the other hand, was found in tumor cells of more central regions and rarely of the invasive front of the tumor. Keratinized areas (dyskeratosis and keratin pearl), as well as mitotic figures, were negative for this antigen.

Figure 3 shows immunostaining for p16^{INK4a} protein and immunohistochemical positivity for Ki-67 antigen.

By comparing the expression profile of p16^{INK4a} protein and the mean tumor volume, we found only a weak inverse correlation between these two variables (R=0.334) (Figure 4a), and no correlation between immunohistochemical expression of this antigen and histological malignancy grade (R=0.143) (Figure 4b). Also, there was no immunoreactivity correlation between the p16^{INK4a} protein and the Ki-67 antigen (R=0.124) (Figure 4c).

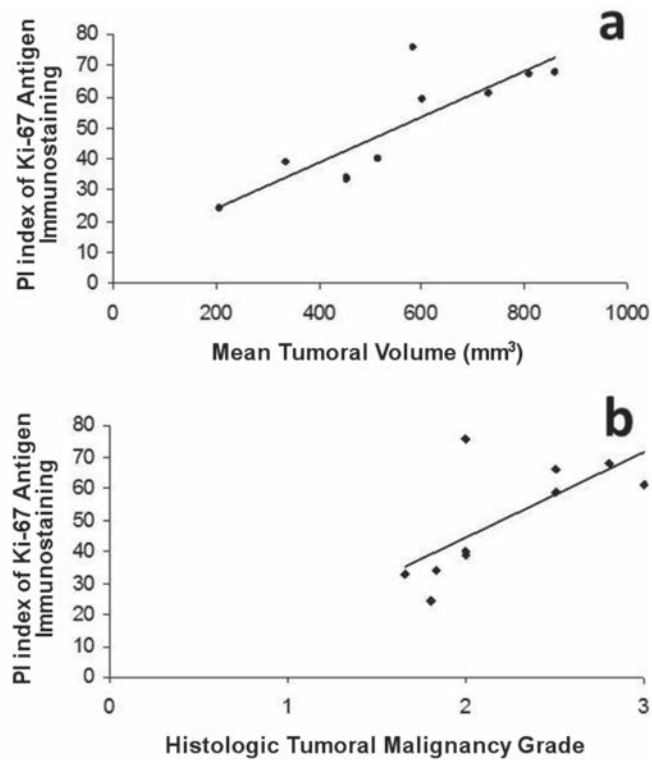

Figure 2. *a) Degree of correlation between the PI index of Ki-67 antigen immunostaining and the mean tumor volume (R=0.702); b) Degree of correlation between the PI index of Ki-67 antigen immunostaining and histological malignancy grade (R=0.486).*

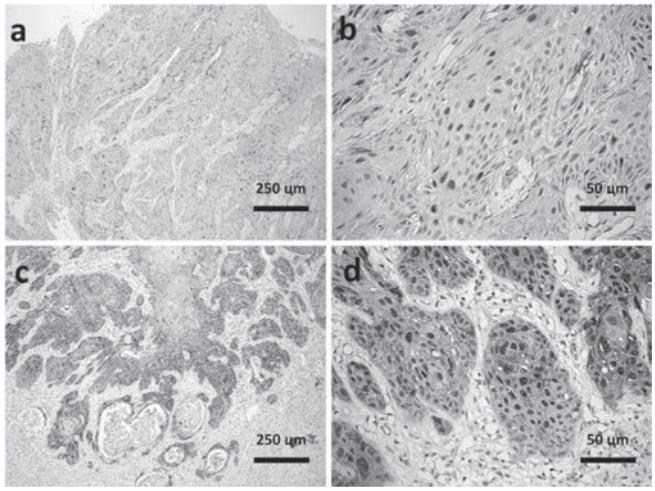

Figure 3 - *a) Immunostaining for p16INK4a protein in the surface areas of the tumor; b) Nuclear and cytoplasmic immunostaining pattern for p16INK4a; c) Immunohistochemistry positivity for Ki-67 antigen predominantly in basal and parabasal layers of nests and neoplastic sheets; d) Ki-67 antigen nuclear pattern.*

DISCUSSION

In this study, there was a strong direct correlation between the mean tumor volume and tumor malignancy grade, ie, the lesions classified as high grade showed the highest rates of tumor growth when compared with the low-grade ones, showing that morphologically undifferentiated cells are genetically unstable and easily escape from cell cycle control mechanisms, with a tendency to have high cell proliferation rates, in agreement with other studies[16,17].

The Ki-67 immunoreactivity relates to evidence of cell proliferation, so that it is expressed in all cell cycle phases but G0, in which cells are quiescent[18]. According to Sousa et al.[19], immunohistochemical analysis of this marker is an effective method for assessing the human malignancies growth fraction, providing valuable information about prognosis.

In the studied sample, we observed that the Ki-67 immunostaining showed varying grades, and in high-grade lesions there was strong expression in diffuse distribution. In some low-grade lesions, with evidence of high mean tumor volume index, we also found strong markings by said antibody, thus confirming the strong direct correlation between Ki-67 and this clinical parameter. Several authors report said correlation, such as Balassiano[20], who analyzed the expression of Bcl-2 markers, p53, mutated p53, caspase-3 and Ki-67 as prognostic factors in proliferative lesions of the oral cavity, such as inflammatory fibrous hyperplasia, actinic cheilitis and squamous cell carcinoma of the lower lip, and found high Ki-67 expression in all lesions.

The Ki-67 positivity kept a weak direct correlation with histological malignancy grade. This is due to the antibody's immunoreactivity instability. According to a published study[21], Ki-67 allows inferences about the time of life of a particular cell, stating only if it is in the cell cycle[21]. Therefore, it is possible that a particular neoplasm has a high proliferation rate and a low percentage of cells positive for this antibody.

Some authors evaluated the expression of PCNA, Ki-67, p53 and bcl-2 in patients with squamous skin carcinoma (n=10) and actinic keratosis (n=10), and confirmed the absence of Ki-67 expression in two cases, confirming the variability in this marker's immunoreactivity[18]. However, it is interesting to note that in several studies the Ki-67 presents a tendency to strong direct correlation with the lesion malignancy degree, being valuable as a prognostic predictor[17,18].

The $p16^{INK4a}$ tumor suppressor gene, encoding the p16 protein, is inactivated by hypermethylation in several types of malignancies, including oral squamous cell carcinoma, constituting a crucial event in the early stages of malignant transformation of the affected tissue.

Using immunohistochemical techniques, it is common to highlight the absence of $p16^{INK4a}$ protein immunoreactivity, a fact that is highly correlated with the findings provided by molecular techniques, which show inactivation of the aforementioned gene[22,23].

In this experimental group, there was little positivity for $p16^{INK4a}$ in high-grade lesions, and moderate to intense staining in low-grade

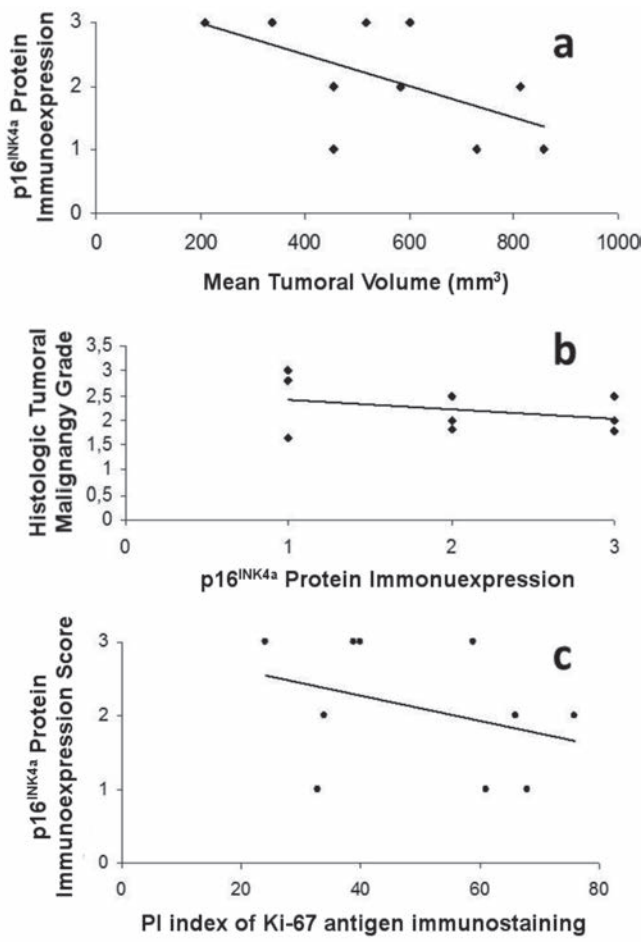

Figure 4 - *a) Degree of correlation between p16INK4a protein immunoexpression and mean tumoral volume (R=0.334); b) Degree of correlation between the p16INK4a protein immunoexpression and histological tumoral malignancy grade (R=0.143); c) Degree of correlation between the p16INK4a protein immunoexpression and the PI index of Ki-67 antigen immunostaining (R=0.124).*

ones. Immunoexpression was strictly nuclear in well-differentiated tumor cells, especially in surface areas, but also in the central areas of the tumor. These findings ar in agreement with other scientific work[16], which reported an immunolocalization trend of p16 in the central and surface areas of the tumor mass, with progressive decrease in regions of the invasion front, which concentrates the most undifferentiated cells, with a higher degree of cell adhesion loss.

Some studies show strong direct relationship between the lack of p16 immunoreactivity and the severity of the histological grading and clinical staging. However, we could not establish such correlation, consistent with another study[16].

We also found a weak inverse correlation between p16^{INK4a} expression and tumor volume, as well as no statistically significant correlation between the immunoreactivity of p16^{INK4a} and Ki-67.

According to the aforementioned authors, this lack of correlation of p16, with important prognostic parameters, is due to the fact that the inactivation of the p16^{INK4a} gene and its related protein would occur in the early stages of oral carcinogenesis and therefore would be more efficient as early markers of malignant transformation than as prognostic markers, being unreliable in predicting the biological behavior of neoplastic lesions.

The results of this study show that tumor volume is an important clinical parameter to measure the aggressiveness of malignant neoplasms, and Ki-67 immunoreactivity was effective as a marker of cell proliferation. Nevertheless, such marker not always displays a significant correlation with the immunoreactivity pattern of proteins that regulate the cell cycle, due to the instability of its expression in the tumor parenchyma.

Moreover, when one intends to correlate the expression of proteins that control the cell cycle with the degree of tumor malignancy, there may be inconsistencies justified by the fact that these proteins either act by independent molecular pathways or at different stages of the cell cycle and of tumor progression, such that their expression may not reflect the proliferative potential of malignant lesions.

In conclusion, this study showed that in the perioral carcinogenesis induced by DMBA in an experimental model, tumor progression is associated with the proliferative fraction of the tumor (Ki-67 positive cells) and with tumor differentiation, but without correlation with the p16^{INK4a} protein expression. New studies are necessary to elucidate the mechanisms of action of genes and proteins involved in the cell cycle.

RESUMO

Objetivo: *avaliar a influência da expressão imuno-histoquímica das proteínas Ki-67 e p16^{INK4a} sobre parâmetros clínico-morfológicos em carcinomas espinocelulares periorais quimicamente induzidos com 9,10-dimetil-1,2-benzantraceno (DMBA) em modelo murino.* **Métodos:** *as lesões foram induzidas topicamente na comissura labial de dez camundongos Swiss durante 20 semanas, sendo determinado o momento de surgimento dos tumores e volume tumoral médio até 26 semanas. Na análise histopatológica, as variáveis estudadas foram gradação histológica de malignidade tumoral e expressão imuno-histoquímica das proteínas Ki-67 e p16^{INK4a}. A correlação entre as variáveis estudadas foi determinada pela aplicação do teste de correlação de Spearman.* **Resultados:** *o tempo médio de surgimento das lesões periorais foi 21,1±2,13 semanas. Volume tumoral médio foi de 555,91±205,52mm3. Dos tumores produzidos, 80% foram classificados como de baixo escore e 20%, alto escore. Evidenciou-se positividade difusa para Ki-67 em 100% das lesões – índice de marcação (PI) de 50,1±18,0. Verificou-se correlação direta forte entre a imunoexpressão do Ki-67 e o volume tumoral (R=0,702) e fraca correlação com o escore de malignidade (R=0,486). A expressão da proteína p16^{INK4a} foi heterogênea, mostrando fraca correlação com o volume tumoral (R=0,334). Não houve correlação entre a expressão imuno-histoquímica das duas proteínas estudadas.* **Conclusão:** *Em modelo experimental de carcinogênese perioral DMBA-induzida, a progressão tumoral está associada à fração proliferativa do tumor (células ki-67 positivas) e com a gradação histológica tumoral, porém não com a expressão da p16^{INK4a}.*

Descritores: *Carcinogênese. 9,10-Dimetil-1,2-benzantraceno. Imuno-Histoquímica. Antígeno Ki-67. Genes p16.*

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Received: 13/10/2015
Accepted for publication: 02/03/2016
Conflict of interest: none.
Source of funding: none.

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DOI: 10.1590/0100-69912016002003

Bladder carcinogenesis in rats subjected to ureterosigmoidostomy and treated with L-lysine

Carcinogênese de bexiga em ratas submetidas à ureterossigmoidostomia tratadas com L-lisina.

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ABSTRACT

Objective: to evaluate the effect of L-lysine in the bladder and intestinal epithelia in rats submitted to vesicosigmoidostomy. **Methods:** we divided forty Wistar rats into four groups: group I – control group (Sham); group II – submitted to vesicosigmoidostomy and treated with L-lysine 150mg/kg; group III – submitted only to vesicosigmoidostomy; and group IV – received L-lysine 150mg/kg. After eight weeks the animals were sacrificed. **Results:** in the bladders of all operated animals we observed simple, papillary and nodular hyperplasia of transitional cells, transitional cell papillomas and squamous metaplasia. As for the occurrence of aberrant crypt foci in the colons of operated animals, we did not observe statistically significant differences in any of the distal, proximal and medium fragments, or in all fragments together (p=1.0000). **Conclusion:** Although statistically there was no promotion of carcinogenesis in the epithelia of rats treated with L-lysine in the observed time, it was clear the histogenesis of bladder carcinogenesis in its initial phase in all operated rats, this being probably associated with chronic infection and tiny bladder stones.

Keywords: Lysine. Carcinogenesis. Urinary Bladder Neoplasms. Epithelium. Therapeutics.

INTRODUCTION

Hammer described the first case of carcinoma in ureterosigmoidostomy in 1929[1]. The risk of cancer in anastomoses areas or bowel of patients undergoing surgery for urinary derivations, bladder expansions or bladder replacements with intestinal segments is known for a long time. Most are adenocarcinomas, but transitional cell carcinomas have also been described. Although the lag time between urinary bypass surgery and the onset of cancer is long, the risk of cancer after ureterosigmoidostomy is estimated at 200 to 500 times compared with the general population. However, the exact pathophysiology of this carcinogenesis process is not known. The earliest pre-neoplastic lesions in colorectal carcinogenesis are the dysplastic aberrant crypts foci (ACF), mucin depleted foci (MDF) and b-catenin-acumulated crypts (BCAC)[2]. Dornelas et al.[3] found that *L*-lysine has promotes bladder chemical carcinogenesis in rats. The objective of this study is to evaluate the effect of this amino acid in the bladder and intestinal epithelia of animals undergoing urinary diversion by vesicosigmoidostomy, a classic experimental surgical carcinogenesis model[4].

METHODS

The research project was conducted at the Department of Pathology and Forensic Medicine of Faculty of Medicine of the Universidade Federal do Ceará (UFC) and developed according to the protocol approved by the Ethics in Animal Research Committee (CEPA) of the Universidade Federal do Ceará.

We divided 40 Wistar rats weighing 150 grams into four groups: Group I (6 animals) was submitted to the opening and closing of the lateral walls of rectum and bladder; Group II (14 mice) was

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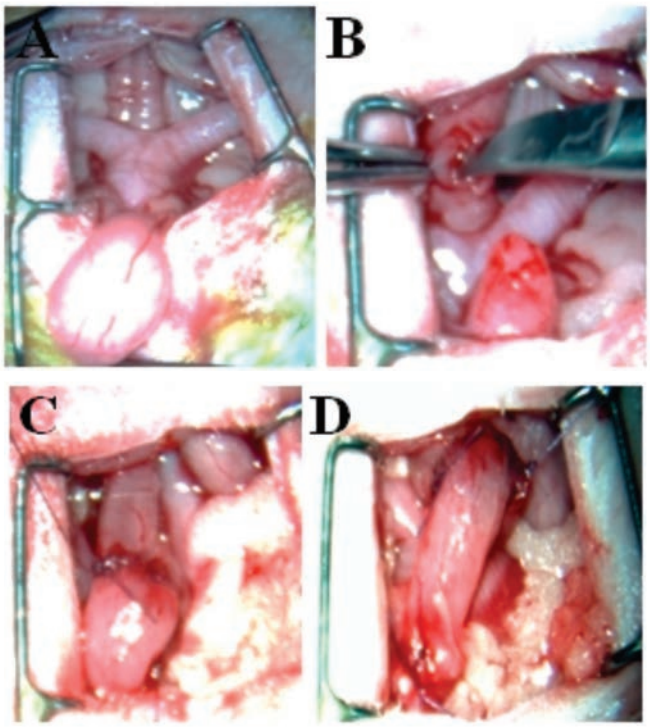

Figure 1. *Surgical steps of vesicosigmoidostomy by the Crissey 1980 technique: A) Bladder exposure, bicornuate uterus and cervix; B) bladder dome opening and longitudinal incision of colon wall; C) 7-0 Vycril continuous running suture of bladder dome to the colon; and D) suture of bladder neck and urethral section.*

subjected to vesicosigmoidostomy according to the Crissey technique[4] (Figure 1), and subsequently treated with *L*-lysine 150 mg/kg diluted in 0.5ml distilled water by gavage; Group III (14 animals) was submitted only to vesicosigmoidostomy; Group IV received only *L*-lysine 150mg/kg diluted in 0.5ml distilled water by gavage. After eight weeks we sacrificed the animals and carried out histological analysis (haematoxylin and eosin) of specimens from the the areas of rectal and bladder anastomosis; The colons was fixed and then stained with 0.1% methylene blue for evaluation of aberrant crypt foci in stereomicroscopy[5,6].

RESULTS

In all operated animals we macroscopically observed polypoid lesions located in the anastomoses regions and bladder epithelium. There were numerous tiny calculi inside the bladders of individuals submitted to vesicosigmoidostomy. In one animal, there was dilation of the right ureter and faceted calculi therein, forming a Stone Street (Figure 2).

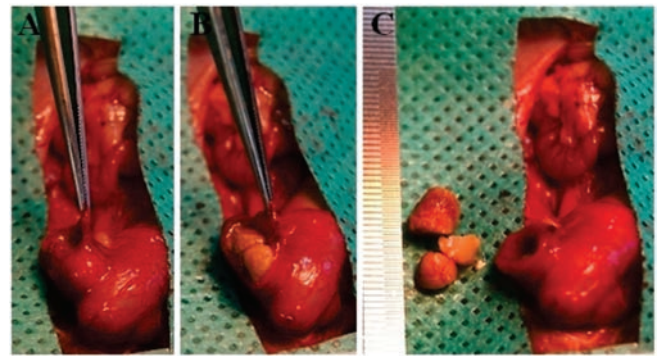

Figure 2. *A) dilated ureter in its near-bladder segment; B) opening of the ureter, displaying the stone street within; C) several multifaceted calculi, the biggest measuring 5mm.*

On histopathology, the bladder segment of all the operated animals displayed transitional cells simple, nodular and papillary hyperplasia, transitional cell papilloma, (Figure 3) and transitional cell papilloma with squamous metaplasia. In the intestinal segment in operated rats, there were rare aberrant crypts and foci of chronic colitis near the anastomosis area, and some animals presented with atrophy of the epithelium with mucin reduction in areas distant from the anastomosis.

Mortality among operated animals was 45% (18 animals). The main causes were kidney and lung abscesses.

Except by group IV, in all other groups, even in the one in which the animals were not operated, the stereoscopic microscopy identified rare ACF. All ACF contained only one crypt. There were no multiplicity of crypts or dysplasia signs in the ACF.

As for the occurrence of ACF in stereoscopic evaluation, when comparing Groups II and III (operated animals) with the Fisher's exact test, we did not find statistically significant differences considering the proximal (p=1.0000), medium (p=1.0000) and distal (p=1.0000) fragments, or all fragments together (p=1.0000) (Table 1).

Regarding the presence or absence of aberrant crypts, the data were expressed as median, interquartile range and minimum and maximum values of measurements made in five animals of Groups I and IV and six rats in Groups II and III. We used the Kruskal-Wallis test to compare the four groups, associated with the multiple comparison test of Dunn, to check for differences between groups in pairs. The

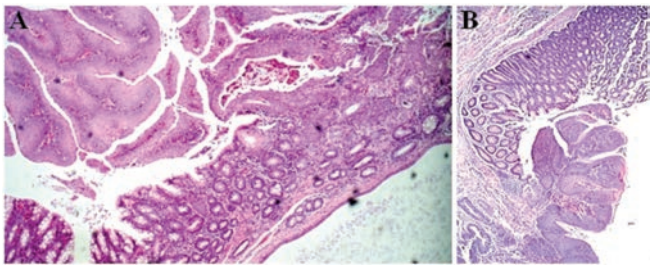

Figure 3. *A) histologic section showing colovesical anastomosis with transitional cell papilloma; and B) transitional cell papillary hyperplasia with squamous metaplasia. Hematoxylin and eosin 10x increase.*

results of the evaluation of the number of aberrant crypts found in the proximal, middle and distal colon segments are described in table 2 and in figure 4.

DISCUSSION

The carcinogenesis of anastomoses in urinary derivations with gastrointestinal segments remains unclear. Some mechanisms have been proposed to explain the pathophysiology, such as chronic inflammation, recurrent infections, hydrogenionic potential changes (PH) and production of carcinogens by bacteria, among other causes[7]. The first urinary diversion was the ureterosigmoidostomy, followed years later by colocystoplasty, ileocystoplasty and gatrocystoplasty. Then, there was the appearance of malignancy in a higher percentage than in the general population in the different derivations and urinary bladder enlargements with gastrointestinal segments. Some authors postulated that patients under 25 years of age undergoing ureterosigmoidostomy had 7,000 times greater risk for developing cancer than the same age population[8].

Many experimental studies were performed in rats using the Crissey vesicosigmoidostomy model[4], searching for histopathologic changes, without using carcinogens, and identifying chronic inflammation, hyperplasia and dysplasia with sulfomucins reduction and increased sialomucins[9]. Gitlin et al.[7] held gastrocystoplasty and ileocystoplasty in dogs and observed overgrowth of transitional epithelium in enterovesical and gastrovesical anastomoses. These cells expressed not only uroplakins (a molecular marker for urothelial differentiation), but also mucosubstance. They then suggested that these anastomotic cells possessed alterations and hybrid characteristics, possibly being vulnerable to neoplastic transformation.

Table 1. *Occurrence of aberrant crypt foci in groups II and III considering the proximal, middle and distal segments.*

Segments	Aberrant Crypt Foci		Significance (Fisher's exact test)
	Group II n / N (%)	Group III n / N (%)	
Proximal	3 / 6 (50)	2 / 6 (33,33)	P = 1,0000
Middle	1 / 6 (16,67)	0 / 6 (0,00)	P = 1,0000
Distal	0 / 6 (0,00)	1 / 6 (16,67)	P = 1,0000
All segments	4 / 6 (66,67)	3 / 6 (50)	P = 1,0000

n: number of rats with aberrant crypt foci in the group; N: number of animals in the group.

In 2012, in a study of 44 different centers between 1970 and 2007 with 17,758 patients undergoing urinary derivations and bladder plasty with intestinal loops, German researchers found 32 tumors[10]. The risk of tumors in ureterosigmoidostomy was 22 times higher, and in cystoplasty, 13 times higher, than in other forms of continent urinary diversion, such as neobladder, with statistical significance ($p < 0.0001$). The risk of tumors in ileocecal derivations (1.26%) and in colon derivation with neobladder was 1.43%, significantly higher ($p = 0.0001$) than in ileal neobladder (0.5%)[10].

Our approach for evaluation of colorectal carcinogenesis was the research of aberrant crypts foci (ACF). The ACF was originally described by Bird[5] in rats subjected to chemical carcinogenesis of the colon. However, some years later the same author suggested, and others have concluded that, the focus of aberrant crypts is in fact part of the sequential evolution of colon carcinogenesis, which can become dysplastic and may cause adenomas and later carcinomas, thus serving as a model of early, or pre-neoplastic, lesion in colorectal carcinogenesis[11-13]. The dysplastic ACF may present microsatellite instability, methylation with epigenetic silencing[14]. For this reason, it has been consecrated as a model for trials of new anticancer molecules, using the model in rats subjected to chemical carcinogenesis[13,15].

Do urinary derivations follow the course of this aberrant crypt foci model? The answer could be interesting if ACF research could be used in the preventive clinical evaluation of patients undergoing urinary derivations. Does ACF occur in vesicosigmoidostomy in rats? There are no reports in the literature. And fur-

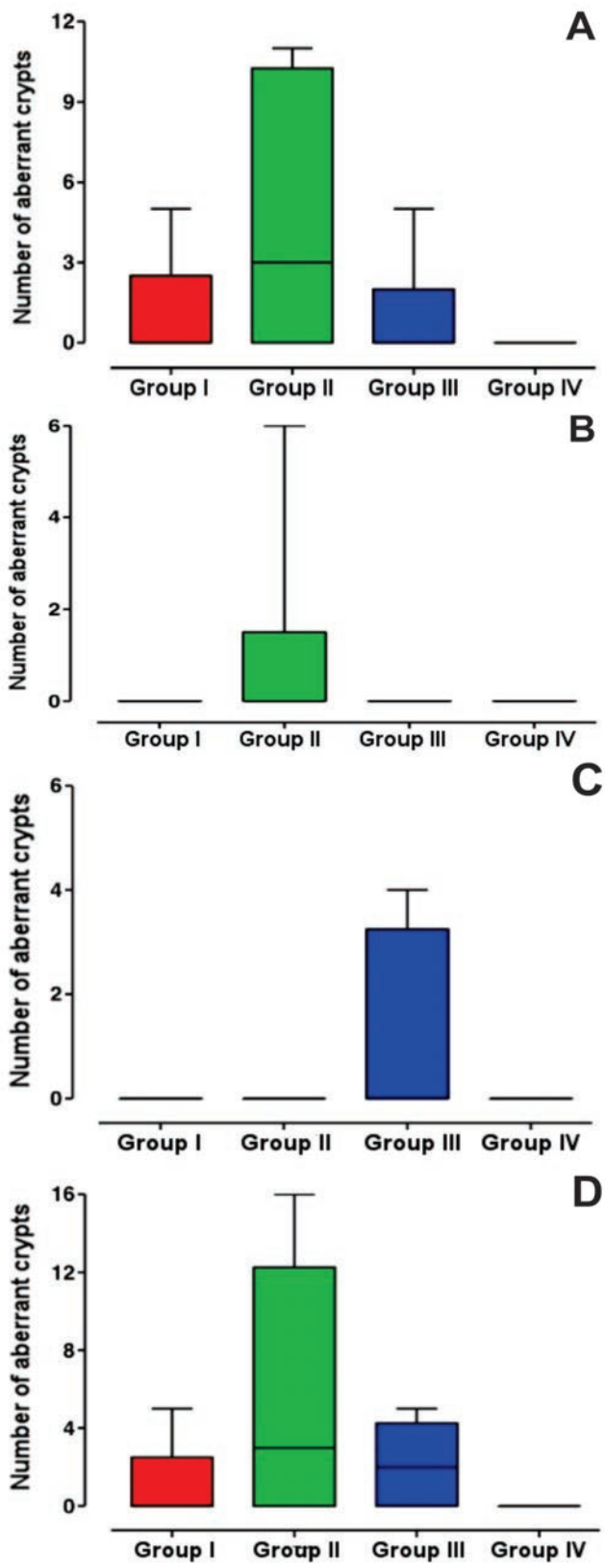

Figure 4. *A) Number of Aberrant Crypts observed in the proximal fragments of groups I, II, III and IV; B) Number of Aberrant Crypts observed in the middle fragments of groups I, II, III and IV; C) Number of Aberrant Crypts observed in the distal fragments of groups I, II, III and I; D) Number of Aberrant Crypts found in groups I, II, III and IV, considering all fragments: proximal, middle and distal.*

ther, can *L*-lysine promote carcinogenesis of the colon and/or bladder submitted to urinary diversion in rats? This occurrence is carried out by promoters in primed cells. Promoters are able to take primed cells to proliferation and, therefore, develop additional mutations. Promoters are not capable of producing mutation, but the condition of maintaining cell proliferation is required so that they can contribute to carcinogenesis[16]. In a recent study, Dornelas et al.[3] found that *L*-lysine has promoting action of bladder carcinogenesis in rats subjected to chemical carcinogenesis by BBN.

In our study we observed rare ACF. There were no multiplicities of aberrant crypts. All ACF contained only one crypt and there were no dysplasias in the ACF. Furthermore, despite a greater number of aberrant crypts in rats submitted to vesicosigmoidostomy, there was no statistically significant difference between groups II and III, so there was no promotion of carcinogenesis in rats treated with *L*-lysine. In addition, we observed ACF in non-operated animals. Although the significance of finding isolated ACF without dysplasias is still unknown, there are reports of ACF involution. There has also been described the spontaneous emergence of ACF in 344 Fisher rats without the use of a carcinogens[17,18]. The commercial diet can promote ACF induction in modified animals[19]. We should note that the observation time in our experiment was eight weeks. Some authors found that advanced age can influence the spontaneous appearance of ACF in humans[20].

When analyzing the graph showing the presence of ACF in the proximal (Figure 4A) segment and in proximal, middle and distal segments together (Figure 4D), we noted that there are greater numbers of ACF in animals undergoing surgery and treated with *L*-lysine than in those who were only operated without *L*-lysine treatment. However, there was no statistically significant difference.

The bladder segment, on the other hand, proved extremely reactive when subjected to derivation. All operated animals showed histological lesions in the bladder epithelium of transitional cell simple, papillary and nodular hyperplasia, transitional cells papilloma and squamous metaplasia, lesions already described as sequential in the bladder histogenesis/carcinogenesis in rodents[21].

Table 2. *Number of Aberrant crypt foci found in all segments in groups I, II, III and IV.*

Segments	Group I Median (Interquartile Range)	Group II Median (Interquartile Range)	Group III Median (Interquartile Range)	Group IV Median (Interquartile Range)	Significance (Kruskal-Wallis)
Proximal	0,0 (0,00 – 2,50)	3,0 (0,00 – 10,25)	0,0 (0,00 – 2,00)	0,0 (0,00 – 0,00)	P=0,2242
Middle	0,0 (0,00 – 0,00)	0,0 (0,00 – 1,50)	0,0 (0,00 – 0,00)	0,0 (0,00 – 0,00)	P=0,4459
Distal	0,0 (0,00 – 0,00)	0,0 (0,00 – 0,00)	0,0 (0,00 – 3,25)	0,0 (0,00 – 0,00)	P=0,1335
All segments	0,0 (0,00 – 2,50)	3,0 (0,00 – 12,25)	0,0 (0,00 – 4,25)	0,0 (0,00 – 0,00)	P=0,1477

Calcium salts precipitate at high pH and urate salts precipitate at low pH. In humans, urolithiasis is very common, but the association between calculi and bladder cancer is rare. However, in rats and mice, crystals and urolithiasis increase the likelihood of bladder carcinogenesis[22,23].

When the bladder epithelium is subjected to mechanical irritative processes (calculi, foreign body) and recurrent infection, it can evolve into reactive histologic lesions and also sequentially to carcinogenesis in humans.

The bladder epithelium is similar among species. However, there are anatomical differences that may pathophysiologically explain the association between calculi and carcinogenesis in animals. During voiding, the urinary system of rodents is in horizontal position, whilst the human is in vertical one. When the bladder contracts, it all "wrinkles" except by the trigone region. When there are stones in the bladder of rats, these loose crystals in the anterior wall promote mucosal damage throughout the bladder with contraction. In humans, foreign objects are located in the trigone region, which does not contract during urination. Mucosal damage is then decreased. For this same anatomical reason, human quickly eliminate the crystals that are within the bladder. When calculi cause obstruction in humans, they cause pain in most cases, what makes treatment and desobstruction to be arranged. Thus, anatomical factors can make rodents more susceptible to bladder carcinogenesis[23,24].

Our animals did not have a functioning bladder trigone. We ligated the bladder neck and sectioned it, but the bladder was still kept in the horizontal position and saccular, not eliminating the bladder contents through the rectum. This occurred in all operated animals of our experiment in a similar way (groups II and III). Urine, feces, repeated infections may have originated the miniature calculi and the histological lesions. All animals presented with miniature calculi in the bladder, and once the bladder of the animal is positioned ventrally due to four-paw ambulation, the irritating stimulus keeps constant and may explain the magnitude of the histological changes observed in bladder epithelium.

In conclusion, although statistically there was no promotion of carcinogenesis in the epithelia of rats treated with *L*-lysine in the observed time, the histogenesis of bladder carcinogenesis is clear in its initial phase in the bladder epithelium in all operated rats, this being probably associated with chronic infection and tiny bladder stones.

ABSTRACT

Objetivo: *o objetivo deste trabalho é avaliar o efeito da L-lisina nos epitélios vesical e intestinal de ratas submetidas à vesicossigmoidostomia.* ***Métodos:*** *quarenta ratas Wistar, foram divididas em quatro grupos: grupo I- grupo controle (Sham); grupo II- submetido à vesicossigmoidostomia e tratado com L-lisina 150mg/kg; grupo III- submetido apenas à vesicossigmoidostomia; e grupo IV- recebeu L-lisina 150mg/kg. Após oito semanas os animais foram sacrificados.* ***Resultados:*** *na bexiga de todos os animais operados observou-se hiperplasia simples, papilar e nodular de células transicionais, papiloma de células transicionais e metaplasia escamosa. Quanto à ocorrência de focos de criptas aberrantes nos colos dos animais operados, não foi evidenciado diferença estatística significante em nenhum dos fragmentos distal, proximal e médio, e todos juntos (P=1,0000).* ***Conclusão:*** *apesar de, estatisticamente, não ter havido promoção de carcinogênese nos epitélios dos ratos tratados com L-lisina, no tempo observado, é nítida a histogênese da carcinogênese de bexiga em sua fase inicial, no epitélio vesical, em todos os ratos operados, estando esta provavelmente associada à infecção crônica e aos diminutos cálculos vesicais.*

Descritores: *Lisina. Carcinogênese. Neoplasias da Bexiga Urinária. Epitélio. Terapêutica.*

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Received: 08/10/2015
Accepted for publication: 16/03/2016
Conflict of interest: none.
Source of funding: none.

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DOI: 10.1590/0100-69912016002004

The influence of nicotine in healing of small bowel anastomoses in rats: angiogenesis and miofibroblasts

A influência da nicotina na cicatrização de anastomoses do intestino delgado em ratos: angiogênese e miofibroblastos

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ABSTRACT

Objective: to know the effect of nicotine on angiogenesis and myofibroblast formation in anastomoses of the small bowel of rats. **Methods:** we randomly divided 60 Wistar rats into the groups Nicotine (N) and control (C), according to the proposed treatment. Each group was subdivided into three subgroups according to the time interval used for the evaluation (7, 14 or 28 days). The N group with 30 animals received nicotine subcutaneously at a dose of 2mg/kg body weight, diluted in 0.3ml of 0.9% saline, twice daily for 28 days prior to the operation, and for more 7, 14 or 28 days, depending on the subgroup. The C group (also 30 animals) received only saline on the same conditions and time intervals. After 28 days we carried out an end-to-end anastomosis 10cm distal to the duodenojejunal flexure in each rat. After 7, 14 or 28 days after surgery, we euthanized ten animals of each group, sent specimens of the anastomosis areas, 1cm proximal to 1cm distal, to counting of blood vessels and myofibroblasts through immunohistochemical staining by the application of monoclonal anti-factor VIII antibodies and anti-smooth muscle alpha-actin. **Results:** the administration of nicotine led to the decrease in the number of blood vessels measured on the 28th postoperative day and the number of myofibroblasts measured on the seventh day following completion of the anastomoses. **Conclusion:** administration of nicotine was deleterious on angiogenesis and myofibroblast formation in rats' small intestine anastomoses.

Keywords: Wound Healing. Nicotine. Intestine, Small. Anastomosis, Surgical. Rats.

INTRODUCTION

With the growing concern and the global debate on the harmful effects of tobacco on the human body, much attention has been directed to the adverse consequences of smoking. The direct and indirect costs related to diseases linked to smoking consume considerable resources for health in our country. Neoplasms, cardiovascular and respiratory diseases associated with smoking are well documented. However, less attention has been paid to surgical complications related to smoking[1-4].

Although tobacco products are consumed for hundreds of years, only in the twentieth century there was a sharp increase in consumption, the cigarette being the most important form of use. Its toxic smoke has more than 5,000 elements, of which nicotine is the primary vasoactive component, considered to cause the smoker's addiction, as it strengthens and enhances the desire to smoke[3,5,6].

The so-called granulation tissue, essential for a healthy scar development, comes at the beginning of the second stage of healing, called proliferative, being mainly composed of newly formed blood vessels (angiogenesis) and modified fibroblasts, called myofibroblasts4. While angiogenesis provides the blood supply suitable for the high level of metabolic activity that the healing process requires, myofibroblasts, through their contractile force, approach the damaged tissue edges[7].

In 1977, Mosely and Finseth[8] drew attention in a pioneering way to the undesirable effects of nicotine on tissue healing. Since then, several clinical and experimental studies have tried to explain these effects, demonstrating that this substance causes deficiencies in several factors involved in the healing process, in areas as diverse as Plastic Surgery and Orthopedics[9].

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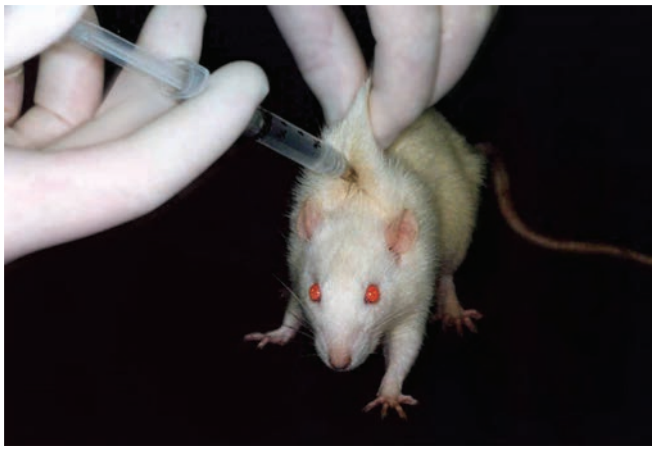

Figure 1 - *Nicotine Subcutaneous administration.*

Very little is known about the influence of nicotine on healing of the digestive tract, as well as the possible mechanisms involved10-13.

The aim of this study is to analyze the effects of nicotine on the healing process of anastomoses of the small intestine of rats as for the number of blood vessels and myofibroblasts present in the scar tissue.

METHODS

We performed the experimental procedures in the Surgical Research Center of the Post-Graduation Program in Surgery of the Universidade Federal of Paraná, and the immunohistochemical study at the Pathology Department, Hospital de Clínicas, Universidade Federal do Paraná, and at the Instituto de Pesquisas Médicas (Ipem) of the Hospital Universitário Evangélico de Curitiba, being duly previously reviewed and approved by ethics in research review committees of the aforementioned institutions.

This study used 60 male Wistar rats aged 160-200 days (average 180) and weighting between 270 and 290g, coming from the Instituto de Tecnologia do Paraná (TECPAR), being held in 12-hour day / night cycles and constant room temperature of 24oC. The animals fed on chow proper for the species and had free access to water throughout the experiment.

We randomly divided the rats into two groups: Group N, with 30 animals submitted to the application of nicotine; and Group C, also with 30 rats, which served as a control. Each group was divided into three subgroups of ten rats each. They were thus named N7, N14, N28, C7, C14 and C28, according to the postoperative evaluation time.

In group N nicotine was administered (Nicotine dihydrogen tartrate salt – Sigma, Saint Louis, Missouri, USA) subcutaneously (Figure 1) at the dose of 2 mg per kg bodyweight twice a day (12/12 hours) diluted in 0.3 ml 0.9% saline and adjusted to pH 7.4, for an initial period of 28 days prior to the surgical procedure. In group C we proceeded in an identical manner, though substituting the nicotine for 0.9% saline solution. The rats were weighed weekly, and the dose of nicotine adjusted when necessary. This dosage was established after calculation so that the amount of nicotine were equivalent to two cigarette cards per day in an adult human.

On the 28th day of application we withdrew feeding for 12 hours prior to the operation, maintaining free access to water. The surgical procedure took place the next day.

The rats were subjected to inhalation anesthesia with halothane in a fume cupboard. We aseptically held a 4 cm long laparotomy and performed an intestinal anastomosis 10 cm distal to the duodenojejunal flexure, performing a cross section of the isolated bowel loop and an end-to-end anastomosis, with 6-0 polypropylene suture in a single plane with separated full-thickness stitches, in a total of eight stitches (Figure 2). The wall of the abdomen was closed in two planes, with a continuous running suture with 3-0 multifilament polyglactin. The animals received free access to water immediately, and to food, 12 hours after the procedure.

The administration of nicotine or saline solution remained even on the day of operation and for more 7, 14 or 28 days according the animal's subgroup, and on the same already reported conditions, when we held a new inhalational anesthesia and harvested the anastomosed segments considering 1 cm proximal to 1 cm distal to the anastomotic line, preserving the specimen in buffered formalin.

For immunohistochemical staining we applied anti-factor VIII monoclonal antibodies (polyclonal, Code 0082 - DakoCytomation - Carpin-

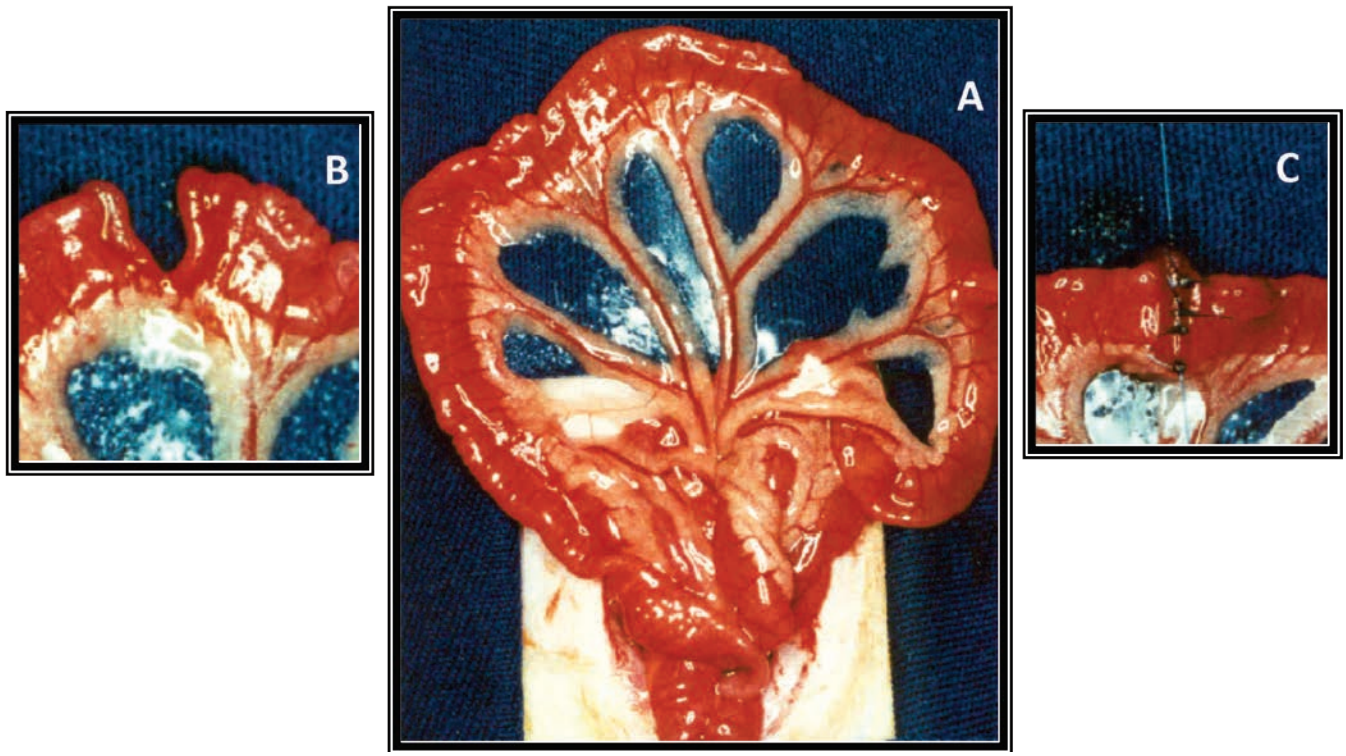

Figure 2. *Anastomosis construction in the small intestine. A) intact intestine; B) section of the intestinal loop; C) anastomosis.*

teria, USA) and anti-smooth muscle alpha-actin (Monoclonal Antibody, M0851 Code - DakoCytomation - Carpinteria, USA). We identified the myofibroblasts positivity in the areas of brownish pigmentation and we used positive and negative controls.

We identified the number of blood vessels by counting the circular structures positively stained by the anti-factor VIII antibody, which reveals the endothelial cells of the vessels' tunica intima. Counting was done in the area of the anastomosis, in a extension 10 mm proximal and 10 mm distal to it (total 20mm included with the anastomosis) with 10x magnification.

As for the quantification of myofibroblasts, we counted the perianastomotic cells positively stained for anti-smooth muscle alpha-actin antibody in a high-power field (40X objective). The scanned images were captured and analyzed by Image Pro Plus (Media Cybernetics, California, USA), through the "Measures" tool.

For each of the quantitative variables we adopted the Mann-Whitney nonparametric test, at a 5% significance level.

RESULTS

The evaluation of the number of blood vessels in the area of the anastomosis in the various phases of the postoperative period can be seen in Table 1. The administration of nicotine lead to a decrease in the number of blood vessels measured on the 28th postoperative day and in the number of myofibroblasts measured on the seventh day after the anastomoses.

The microscopic appearance of blood vessels with positivity for anti-factor VIII in shown in Figure 3.

The quantification of myofibroblasts in the various postoperative stages is shown in Table 2. An image of a myofibroblast stained by anti-alpha-actin antibody is seen in Figure 4.

DISCUSSION

In this experiment we observed that the use of nicotine led to a statistically significant decrease in the number of blood vessels on the 28th postoperative day and in the number of myofibroblasts on

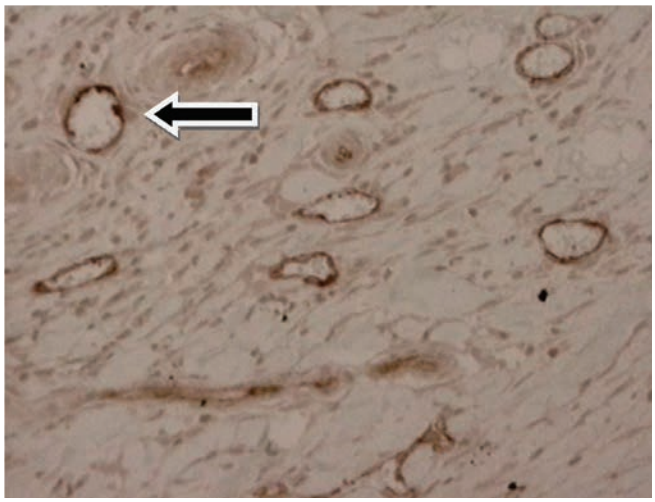

Figure 3. *Blood vessels with intima stained in brown, showing positivity for anti-factor VIII (original magnification 100X).*

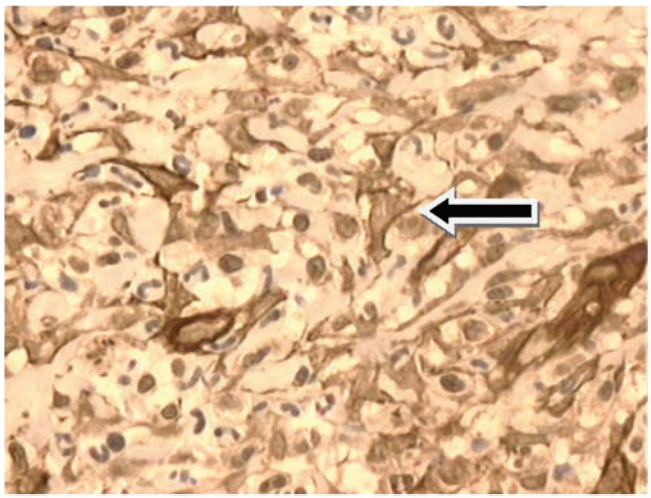

Figure 4. *Myofibroblasts stained in brown (anti-smooth muscle alpha-actin antibody), showing polygonal shape with cytoplasmic projections and vesicular nuclei (400X original magnification).*

the seventh day following completion of the anastomoses. Similarly, many previous studies determined that this drug acts negatively on the healing process in various tissues, leading to tissue vasoconstriction, decreased proliferation of lymphocytes, fibroblasts and collagen, among others14-21. The lymphocytes are an important source of angiogenesis-inducing cytokine production. Therefore, reduction of lymphocytes by nicotine18 can lead to decreased formation of new blood vessels in the scar tissue, as observed in this study. It is important to remember that the oxygen carried through the new vessels is a key factor in the synthesis of collagen, mainly responsible for the resistance in the scar tissue. Thus, decreased angiogenesis, with consequent deficit of oxygen in the scar tissue, may be one explanation for the decrease in collagen production and rupture strength in the region of intestinal anastomoses identified by previous studies3,7.

Therefore, nicotine may exert inhibiting effect on angiogenesis through several mechanisms: a- inhibition of angiogenic factors, either by direct negative action in their release mechanism or by deleterious effects on the cells that produce them, such as lymphocytes and fibroblasts5,13,18,19; b- direct deleterious effect on the endothelial cells5,11,19.

Nicotine also reduces the formation of fibroblasts in the healing process of intestinal anastomoses, which explains the deficit of collagen, mainly responsible for local rupture strength5.13, since these cells are the main source of production of that protein. As fibroblasts are the precursor cells of myofibroblasts, whose main function is to promote the approximation of the healing wound edges, the very formation of the latter would also be compromised.

Nicotine may therefore have a negative effect on the proliferation of myofibroblasts through different mechanisms: a- inhibition of the fibroblast precursors of

Table 1. *Number of blood vessels in the 7th, 14th and 28th postoperative days*

Number of vessels	Group	Mean ± SD	p value
7 days	Control	170.89 ± 57.74	1.0000
	Nicotine	159.33 ± 72.46	
14 days	Control	177.56 ± 126.66	0.2973
	Nicotine	107.89 ± 59.24	
28 days	Control	118.67 ± 71.48	0.0027
	Nicotine	62.11 ± 57.26	

Table 2. *Quantification of myofibroblasts number on the 7th, 14th and 28th postoperative days*

Number of myofibroblasts	Group	Mean ± SD	p value
7 days	Control	19.67 ± 6.16	0.0206
	Nicotine	13.63 ± 2.62	
14 days	Control	16.75 ± 6.16	0.6730
	Nicotine	17.89 ± 5.99	
28 days	Control	16.67 ± 6.06	0.0027
	Nicotine	14.63 ± 3.89	

myofibroblasts, either by direct action or by decreasing the oxygen level at the injury site, hampering multiplication and the functioning of these cells13; b- inhibition of cells producing fibroblast-proliferation stimulating cytokines, such as macrophages and lymphocytes15.

Many can be the mechanisms by which nicotine can exert harmful effects on the normal healing process. These should be clarified by further studies, as there are not enough previous works in the literature that have done this analysis for comparison.

In conclusion, according the data presented in this study, administration of nicotine was deleterious to angiogenesis and to myofibroblast formation in anastomoses of the small intestine of rats.

R E S U M O

Objetivo: *conhecer o efeito da nicotina sobre a angiogênese e formação de miofibroblastos em anastomoses do intestino delgado de ratos.* **Métodos:** *sessenta ratos Wistar foram divididos de maneira aleatória em grupos Nicotina(N) e Controle (C), conforme o tratamento proposto. Cada grupo foi subdividido em três subgrupos, de acordo com o intervalo de tempo utilizado para a avaliação (7, 14 ou 28 dias). O grupo N, com 30 animais, recebeu nicotina por via subcutânea, na dose de 2mg/Kg de peso, diluída em 0,3ml de solução salina a 0,9%, em duas aplicações diárias, durante 28 dias prévios à operação e por mais 7, 14 ou 28 dias, conforme o subgrupo. O grupo C (igualmente com 30 animais) recebeu somente a solução salina nas mesmas condições e intervalos de tempo. Após 28 dias efetuou-se, em cada rato, anastomose término-terminal a 10cm da flexura duodenojejunal. Após 7, 14 ou 28 dias da cirurgia, os dez animais de cada subgrupo foram eutanasiados, sendo que as áreas anastomosadas, 1cm proximal a 1cm distal, foram encaminhadas para contagem de vasos sanguíneos e miofibroblastos, através de coloração imuno-histoquímica por aplicação dos anticorpos monoclonais antifator VIII e anti-alfa-actina muscular lisa.* **Resultados:** *a administração de nicotina levou à diminuição do número de vasos sanguíneos aferidos no 28º dia pós-operatório e do número de miofibroblastos aferidos no sétimo dia após a realização das anastomoses.* **Conclusão:** *a administração de nicotina foi deletéria sobre a angiogênese e formação de miofibroblastos em anastomoses do intestino delgado de ratos.*

Descritores: *Cicatrização. Nicotina. Intestino Delgado. Anastomose Cirúrgica. Ratos.*

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Received in: 23/11/2015
Accepted for publication: 13/03/2016
Conflict of interest: none.
Source of funding: none.

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DOI: 10.1590/0100-69912016002005

Extended pelvic resections for the treatment of locally advanced and recurrent anal canal and colorectal cancer: technical aspects and morbimortality predictors aftet 24 consecutive cases

Ressecções pélvicas alargadas no tratamento do câncer colorretal e de canal anal localmente avançado ou recidivado: análise dos aspectos técnicos e fatores de morbimortalidade em 24 casos consecutivos

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ABSTRACT

Objective: to evaluate the profile of morbidity and mortality and its predictors related to extensive pelvic resections, including pelvic exenteration, to optimize the selection of patients and achieve better surgical results. **Methods:** we performed 24 major resections for anorectal pelvic malignancy from 2008 to 2015 in the Instituto do Câncer do Ceará. The factors analyzed included age, weight loss, resected organs, total versus posterior exenteration, angiolymphatic and perineural invasion, lymph node metastasis and overall and disease-free survival. **Results:** the median age was 57 years and the mean follow-up was ten months. Overall morbidity was 45.8%, with five (20.8%) serious complications. There were no deaths in the first 30 postoperative days. The median overall survival was 39.5 months, and disease-free survival, 30.7 months. Concomitant resection of the bladder was an isolated prognostic factor for higher risk of complications (87.5% vs. 26.7%, p = 0.009). Angiolymphatic invasion and lymph node metastasis did not reach significance with respect to disease-free survival. **Conclusion:** treatment of advanced anorectal tumors is challenging, often requiring combined resections, such as cystectomy and sacrectomy, and complex reconstructions. The magnitude of the operation still carries a high morbidity rate, but is a procedure considered safe and feasible, with a low mortality and adequate locoregional tumor control when performed in referral centers.

Keywords: Neoplasms. Rectal Neoplasms. Recurrence. Anal Canal. Pelvic Exenteration.

INTRODUCTION

Colorectal cancer (CRC) is the most common malignancy of the digestive tract1. For epidemiological purposes, included in this group are also the malignant anal canal neoplasms (ACN).

In recent decades, there has been great progress in treating these malignancies, with the incorporation of multimodal therapy with chemotherapy and radiotherapy. In ACN, for example, the exclusive chemoradiation therapy is curative in up to 85% of cases, reserving surgery only for recurrence or persistent disease2. For CRC, although radiotherapy and chemotherapy do not have a exclusive curative role, they have shown benefit in the context of adjuvant or neoadjuvant therapy. Surgery remains as the only well-established treatment with curative potential, also representing a rescue option in recurrence cases3. Extensive pelvic resections rise as the only possibility of cure for patients with extensive locoregional recurrence despite conservative surgical treatment, and in cases with invasion of adjacent organs. They comprise genito-urinary tract resections (pelvic exenteration – PE) and composite resections, including bone segments or soft tissue4.

PE was first described by Brunschwig 5, in 1948, being set as a high mortality surgery that consisted of removing all the pelvic organs, including the urinary system (bladder, distal urethra and prostate gland), the reproductive one (uterus, vagina) and rectosigmoid. Combined resections are characterized by the removal of organs or segments that do not include the ones mentioned above, such as the sacrum or coccyx, perineal skin, vulva etc.

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Table 1. *Degree of complications according to the Dindo-Clavien scale*

Grade	Definition
0	No complicator observed
1	Any deviation from the normal course without the need for pharmacological treatment or surgical, endoscopic and radiologic interventions Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics, electrolytes and physiotherapy. This grade also includes wound infections opened at the bedside
2	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions* and total parenteral nutrition are also included
3a	Requiring surgical, endoscopic or radiological intervention not under general anesthesia
3b	Requiring surgical, endoscopic or radiological intervention under general anesthesial
4	Death-threatening complication that needs intensive care support, with dysfunction of one organ
5	Death

** Neste trabalho, assim várias publicações mais recentes, modificou-se a classificação de Dindo-Clavien e hemotransfusão não foi considerada como complicação pós-operatória.*

Today, pelvic resections are carried out safely and with low mortality in many centers around the world, while their morbidity persists, ranging around 60% in cases requiring bladder resection4.

The objective of this study is to evaluate clinical and epidemiological aspects, technical details and prognostic factors of complications related to such large resections, to optimize treatment and selection of candidates for these procedures, providing a greater chance of cure, less morbidity and better quality of life.

METHODS

We conducted a retrospective cohort study including all cases of enlarged pelvic resections of the Departamento de Oncologia Digestiva in the period from January 1st, 2008 to October 1st, 2015, at the Hospital Haroldo Juaçaba of the Instituto do Câncer do Ceará (HHJ-ICC). We included only patients matching the following eligibility criteria: a) surgery conducted by HHJ-ICC staff; b) rectosigmoidectomy with or without abdominoperineal amputation of the rectum, associated with one or more procedures required due to direct tumor invasion: hysterectomy, cystectomy, prostatectomy, sacrectomy, coccyx resection vulvectomy, colpectomy, extensive perineal resection of soft tissue requiring flap rotation; and c) primary site set to rectosigmoid or anal canal. All data were recorded in a specific protocol form.

The variables analyzed were: gender, age, diagnosis, symptoms and weight loss, body mass index (BMI), type of surgery, blood transfusion, admission to the intensive care unit, method of reconstruction of the gastrointestinal and urinary tract, mortality and morbidity, lymph node involvement, angiolymphatic and perineural invasion, overall survival (OS) and disease-free survival (DFS).

The type of resection has been classified as: a) classic total pelvic exenteration (TPE): removal of the rectosigmoid with the bladder (in man) and the uterus (in female); b) classic posterior pelvic exenteration (PPE): resection of the rectosigmoid and uterus in women; c) other extended resections (OER): other resections not classified in the previous classifications.

For analysis of morbidity, when the OER cases also included pelvic exenteration, we classified them as TPE or PPE plus the specification of the additional segment removed.

Operative mortality was defined as death within 30 days of surgery. Morbidity was rated according to the scale of Dindo-Clavien6, with modifications, taking into account the clinical outcome and the therapy employed for resolution (Table 1).

For grouping of categorical variables, the complications were defined as minor (Clavien grades

1 or 2) and major (grades 3 or 4). We also split the category of major complications, separating them into non-severe (grade 3a) and severe (grades 3b and 4). The inclusion of this subdivision is justified by the fact that there is an important practical difference in the management of complications grades 3a and 3b.

We present results as median, mean and standard deviation, absolute and relative frequency. Categorical variables were compared within groups using the chi-square and Fisher's exact tests, as appropriate. All statistical analysis was performed with SPSS Statistics® software19. We considered a p value of 0.05 as significant. We also used a spreadsheet software to assist in the tabulation of data and preparation of graphics.

The work was approved by the Ethics in Research Committee of the Hospital Haroldo Juaçaba-Instituto do Câncer do Ceará, Opinion No 006/2011 of 24/02/2011.

RESULTS

From January 1st, 2008 to October 1st, 2015 we held 24 extended pelvic resections in the Departamento de Oncologia Digestiva of the Instituto do Câncer do Ceará for the treatment of colon, rectum and anus cancer exclusively. The same surgical team performed all procedures.

Twenty patients (83.3%) were female and four (16.7%) were male. The age ranged from 35 to 74 years, with a median of 57 years. In 14 (70%) cases we could obtain anthropometric data of the first hospitalization. The weight ranged from 37 to 65.5 kg (mean 51.7 and median 51.5), and the BMI, 16.23 to 31.57 kg/m2 (average 23.44 and median 22.5) (Table 2).

All patients were symptomatic, the period of time between onset of symptoms and the first visit ranging from two to 120 months. The most common symptom was rectal bleeding, reported by 19 (79.2%) patients, followed by pain in 17 (70.8%) and weight loss in 14 (58.3%).

In relation to multimodal therapy, all 24 (100%) patients underwent radiotherapy or chemotherapy during the course of treatment. Eighteen

Table 2. *Characteristics of the 24 patients who underwent pelvic exenteration.*

Total of patients (n)	24 (100%)
Gender	
Female	20 (83,3%)
Male	4 (16,7%)
Age (median)	57 (35-74)
BMI (n)	14
Malnourished (< 18,5 kg/m2)	2 (14,2%)
Eutrophic (18,5-24,9)	7 (50%)
Overweight (25-29,9)	3 (21,4%)
Obesity (>29,9)	1 (7,1%)
Tumor site	
Superior rectum	1 (4,2%)
Middle rectum	7 (29,2%)
Lower rectum	8 (33,3%)
Sigmoid	1 (4,2%)
Multicentric (colon e rectum)	1 (4,2%)
Anal canal	6 (25%)
Symptomatology	
Time of symptoms	6 (2-120)
Abdomino-pelvic pain	17 (70,8%)
Visible tumor	2 (8,3%)
Asthenia	3 (12,5%)
Weight loss (>10% in 6 months)	14 (58,3%)
Hematochezia	19 (79,2%)
Rectovaginal fistula	3 (12,5%)
Rectovesical fistula	1 (4,2%)
Complementary treatment	
Neoadjuvant radiochemotherapy	18 (75%)
Preoperatively CEA (n = 14)	3,55 (1,05-122,7)
Complications	
Respiratory complications	4 (16,7%)
Surgical site infection	6 (25%)
Abdominal wall dehiscence	4 (16,7%)
Acute renal failure	2 (8,3%)
Prolonged ileum	1 (4,2%)
Urinary retention	3 (12,5%)
Fistula of the urinary reconstruction	1 (4,2%)
Colostomy necrosis	1 (4,2%)

Source: HHJ-ICC (2008-2012).

(75%) patients underwent concomitant chemoradiotherapy before surgery, complementing systemic therapy postoperatively. Six (25%) underwent only adjuvant chemotherapy. The regimens were 5-fluorouracil (5-FU) with leucovorin (LV), with or without oxaliplatin, 5-FU with cisplatin (CDDP) and capecitabine alone.

Regarding the extent of resection, each case is detailed in Table 3. Additional organs removed in extended resections were: uterus and appendages, vagina, prostate and bladder, sacrum in the S3 level and below, coccyx, perineum and buttocks skin, vulva, right colon and kidney. The most commonly used soft tissue reconstruction method was the vertical myocutaneous flap of the rectus abdominis (VRAM) in seven cases, with construction of neovagina in two patients.

As for the reconstruction of the gastrointestinal tract, the preservation of the anus sphincter with a primary anastomosis was possible in nine (37.5%) cases. We performed a double-barreled wet colostomy (DBWC) in five (20.8%) patients and isolated terminal colostomy in ten (41.6%).

Seven (29.1%) patients received intraoperative blood transfusion, with a number of packed red blood cells that ranged from two to four.

The overall morbidity was 45.8%, with 17 postoperative complications in 11 patients; of these, five (20.8%) were severe complications (grades 3b and 4). There were no deaths in the first 30 days after surgery (Table 2).

Postoperative histopathologic study showed the size of the main injury ranging from 0.1 to 12 cm, number of lymph nodes resected from zero to 32 (mean 13, median 14.5) and residual margins free of disease in all cases.

The mean follow-up was ten months, ranging from one to 56. Mean overall survival was 39.5 months, and disease-free survival, 30.7, being 32.7 months for patients with CRC and 15.7 for the ones with ACN, without statistical significance ($p = 0.319$). Blood transfusion, angiolymphatic invasion and lymph node metastasis did not reach significance with respect to overall or disease-free survival (Figure 1).

Table 3. *Location of the primary tumor and the type of surgery performed.*

Cases	Primary site	Performed resection
1	Lower rectum	PPE + posterior colpectomy
2	Middle rectum	PPE
3	Anal canal	TPE
4	Lower rectum	PPE + posterior colpectomy
5	Lower rectum	TPE + colpectomy + vulvectomy + sacrectomy
6	Right colon	TPE + right colectomy
7	Middle rectum	TPE
8	Anal canal	TPE + VRAM reconstruction
9	Middle rectum	TPE + posterior colpectomy
10	Lower rectum	PPE + posterior colpectomy
11	Sigmoid	PPE + unilateral distal ureterectomy with psoas bladder
12	Anal canal	PPE + posterior colpectomy + VRAM
13	Lower rectum	PPE + posterior colpectomy + VRAM
14	Middle rectum	PPE
15	Lower rectum	PPE + posterior colpectomy
16	Middle rectum	PPE + posterior colpectomy + right colectomy
17	Middle rectum	PPE
18	Sigmoid	PPE
19	Lower rectum	TPE + left nephrectomy
20	Lower rectum + colon	PPE + total colpectomy + sacrococcygeal resection + total colectomy
21	Anal canal	PPE + posterior colpectomy + VRAM and neovagina
22	Anal canal	PPE + posterior colpectomy + VRAM and neovagina
23	Middle rectum	TPE + sacrococcygeal resection + VRAM
24	Anal canal	PPE + posterior colpectomy + VRAM

Source: *HHJ-ICC (2008-2012).*

TPE- Total pelvic exenteration; PPE- posterior pelvic exenteration; VRAM- vertical myocutaneous flap of the rectus abdominis

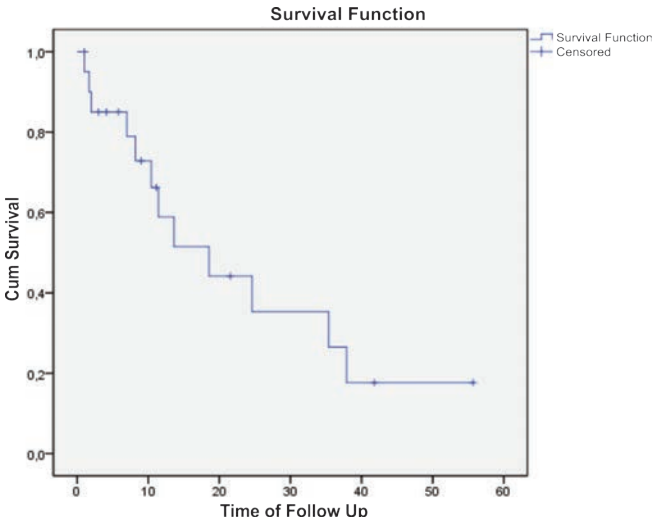

Figura 1. Curva de sobrevida calculada pelo método de Kaplan-Meier.

In the analysis of morbidity clinicopathological factors, we ovserved concomitant cystectomy (TPE) as the only factor that reached statistical significance for the presence of major complications, with a complication rate of 87.5% (p = 0.009, OR 19.5 [95% CI 1.7-209.5]). Age greater than 63 years (p = 0.659), intraoperative transfusion (p = 0.278) and weight loss greater than 10% in six months (p = 0.197) did not reach statistical significance as to overall morbidity (Table 4).

DISCUSSION

Large pelvic resections may represent the only possible cure treatment for advanced colorectal cancer, as well as for selected ACN cases. In general, surgery is the en bloc removal of the rectosigmoid together with the compromised adjacent organs, most commonly the genito-urinary tract (uterus and appendages, bladder and prostate) but often even more complex resections are required, such as colpectomy, vulvectomy or sacrectomy.

The first series of enlarged pelvic resections were published in the mid-twentieth century and focused specifically on PE. At the time, they displayed very high mortality, ranging from 14 to 26.9% in patients undergoing TPE for primary CRC7,8.

In recent years, however, there was a drastic reduction in surgical mortality, and today this ranges from 0-10% in specialized centers9,10. Among the main factors that contributed to the improvement in surgical mortality, we highlight the better patients selection, the adequacy of the urinary reconstruction technique and the better perioperative care.

Despite the marked improvement in mortality over the years, depending on the radicality required in the enlarged pelvic resections, these can also become mutilating procedures, carrying a high average morbidity rate of 30 to 60%, with a possible decrease in patients' quality of life11.

Nonetheless, when considering other types of treatment for advanced or recurrent anorectal tumors, the results are even more disappointing, with palliative radiotherapy associated with a median survival of 14 months and an average pain control time of only three months for CRC4. PE, on its turn, as a treatment for such cases, exhibits excellent symptomatic control, and five-year OS ranging from 10 to 30%11,12. Reported results from the Memorial Sloan-Kettering Cancer Center (MSKCC) in patients undergoing TPE for CRC showed specific cancer survival of 49 months, with a 40% survival at five years, ranging from 77% for locally advanced primary CRC cases and 28% for relapses 13,14.

In this study, the median overall survival was 39.5 months, similar to that reported by another Brazilian study12, of 37.7 months, although we can not make a direct comparison, since six (25%) of this study's cases are primary of the anal canal. Still, the benefit of extended pelvic resection is evident in the locoregional treatment of locally advanced or recurrent anorectal cancer, especially when compared to other treatment modalities.

In this study, six (25%) patients had relapsed ACN, a higher frequency than that reported in other national series, in which the rescue extended surgery was indicated due to ACN recurrence in only 4.3% of cases15.

The age range was similar to other previous publications, national and international, with medians ranging from 52 to 57.1 years9,12,13. The mean age of 60 years is a constant in various reported series, which can be explained by the clear selection of patients with better performance status to undergo a procedure of this magnitude, enhanced by high-

Table 4. *Morbidity factors associated with pelvic exenteration*

Clinicopathological factor	N	Absence or mild complications	Severe complications	
		N (%)	N (%)	*Valor p*
Age*				
< 63 years	17	10 (58.8%)	7 (41.2%)	0.659
≥ 63 years	7	3 (42.9%)	4 (57.1%)	
Intraoperative transfusion				
Yes	8	3 (37.5%)	5 (62.5%)	0.278
No	15	9 (60%)	6 (40%)	
Weight loss >10%				
Yes	14	6 (42.9%)	8 (57.1%)	0.197
No	9	7 (77.8%)	2 (22.2%)	
Total exenteration				
Yes	8	1 (12.5%)	7 (87.5%)	0.009
No	15	11 (73.3%)	4 (26.7%)	
Primary Tumor				
Anal canal	6	3 (50%)	3 (50%)	0.59
Colorectal	18	10 (55.6%)	8 (44.4%)	

Source: HHJ-ICC (2008-2012).

** we used the cutoff age of 63 years to match the 75th percentile of the sample.*

er surgical mortality observed in elderly patients as depicted in some older studies7. Being a major surgery, often leaving important consequences for life, such as colostomy or definite urostomy and urinary incontinence, there is always a selection bias of patients who will be submitted to this procedure, the procedure's risk-benefit and life expectancy being weighed, and the age often continuing as a limiting factor.

Regarding nutritional status, we obtained anthropometric data from medical records of 14 patients, of whom 50% were eutrophic. Two patients (14.2%) had malnutrition, while other four (28.5%) were characterized as overweight or obese by the BMI.

Although the proportion of patients with malnutrition according to the World Health Organization classification16 is small in this study, it is important to highlight the fact that in 14 (58.3%) cases there was weight loss higher than 10% in six months, which, as Waitzberg17, is indicative of severe malnutrition, increasing the incidence of fistulae and decreased cellular immunity function, with greater susceptibility to infectious complications. In this work, there were 57.1% of complications in the group with weight loss versus 22.2% in patients who did not have significant weight change, although this did not reach statistical significance (p = 0.197), possibly due to the small sample size .

All patients were symptomatic, with objective complaints such as chronic pain, severe bleeding and fistula, the time between the symptoms and the first visit to the reference service varying from two to 120 months, with a median of six months. Costa et al. showed similar results, with a total of 15 (100%) symptomatic patients, seven of whom have two or more symptoms18. Two of our patients had fistulas (rectovaginal or rectovesical). Both were female and had communication of the fistula path to the buttocks skin. In both cases, as also reported by Costa et al.18, the operation solved the symptoms of pain, bleeding, and fistula, with a clear improvement in quality of life (Figure 2).

As for the technical aspects, it is important to note that although Brunschwig5 has described and detailed TPE, radical pelvic resections can never be consid-

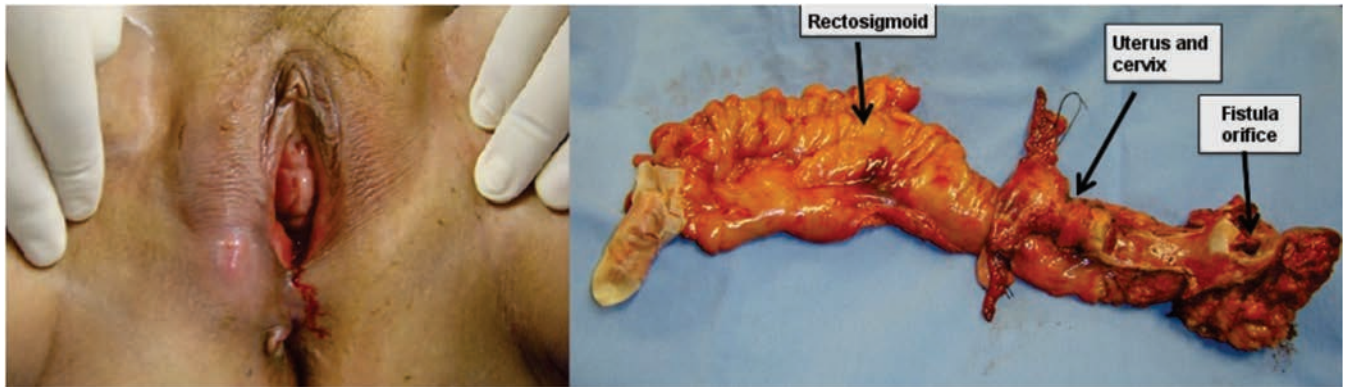

Figure 2 - *Rectovaginal fistula. B) Specimen with fistula orifice.*

ered fully standardized surgeries, since wider resections may be required to achieve absence of residual disease, including dissections ouside the conventional anatomical planes, with, for example, ureterectomy with bladder flap, sacrectomy, vulvectomy, etc. (Figure 3).

In this work, we carried out 16 PPEs and eight TPEs. Furthermore, additional resection was required in 12 patients, as described in Table 3. Importantly, in seven (29.1%) cases there was need of a myocutaneous flap rotation with perineal reconstruction, a well-established technique for the synthesis of large perineal defects and filling of the pelvic hollow. In two patients, reconstruction with VRAM flap provided the maintenance of sexual ability through the making of a neovagina. All procedures were performed in the same surgical time by a single team.

Even with all perioperative quality evolution, in this study the overall morbidity resulting from necessary resections and reconstructions still persisted at a level of 45.8%. In univariate analysis, the only factor that showed significance for the increase in complications was the realization of total pelvic exenteration, with a complication rate of 87.5% (p = 0.012). Similar results were previously described19, with 76.9% morbidity related to radical cystectomy in patients with irradiated pelvis.

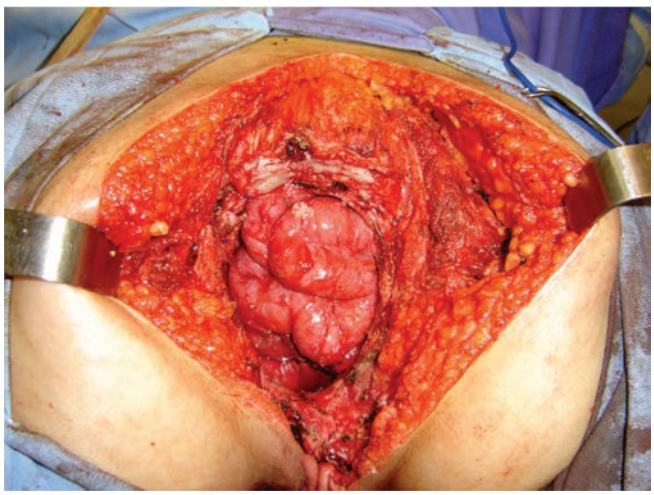

Figure 3 - *Surgical field after total pelvic exenteration with sacrectomy (posterior view)*

The morbidity difference between TPE and PPE found in this study is a well-established fact, the PPE morbidity being close to one of classical rectosigmoidectomy. Lohsiriwat et al. compared a group of patients treated with PPE with patients undergoing rectosigmoidectomy or rectal abdominoperineal resection20. There was no difference between the number of complications and hospital length of stay, the operative time (274 vs. 157min, p <0.001) and blood loss (769 vs. 203, p = 0.008) being higher for PPE. Thus, it is evident that there is a clear difference in surgical morbidity when adding resection of urinary tract.

Regarding follow-up, 13 patients were free of disease at the last follow-up visit, and nine had recurrence diagnosis (including deaths by relapse). In the literature, the main determinants of survival are the presence of lymph node metastasis and angiolymphatic invasion. Although there is a difference in survival dependent on these factors, there was no statistical significance, possibly due to the limiting sample and short follow-up time.

In conclusion, surgical treatment of locally advanced or recurrent colorectal cancer and anal canal is a complex surgical procedure, which has on average 50% morbidity, reaching levels higher than 80% when adding the resection of the urinary tract

in an already irradiated pelvis. The evolution of the surgical technique and the quality of perioperative care associated with a careful selection of patients have been able to reduce surgical mortality to values lower than 5% in specialized centers. Still, such procedures can present very high morbidity, especially when associated with concomitant cystectomy, which greatly affects the profile of complications.

Candidates for surgery should be evaluated with a careful selection, since additional resections are often necessary, followed by complex reconstruction procedures. Although there are few national publications on the subject, it is important to note that reported Brazilian series have satisfactory results, making extended pelvic resections feasible procedures in large specialized centers.

R E S U M O

Objetivos: *avaliar o perfil de morbimortalidade e seus fatores preditivos relacionados às ressecções pélvicas extensas, incluindo a exenteração pélvica, com o intuito de otimizar a seleção dos pacientes e obtenção de melhores resultados cirúrgicos.* ***Métodos:*** *foram realizadas 24 grandes ressecções pélvicas por neoplasia maligna anorretal de 2008 a 2015 no Instituto do Câncer do Ceará. Os fatores analisados incluíram idade, perda de peso, órgão ressecados, exenteração total versus posterior, invasão angiolinfática e perineural, metástase linfonodal e sobrevida global e livre de doença.* ***Resultados:*** *a mediana de idade foi 57 anos e o tempo médio de seguimento foi dez meses. A morbidade global foi 45,8%, com cinco (20,8%) complicações graves. Não houve óbito nos primeiros 30 dias de pós-operatório. A sobrevida global média foi 39,5 meses e a sobrevida livre de doença foi 30,7 meses. A ressecção concomitante da bexiga foi fator prognóstico isolado com maior risco para complicações (87,5% vs. 26,7%, p=0.009). Invasão angiolinfática e metástase linfonodal não alcançaram significância com relação à sobrevida livre de doença.* ***Conclusão:*** *o tratamento dos tumores anorretais avançados é desafiador, necessitando frequentemente de ressecções combinadas, como a cistectomia e sacrectomia, além de reconstruções complexas. A magnitude da cirurgia ainda carrega uma elevada taxa de morbidade, porém é um procedimento considerado seguro e factível, com uma baixa mortalidade e adequado controle locorregional tumoral quando realizado em centros de referência.*

Descritores: *Neoplasias. Neoplasias Retais. Recidiva. Canal Anal. Exenteração Pélvica.*

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Recebido em: 30/09/2015
Aceito para publicação em: 07/03/2016
Conflito de interesse: nenhum.
Fonte de financiamento: nenhuma.

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DOI: 10.1590/0100-69912016002006

Impact of neoadjuvant therapy in downstaging of lower rectal adenocarcinoma and the role of pelvic magnetic resonance in staging

Impacto da terapia neoadjuvante na diminuição do estádio no adenocarcinoma de reto baixo: papel da ressonância magnética da pelve na determinação do estádio

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A B S T R A C T

Objective: to evaluate the effect of neoadjuvant therapy on the stage (TNM) of patients with rectal adenocarcinoma and validate the use of MRI as a method of determining locoregional stage. **Methods:** we conducted a retrospective study of 157 patients with lower rectum adenocarcinoma, whom we divided into two groups: Group 1, 81 patients (52%) who had undergone surgical treatment initially, with the purpose to analyze the accuracy of locoregional staging by pelvic magnetic resonance imaging throug the comparison of radiological findings with pathological ones; Group 2, 76 patients (48%), who had been submitted to neoadjuvant therapy (chemotherapy and radiation) prior to definitive surgical treatment, so as to evaluate its effects on the stage by comparing clinical and radiological findings with pathology. **Results:** In group 1, the accuracy of determining tumor depth (T) and lymph node involvement (N) was 91.4% and 82.7%, respectively. In group 2, neoadjuvant therapy decreased the T stage, N stage and TNM stage in 51.3%, 21% and 48.4% of cases, respectively. **Conclusion:** neoadjuvant therapy in patients with rectal adenocarcinoma is effective in decreasing disease stage, and pelvic magnetic resonance imaging is effective for locoregional staging.

Keywords: Adenocarcinoma. Rectal Neoplasms. Neoadjuvant Therapy. Magnetic Resonance Imaging. Neoplasm Staging.

INTRODUCTION

Surgical resection of rectal cancer is still the only possibility of cure and is still regarded as the main form of treatment for many authors[1,2]. The evolution of the surgical technique, except for the access routes, reached its peak after finding that the total mesorectal excision and resection of the circumferential margin significantly decrease local recurrence[3].

In the 90s, it has become consensus that the treatment of adenocarcinoma of the rectum stages II and III would require, in addition to the operation, complementary chemotherapy and radiotherapy, after the discovery of their beneficial effects in reducing disease recurrence and increasing long-term survival rates[4]. Despite this evolution, the treatment of rectal cancer remains challenging, since long-term survival has not evolved consistently[5]. Eight large clinical series were published that analyzed neoadjuvant therapy for rectal cancer. All these studies demonstrated a superiority of this therapeutic modality when compared with surgery performed in an exclusive manner, as well as in relation to the adjuvant therapy[2].

Among the benefits of neoadjuvant radiotherapy and chemotherapy, there are: increased preoperative radiosensitivity of tissues due to the absence of surgical fibrosis, lower exposure of the small intestine to radiation, lower systemic toxicity, and decrease in lesions' size, which increase resectability and the sphincter preservation rate[6]. As disadvantages we have: the potential deficiency in the accurate determination of pathologic stage, which may result in failure of the postoperative planning, the postponement of definitive surgical treatment, and possible increase in morbidity and operative mortality[7]. Currently, patients with resectable lower

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rectal cancer in stages II and III should be submitted to neoadjuvant therapy provided they do not have medical contraindications[8].

Thus, the precise determination of the stage (TNM) is essential for the treatment to be well indicated[2]. As a general rule, Computerized Tomography of the chest and abdomen is the choice for the detection of metastatic disease (M), and the pelvic magnetic resonance imaging (MRI) or the transrectal ultrasonography are better to determine the locoregional stage (T and N)[9]. Conceptually, the ultrasound exam is superior in the analysis of smaller and more superficial tumors when compared with MRI, which has better accuracy in larger tumors that extend beyond the circumferential margin[10,11].

The objectives of this study were to evaluate the effect of neoadjuvant therapy on the stage of patients with low rectal adenocarcinoma and to validate the use of MRI as a method of determining locoregional stage.

METHODS

We held a retrospective analysis of 157 medical records of patients diagnosed with lower rectal adenocarcinoma during the period from February 2005 to October 2012. This study was approved by the Ethics in Research Committee of the Irmandade da Santa Casa de Misericórdia de São Paulo, under number 109,338.

We divided patients into two groups according to the initial therapeutic approach: Group 1, patients initially referred to surgical treatment, on an elective basis, after preoperative staging; Group 2, patients who, after having their stage determined, were referred to neoadjuvant therapy prior to definitive surgical treatment. The operation in these cases was performed eight weeks after completion of the neoadjuvant therapy, without further staging by imaging methods.

We performed preoperative staging by physical, proctologic and radiological examination, CT scan of the chest and upper abdomen to assess systemic disease (distant metastases) and pelvic MRI to evaluate locoregional involvement. The final stage was determined by the pathological examination of surgical specimens, associated with pre- and intraoperative findings. For the stage description, we adopted the system described by the American Joint Committee on Cancer[12].

All imaging tests in this series were performed at the Radiology Service of the Irmandade da Santa Casa de Misericórdia de São Paulo, using MRI machines models Philips Intera 1.0T or Philips Achieva 1.5T SE.

Depending on tumor location and intraoperative conditions, the performed procedures were abdominal rectosigmoidectomy or rectal amputation with total mesorectal excision.

The chemotherapy regimen employed in patients undergoing neoadjuvant therapy was 5-fluorouracil at a dose of 380 mg/m^2 and Leucovorin 20 mg/m^2 for five consecutive days (D1 to D5) concurrent with the first and fifth week of radiation therapy. The body surface area was obtained from the formula: Weight (kg)$^{0.425}$ x Height (cm)$^{0.725}$ x 71.84 /10.000.

Radiotherapy consisted of 28 sessions in five weeks and three days of 180cGy per session, total 5040 cGy.

We excluded from the study patients with history of colorectal cancer surgery, those operated in the emergency department or undergoing palliative surgery, and those who abandoned treatment.

We analyzed the variables gender, age at diagnosis, depth of tumor invasion in the rectal wall (T), lymph node involvement (N), presence of metastases (M), preoperative and final stages (TNM).

For the statistical analysis of the results we applied the Wilcoxon and McNemar tests to verify possible differences between variables T, N, M and the stage of both groups. We did not compare Groups 1 and 2. We used a spreadsheet software for data organization and the IBM SPSS (Statistical Package for Social Sciences), version 21.0, to obtain the results.

RESULTS

Of the 157 patients, 81 (52%) correspond to Group 1, in which the surgery was performed first,

and 76 (48%) to Group 2, in which the neoadjuvant therapy was performed before the operation.

The average age of Group 1 patients was 58.27 years ± 13.15, while in Group 2 it was 59.96 years ± 11.81.

In Group 1, 33 (41%) individuals were women, with a mean age of 58.63 years ± 13.44, and 48 men were (59%), mean age 58.02 years ± 13.09.

As for Group 2 patients, 37 (49%) were women, with a mean age of 59.56 years ± 12.62, and 39 men (51%), mean age of 60.33 years ± 11.15

Group 1 Results

The analysis of radiological and pathological correlation of the T variable detected no statistically significant variation, with accuracy of 91% (Figure 1).

The analysis of radiological and pathological correlation of the N variable detected no statistically significant variation, with accuracy of 83% (Figure 2).

The variable M remained constant, both pre and postoperatively. The correlation between clinical stage and the final stage showed agreement in 84% of cases. In 11% of cases, the stage was initially underestimated, and in 4%, overestimated. There was one case in which the lesion was not detected (Figure 3).

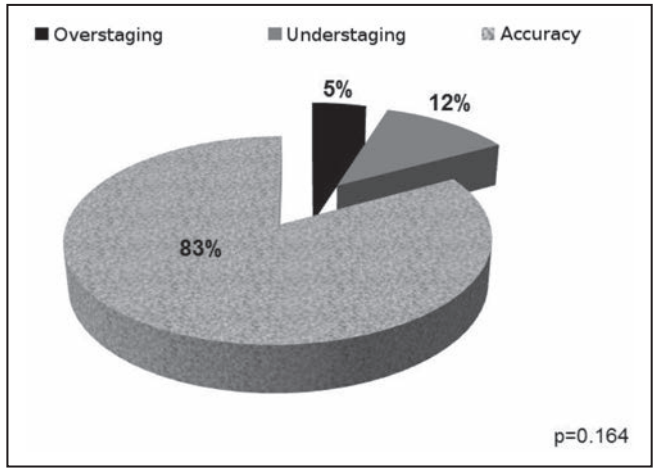

Figure 2: *Radiological and pathological correlation of changes in variable N in the 81 Group 1 patients. Source: ISCMSP, 2013.*

Group 2 Results

The analysis of the effect of neoadjuvant therapy on the variable T showed that there was regression in 51% of cases, and the pathological response (T0) occurred in 17% of cases (Table 1).

The analysis of the effect of neoadjuvant therapy on the variable N showed regression in 21% of cases (Table 2).

The analysis of the effect of neoadjuvant therapy on the variable M demonstrated that there was an increase in the occurrence of distant metastases of around 7%, with no statistical significance.

The analysis of the effect of neoadjuvant therapy on stage displayed a regression of 48.5%

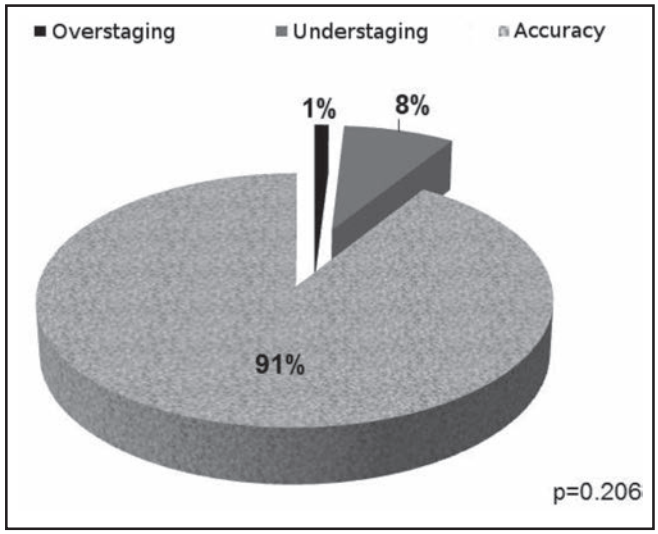

Figure 1*: Radiological and pathological correlation of changes in variable T in the 81 Group 1 patients. Source: ISCMSP, 2013.*

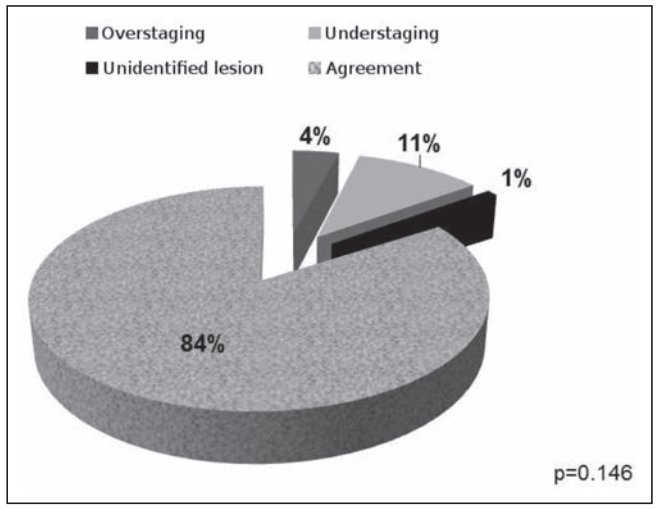

Figure 3: *Correlation between clinical and final stages in the 81 Group 1 patients. Source: ISCMSP, 2013.*

Table 1: *Variable T: comparison between radiological determination and histopathology in Group 2.*

Group	cT	ypT					Total	Sig. (p)
		0	1	2	3	4		
Neoadjuvancy	1	1	0	0	1	0	2	< 0,001
		1.3%	0%	0%	1.3%	0%	2.6%	
	2	2	1	4	6	0	13	
		2.6%	1.3%	5.3%	7.9%	0%	17.1%	
	3	8	1	13	21	1	44	
		10.5%	1.3%	17.1%	27.6%	1.3%	57.9%	
	4	2	0	1	10	4	17	
		2.6%	0%	1.3%	13.2%	5.3%	22.4%	
	Total	13	2	18	38	5	76	
		17.1%	2.6%	23.7%	5%	6.6%	100%	

Source: ISCMSP, 2013. Teste dos Postos Sinalizados de Wilcoxon.

and a 20% increase. We observed a complete pathological response rate of 14.5%, which corresponds to 11 cases (Table 3). The exclusion of stages I and IV shows that stage regression occurs in 56% of cases, with complete pathological response in 16%.

DISCUSSION

The first large prospective randomized study that demonstrated the effectiveness of neoadjuvant chemotherapy and radiotherapy came from Germany in 2004. It randomized 823 patients to receive chemotherapy and radiation preoperatively (421 cases) and postoperatively (402 cases). The authors found that the incidence of local recurrence at five years was 6% versus 13%, respectively. There was no significant increase in survival in five years between the groups[13].

Although the optimal regimen of neoadjuvant treatment is not yet well defined, there is no doubt of its effectiveness, especially in the control of local recurrence, and hence the increase in disease-free interval [2]. There is a polarization between the European and US institutions. In European publications, preference is mainly for short cycles of neoadjuvant radiotherapy, because they have lower morbidity. In American studies, similar to what was done in this study, preference is given to cycles with longer duration, arguing that the reduction of tumor size is more efficient.

The intent of this study was to analyze, in a stratified manner, the effects of neoadjuvant therapy on the stage (TNM) and on their individual variables in Group 2 patients. These variables were determined in two specific moments: preoperatively, with the aid of pelvic MRI (for the determination of locoregional stage – T and N) and chest and abdomen CT scan (for detecting distant metastases – M); and in the postoperative period, through histopathology data.

One might question maintaining the M variable in this study, since neoadjuvant therapy has essentially locoregional effects. However, its analysis

Table 2: *Variable N: comparison between radiological determination and histopathology in Group 2.*

Group	cN	ypN			Total	Sig. (p)
		0	1	2		
Neoadjuvancy	0	42	2	5	49	0,036
		55.3%	2.6%	6.6%	64.5%	
	1	11	7	1	19	
		14.5%	9.2%	1.3%	25%	
	2	2	3	3	8	
		2.6%	3.9%	3.9%	10.5%	
	Total	55	12	9	76	
		72.4%	15.8%	11.8%	100%	

Source: ISCMSP, 2013.Teste de McNemar.

Table 3. *Comparison between clinical and final stages in Group 2.*

Group	Clinical stage	Pathological stage								Total	Sig. (p)
		0	I	IIA	IIB	IIIA	IIIB	IIIC	IV		
Neoadjuvancy	I	1	3	5	0	1	0	0	0	10	0.012
		1.3%	3.9%	6.6%	0%	1.3%	0%	0%	0%	13.2%	
	IIA	6	8	10	1	0	1	2	2	30	
		7.9%	10.5%	13.2%	1.3%	0%	1.3%	2.6%	2.6%	39.5%	
	IIB	2	1	3	1	0	0	0	1	8	
		2.6%	1.3%	3.9%	1.3%	0%	0%	0%	1.3%	10.5%	
	IIIA	0	2	1	0	1	0	0	0	4	
		0%	2.6%	1.3%	0%	1.3%	0%	0%	0%	5.3%	
	IIIB	1	4	3	0	1	5	0	1	15	
		1.3%	5.3%	3.9%	0%	1.3%	6.6%	0%	1.3%	19.7%	
	IIIC	1	0	0	1	0	2	2	1	7	
		1.30%	0%	0%	1.3%	0%	2.6%	2.6%	1.3%	9.2%	
	IV	0	0	0	0	0	0	0	2	2	
		0%	0%	0%	0%	0%	0%	0%	2.6%	2.6%	
	Total	11	18	22	3	3	8	4	7	76	
		14.5%	23.7%	28.9%	3.9%	3.9%	10.5%	5.3%	9.2%	100%	

Source: ISCMSP, 2013. Teste dos Postos Sinalizados de Wilcoxon.

is mainly for the correct determination of the stage, which depends critically on the three variables (T, N and M). Furthermore, despite its systemic effects are still scarcely mentioned, some authors demonstrated that neoadjuvant chemotherapy may start the early systemic treatment of metastases, and be used as a marker of tumor response, which may enhance subsequent treatment[14].

Group 1 corresponds to a period in which neoadjuvant therapy was not yet established in the service. From the end of 2007 on, patients who presented in clinical stage II or III (T3 N0 M0 or T1,2,3 N1,2 M0) have been submitted to neoadjuvant therapy.

The reason for analyzing Group 1 patients, of different treatment, was primarily to assess the quality in determining the clinical stage in our service, as it was the same throughout the sample of this series, both in Groups 1 and 2. Thus, the bias of overstaging or understaging, that MRI may potentially present, was eliminated. In Group 1, there was a significant correlation between the clinical and pathological stages, which occurred in 91% of cases for the variable T and 83% for the variable N.

For many authors, pelvic MRI is considered the most suitable technique for determining locoregional stage, due to its high sensitivity and specificity in the analysis of structures adjacent to the rectum, including the mesorretal fascia[15]. Likewise, it is the only available technique for the proper assessment of the circumferential margin (CRM), currently considered one of the most important prognostic factors of local recurrence. In a recent American publication, the authors concluded that CRM ≤ 1mm is an independent risk factor for local recurrence, equivalent to surgical safety margin; CRM≤2mm, on its turn, is associated with the occurrence of distant metastases, regardless of tumor depth (T) and lymph node involvement (N)[16].

According to Mortensen *et al*.[17], the MRI accuracy in determining tumor depth varies with the level of rectum wall involvement, as follows: T1 lesions – 75%; T2 – 54%; T3 – 87%; and T4 – 86%. As for lymph node involvement, MRI's accuracy is up to 85%[15].

A meta-analysis published in 1997, involving 26 publications with 1,976 patients, found that endorectal ultrasound has an accuracy of 88% in stage determination[17]. Among the disadvantages related to ultrasound, we can mention that is an operator-dependent examination; It has low sensitivity in distinguishing inflammatory thickening from trans-

mural tumor extension itself; bulky and stenotic lesions are technically difficult to assess; its application in patients undergoing neoadjuvant therapy is still being determined, but the initial data are favorable to pelvic MRI[9].

MRI is indeed an excellent method for assessing tumor invasion in the rectal wall, but the same can not be said with respect to lymph node involvement, since the literature data are not so encouraging. It has increasingly been given importance to the morphological characteristics of perirectal lymph nodes, such as their heterogeneity and jagged edges, which are more predictive than their dimensions[18]. We should point out that 18% of lymph node metastases occur in lymph nodes smaller than 5mm[19].

This topic becomes even more important regaring the new stage determination after the neoadjuvant therapy. The literature reveals that the current diagnostic methods, such as positron emission tomography[20] and high-resolution MRI[21], are still inconsistent in the evaluation of residual, clinically undetectable disease. In this series, we did not have a new stage determination because we believe that the ideal cancer treatment should be based on the initially set clinical stage, so that there would be no change in surgical planning. Nevertheless, we found 14.5% of complete pathological response, which makes us think about new treatment perspectives. Another very relevant aspect is the great complexity of performing MRI in the service where the study was conducted, due to high demand and high cost.

There is a lot of controversy in the literature regarding the ideal time interval to perform the operation. Those defending shorter time intervals suggest that the operative difficulties are smaller due to lower incidence of adhesions and fibrosis arising from the pelvic radiation, allowing the realization of a more radical procedure; they also argue that the risk of disease dissemination would be lower. Those defending longer time intervals believe that the incidence of complete pathological response is higher. Tulchinsky *et al*.[22] found complete pathological response rates of 35% in patients operated after seven weeks, compared with 17% in those operated before this period. In our sample, the time interval between the completion of neoadjuvant therapy and surgery was eight weeks, and the complete pathological response rate was similar to the study of Rödel *et al*.[23], which reached 17%. However, stage regression in our study was 48.5%, an index similar to the one published by Kurių *et al*.[24], of 40%.

This relatively below average index, with regards to the complete pathological response we obtained in our study, can be attributed to some existing naming discrepancies in the literature to describe the tumor behavior to neoadjuvant therapy. It is clear that often used terms, such as "downstaging", "downsizing", "tumor regression", may be wrongly employed. Isolated alterations in variables T, N or M may not necessarily be interpreted as stage reduction. Stage regression shall be determined by the combined analysis of variables (TNM)[2]. WE should note that, by definition, the concept of complete pathological response should be translated as T0N0M0, ie no identification of tumor in the surgical specimen. The concept of complete clinical response, on its turn, is the absence of clinically detectable residual disease after neoadjuvant treatment[25].

We observed that neoadjuvant therapy regressed T stage in 51% of cases, and for variable N this index amounted to 21%. Despite this difference, both were significant from a statistical point of view. The relatively low response of N in relation to T is the one responsible for the great discussion generated around the therapeutic modality of expectant management in the face of complete clinical response[26,27].

There is no doubt that neoadjuvant therapy brings concrete benefits for patients with rectal adenocarcinoma, such as increased incidence of operations with sphincter preservation[28], although many questions are still far from being answered. Among them, how to identify non-responders? Despite the lack of statistical significance in our series, we found stage progression in 20% of cases. The importance of early identification of patients who will not respond to this treatment modality would probably prevent disease progression, since the time relapsed between the end of therapy and surgery is not negligible.

With the evolution of diagnostic imaging methods and advances in molecular biology, new neoadjuvant therapy protocols will emerge in the near future to guide more individualized treatment modes, reducing adverse effects and not delaying surgical treatment, which is undoubtedly still the only curative therapy.

In conclusion, in patients with rectal adenocarcinoma neoadjuvant therapy and magnetic resonance imaging of the pelvis are both effective, the former in stage reduction, and the latter as a method of determining locoregional stage.

R E S U M O

Objetivo*: avaliar o efeito da terapia neoadjuvante, nos pacientes portadores de adenocarcinoma de reto, sobre o estádio (TNM) e validar o emprego da ressonância magnética como método de determinação do estádio locorregional.* **Métodos***: estudo retrospectivo de 157 pacientes com diagnóstico de adenocarcinoma de reto baixo, que foram divididos em dois grupos: Grupo 1, 81 pacientes (52%), submetidos ao tratamento cirúrgico de princípio, cuja finalidade foi analisar a acurácia da determinação do estádio locorregional pela ressonância magnética da pelve, através da comparação entre os achados radiológicos e os achados anatomopatológicos; Grupo 2, 76 pacientes (48%), encaminhados à terapia neoadjuvante (quimioterapia e radioterapia), antes do tratamento cirúrgico definitivo, com o intuito de avaliar seus efeitos sobre o estádio, através da comparação dos achados clínico-radiológicos com os anatomopatológicos.* **Resultados***: no grupo 1, a acurácia da determinação da profundidade da lesão (T) e do comprometimento linfonodal (N), foram de 91,4% e 82,7%, respectivamente. No grupo 2, a terapia neoadjuvante diminuiu o estádio T, estádio N e o estádio TNM em 51,3%, 21% e 48,4% dos casos, respectivamente.* **Conclusão***: a terapia neoadjuvante nos pacientes com adenocarcinoma de reto é efetiva na diminuição do estádio e a ressonância magnética da pelve é eficaz na determinação do estádio locorregional.*

Descritores*: Adenocarcinoma. Neoplasias Retais. Terapia Neoadjuvante. Imagem por Ressonância Magnética. Estadiamento de Neoplasias.*

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Received: 01/1022015
Accepted for publication: 08/03/2016
Conflict of interest: none.
Source of funding: none.

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DOI: 10.1590/0100-69912016002007

The collagen, fibrinogen and thrombin biological adhesive is effective in treating experimental liver injuries

O adesivo biológico de colágeno, fibrinogênio e trombina é eficaz no tratamento de lesões hepáticas experimentais

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ABSTRACT

Objective: to evaluate the effectiveness of an collagen-based adhesive associated with fibrinogen and thrombin in experimental liver injuries in rats. **Methods:** we randomly divided 30 Wistar rats into three groups: A, B and C. All underwent a standard liver traumatic injury. In group A, the lesion was treated with the adhesive; in group B, with conventional, absorbable suture; group C received no treatment. We analyzed the time of hemostasis, mortality, occurrence of adhesions and any histological changes. **Results:** there was no statistical difference in relation to mortality (p=0.5820). The adhesive treated group showed the lowest hemostasis times (p=0.0573, odds ratio 13.5) and lower incidence of adhesions (p=0.0119). The histological alterations of the Groups A and B were similar, with foreign body granuloma formation separating the adhesive material and the hepatic stroma suture. **Conclusion:** the collagen adhesive associated with fibrinogen and thrombin was effective in treating experimental hepatic injury, providing a lower incidence of adhesions between the liver and surrounding structures.

Keywords: Wounds and Injuries. Liver. Hemostatics. Thrombin. Tissue Adhesives.

INTRODUCTION

The surgical techniques to approach liver bleeding include local compression, cauterization, bandages, sutures, resections and drainage[1,2]. In complex liver lesions accompanied by hemodynamic instability, laparotomy is indicated for bleeding control with eventual Pringle maneuver[2-4], ligation of affected vessels and ducts, as described by Patcher[2], and even damage control surgery[5].

The development of a wide variety of hemostatic agents and tissue adhesives that occurred in recent years[6] offers surgeons the opportunity to use these products in order to achieve quicker and easier bleeding control. The seriousness and the difficulty in managing certain cases of liver trauma motivate the search for new therapeutic alternatives, especially for bleeding control. The efficiency of the new hemostatic lead to the hypothesis to test the efficacy of collagen adhesives associated with fibrinogen and thrombin, compared with the conventional suture in the treatment of experimental traumatic liver injury.

METHODS

This experimental study was conducted in the Surgical Technique Laboratory of the Faculdade de Medicina de Jundiaí, Jundiaí-SP, and was approved by the Ethics Committee for Animal Use with number 81/110.

We included 30 adult, male, Wistar rats, with a mean age of 3.55 months, weighing on average 442,80g (342g-527g). The animals were randomly divided into three groups, A, B and C, ten subjects in each.

All rats received premedication with atropine at a dose of 0.05 mg/kg subcutaneously in the dorsal region and acepromazine (Acepran® 1% – Univet, São Paulo) 1mg/kg by the same route. After 15 minutes of application of premedication, they received an association of tiletamine and zolazepan (Zoletil® 50 – Virbac, São Paulo) 20mg/kg intramuscularly. We initiated the operative procedure after full action of the anesthetic drugs, monitored by loss of corneal and eyelid reflexes and limbs flexion.

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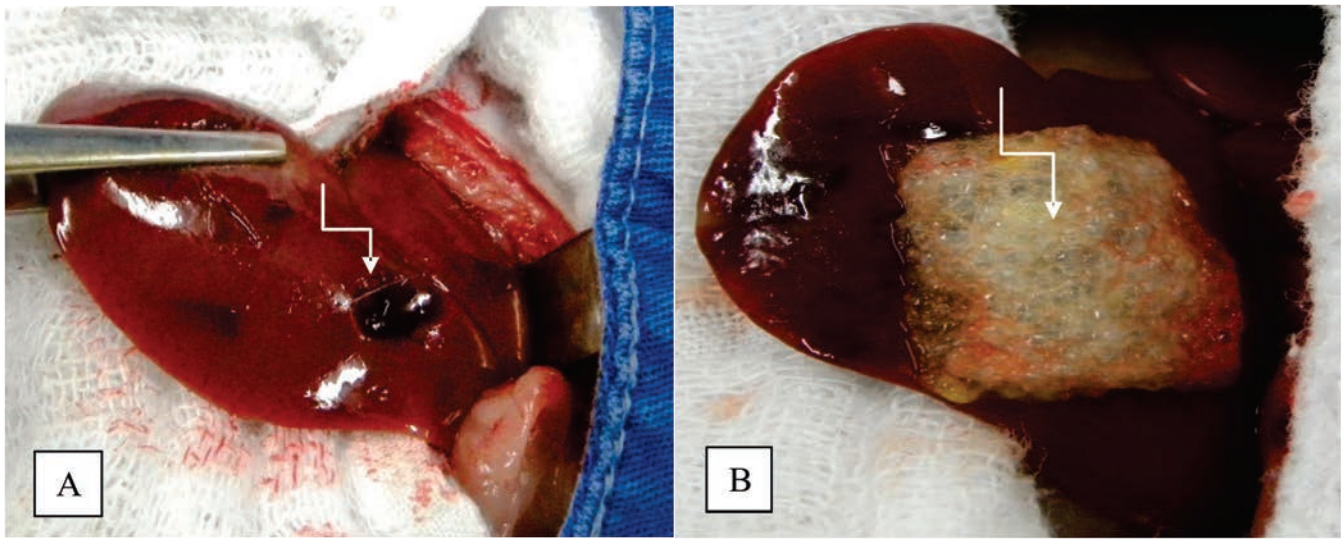

Figure 1. *A) Hepatic Injury (2x magnification); B) Final aspect of the adhesive, indicated by the arrow on the liver injury (2x magnification)*

All rats underwent laparotomy under aseptic technique, started from the xiphoid, approximately 3cm long. After opening the abdominal wall, we positioned a small orthostatic retractor and identified the liver, the organ chosen to perform the standardized injury with a biopsy surgical instrument (Punch Keyes® – ABC Surgical Instruments, Brazil) 5mm in diameter, introduced 5mm in depth into the parenchyma (Figure 1A).

From then on, we treated the animals according to the group to which they belonged. In Group A, after one minute of bleeding we performed treatment of injury using the surgical collagen adhesive associated to fibrinogen and thrombin (Tachosil® – Nycomed, Austria), previously activated in 0.9% saline (Figure 1B), with subsequent cleaning of the cavity and abdominal wall closure. In Group B, one minute after bleeding, we performed treatment of the injury with parenchymal liver suture using 3-0 polyglactin-910 (Vicryl® – Ethicon, USA) and subsequent cleaning of the cavity and the abdominal wall closure. In Group C, control group, we did not carry out any treatment of the hepatic injury, and only closed the abdominal wall.

In the experiments in groups A and B were recorded the hemostasis times for further analysis. Postoperatively, all rats received analgesia with dipyrone drops added to water and diet with appropriate chow at will. After eight weeks, the surviving rats were euthanized in a carbon dioxide chamber, with immediate necropsy for observation of intra-abdominal conditions and removal of the liver for histological analysis.

The study variables were the time to hemostasis, the occurrence of deaths, the occurrence of adhesions and any histological changes.

The hemostasis time was the time required to control bleeding are noted in the groups A and B. In group C, we did not record the time to hemostasis, immediately closing the abdominal wall after the liver injury. In the study design, we opted not to interfere in any way in the hemostasis of the induced injuries of the control group. We feared that, during the bleeding observation to note the time of hemostasis, if the bleeding was heavy the researcher might fell motivated to interfere with gauze compression or absorbing the blood with gauze. Attitudes like these would interfere with the results, with a tendency to decrease adhesions.

We classified adhesions into five grades, adapting the classification described in 1964 by Mazuji *et al*.[7]: Grade zero – absence of adhesion; Grade I – adhesion in the liver injury site to the abdominal wall, small and irregular; Grade II – in the liver injury site to the abdominal wall and to the omentum, of medium intensity and easy separation; Grade III – adhesion in the liver injury site to the abdominal, to the omentum and to the intestinal loops, intense and of difficult separation; Grade IV – adhesion in the injury site to any other region, very intense, homogeneous and difficult to separate. After analysis of

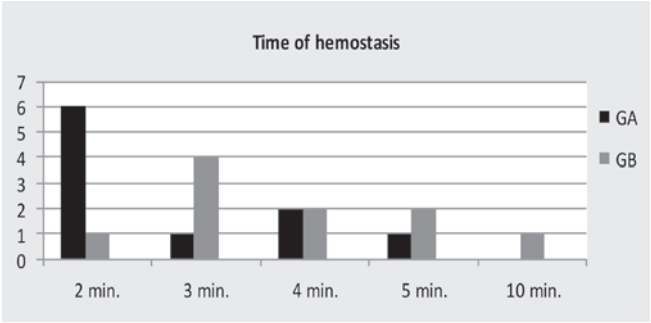

Figure 2. *Distribution of the lesions repair times between groups A and B. Vertically, the number of rats, and horizontally, the time of hemostasis*

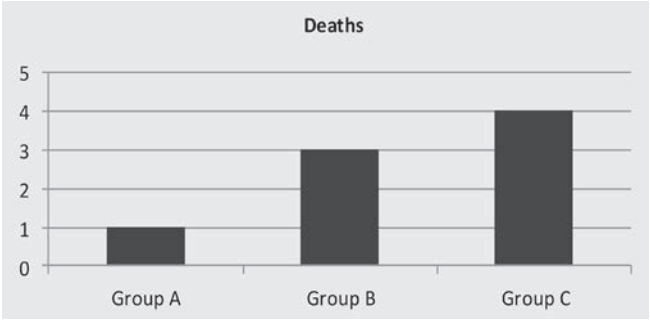

Figure 3. *Distribution of deaths between groups A, B and C. Vertically the number of deaths, and horizontally, the Groups.*

adhesions, we removed the rats livers and placed them in 10% formalin with subsequent preparation of slides with hematoxylin-eosin and picrosirius for microscopic analysis.

Statistical analysis was performed with the presentation of absolute (n) and relative (%) frequency distribution tables for all variables.

We analyzed the variables death, hemostasis time and the occurrence of adhesions with the Fisher's exact test. For the qualitative death variable, we made the comparison using the Fisher's exact test because the conditions of application of the chi-square test were not met. For the variable time of hemostasis, we compared the occurrence of the shorter time, which was two minutes between the two groups (adhesive and suture), using the Fisher's exact test, because it is a qualitative variable; we also calculated the odds ratio with its respective confidence interval. The significance level for the statistical tests was 5%.

RESULTS

Hemostasis Time

The overall average was 3.5 minutes, ranging between two and ten minutes. In Group A, the average time was 2.4 minutes, with the shortest time two, and the longest, five. In Group B, the average time was 4.2 minutes, ranging from two to ten minutes.

The distribution of the occurrence of hemostasis time of each group is shown in Figure 2.

When we grouped and analyzed the results with time equal to two minutes and longer than two minutes, in groups A and B (Table 1) we obtained a borderline significance between them by the Fisher exact test (p=0.0573). The *odds ratio* was 13.5 (range 1.20 to 15.2), which means that the animals of group B are 13.5 times more likely to have greater hemostasis time than two minutes. Therefore, this data shows statistical significance.

Death

Group A showed mortality of 10% (1/10 animals), group B had mortality of 33.3% (3/10 animals) and group C, 40% (4/10 animals). Overall mortality was 26.67% (8/30 animals). Table 2 and Figure 3 show the distribution of the number of deaths in each group.

The Fisher's exact test did not identify difference with statistical significance when comparing Group A with Group B (p=0.5820), Group A with Group C (p=0.3034) and Group B with Group C (p=1.0000).

Table 1. *Distribution of hemostasis time equal to two minutes and greater than two minutes in groups A and B in absolute numbers and percentages (in parentheses)*

	2 minutes	> 2 minutes	Total
Group A	6 (60%)	4 (40%)	10 (100%)
Group B	1 (10%)	9 (90%)	10 (100%)
Total	7 (35%)	13 (65%)	20 (100%)

Hemostasis time – animals of Group A versus Group B – Fisher exact test, p = 0.0573, Odds Ratio = 13.5.

Table 2. *Distribution of deaths in each group in absolute numbers and percentages.*

	Group A	Group B	Group C	Total
Death (n)	1	3	4	8
Death (%)	10	33.3	40	26.67

Adhesions

Group A had three rats with Grade 0 adhesions and six with Grade I. Group B had two rats with Grade I adhesions, three with Grade II and two with Grade III. The C group had one mouse with Grade I adhesions, four with Grade II and one with Grade III. No rat showed Grade IV adhesions.

Table 3 shows the distribution of the degree of adhesions in each study group.

When analysed the adhesions variable, we found that Group A had a lower incidence than Group B, with statistical significance (p=0.0119 – Fisher's exact test). A similar result occurred when comparing group A with group C (p=0.0069). When comparing Groups B and C, we found no statistically significant difference (p=1.0000).

Histological Changes

Histological changes found in the slides of the rats' livers of Group A were reaction to the foreign body with formation of histiocytes palisades, separating amorphous material (adhesive) from stromal liver cells (Figure 4) and plasma cell infiltrate and bilirubin extravasation due to ductal injury. We also observed intense collagen deposition (Figure 5), with dense fibrosis. Histological changes found in the slides of Group B rats' livers were foreign body, granuloma-type inflammatory reaction around the suture fragments, with giant cells and absent fibrosis. The slides of the rats in Group C showed extravasation of red blood cells, without formation of inflammatory tissue.

DISCUSSION

The induced liver injuries tried to reproduce intermediate lesions that correspond to grade III lesions when compared to liver trauma classification of the American Association for the Surgery of Trauma (AAST)[1,3,8].

For the choice of tissue adhesive, we looked for a product that could take advantage of the properties of bleeding, barrier offered by mechanical hemostatic agents, associated with direct action on blood clotting, offered by active hemostatic agents. Thus, the choice fell on a combination of products already on the market, represented by the combination of collagen associated to fibrinogen and thrombin[9-13]. This is a totally biological product, without synthetic components. This adhesive was evaluated in clinical studies as support to hemostasis in different kinds of surgery, most often in elective situations, especially on parenchymatous organs, showing effectiveness in controlling bleeding[9-13].

Frilling, in 2005, reported the adhesive superiority compared with the argon beam during liver resection with respect to homeostasis time[12]. We obtained similar

Table 3. *Distribution of Adhesions in Groups A, B and C.*

	ADHESIONS		
	Group A	Group B	Group C
Grade zero	3	0	0
Grade I	6	2	1
Grade II	0	3	4
Grade III	0	2	1
Grade IV	0	0	0

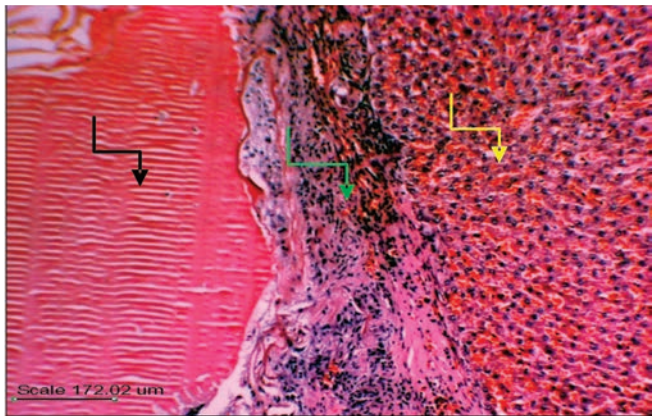

Figure 4. *Photomicrograph of histological section stained with hematoxylin-eosin, showing a Group A rat liver. The black arrow points to the adhesive amorphous material; the green arrow points to the foreign body, granuloma-type inflammatory process area, with histiocytes distributed in palisade, separating the adhesive material from the liver stroma.*

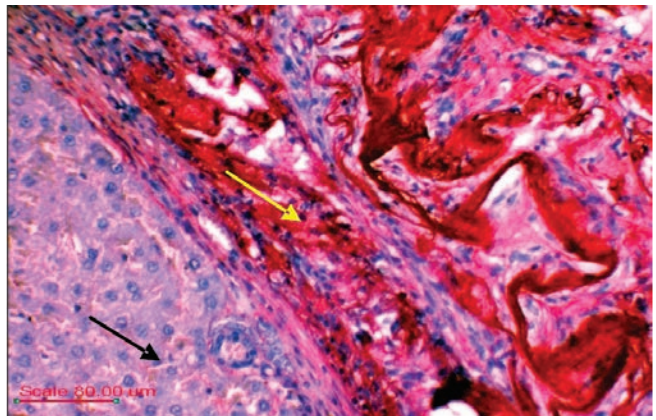

Figure 5. *Photomicrograph of histological section of the Masson's trichrome biochemical reaction, showing a Group A rat liver. The yellow arrow points to collagen fibers stained in red permeating inflammatory lymphonuclear and giant cells; the black arrow points to the liver parenchyma.*

findings when evaluating the injury repair time with the use of adhesive compared with conventional suturing. The shorter hemostasis time obtained reflects the easy handling and effectiveness of the material in controlling bleeding, a fact already identified with the use of collagen alone, as demonstrated by Mantovani *et al*.[14], or when combined with fibrinogen and thrombin, as shown by experimental studies using dogs[9] and pigs[10]. It is noteworthy that in some rats treated with injury suture, the extended time to achieve hemostasis was due to the difficulty of manipulation of the liver tissue, which was very frail.

Like the collagen, fibrinogen and thrombin adhesive, other hemostatic agents are also cited as effective in the control of various types of bleeding. In 1990, De la Garza and Rumsey showed effectiveness in controlling bleeding with the use of fibrin glue in two patients suffering from liver trauma[15]. In the same year, Ochsner *et al*. used this product in 26 patients suffering from liver and splenic injuries, also with effective bleeding control[16].

Several experimental studies show the effectiveness of fibrin adhesive in controlling hepatic hemorrhage in dogs[17], pigs[18,19], rats[20] and rabbits[21], with good adhesion to the injured liver, little local inflammatory reaction and few complications. In our study we obtained similar findings to those of the cited works.

The occurrence of adhesions, which can be classified as a complication of surgical treatment, was statistically lower in the group treated with the adhesive compared with the group treated with suture (p=0.0119). This may be due to the animals treated with suture presenting major bleeding and bruising at the site of injury, resulting in greater inflammatory reaction and consequent adhesion.

Frena and Martin[13], in 2006, found the absence of biliary fistulas with the use of this product in elective hepatectomies in humans, which also occurred in our study, even when dealing with liver trauma, which increases the chance of this complication.

The mortality of the group treated with the adhesive (10%) showed no statistically significant difference from the group treated with suture (33.3% / p = 0.5820) and the control group (40% / p=0.3034). In a study of 1,000 patients suffering from liver trauma led by Feliciano *et al*. between 1979 and 1984, the mortality rate found was 10%[22], and in another study, conducted by Saaiq *et al*. in Islamabad, Pakistan, between 2003 and 2010, mortality was 9.73%[23]. Thus, mortality with the adhesive experimental use is similar to those found in liver trauma treatments conventionally performed in humans.

The presence of foreign body inflammatory reaction found in the histological analysis of the rats' livers treated with the collagen adhesive associated with fibrinogen and thrombin was similar to changes found in studies using fibrin glue in rats[24], fibrin glue in rabbits[21] and polyglycolic acid mesh in pigs[25]. We did not observe histological findings suggestive of liver tissue necrosis or vacuolar degeneration, as described with the use of cyanoacrylate[26], or the presence of

abscesses near the adhesive application areas. The intense collagen deposition identified close to the adhesive application areas (Figure 5) is an important fact, if we consider that collagen is essential for the injured tissue repair process[24].

Conservative treatment of isolated liver trauma has been increasing in recent decades, reaching levels of 80% in the present day[27]. This fact, associated with the development of less invasive therapies such as angiography with embolization[28,29], decreases the need for surgery to control liver bleeding. However, in situations of hemodynamic instability or with associated trauma to other organs, particularly in hollow viscus, surgical treatment is often mandatory[1,3,8,22,27,28]. The liver operative approach can be a complex procedure, requiring great skill and experience of the surgeon[2]. This study showed that a collagen adhesive associated with fibrinogen and thrombin was effective in the treatment of traumatic liver injury in rats and has the potential to be used by surgeons during the same approach in humans. Its ease of handling when compared with liver tissue suturing, leading to diminished bleeding control time and low complications rates, are the main points favorable for this material.

We conclude that the treatment with collagen adhesive associated with fibrinogen and thrombin was effective in experimental hepatic injury, opening new perspectives for use in liver injuries in humans.

ACKNOWLEDGEMENT

We thank Professor Siani Sirlei Morais for the statistical analysis of this study.

R E S U M O

Objetivo: *avaliar a eficácia de um adesivo a base de colágeno associado ao fibrinogênio e trombina, no trauma hepático experimental em ratos.* **Métodos:** *toram incluídos no estudo 30 ratos Wistar, igualmente divididos aleatoriamente em três grupos: A, B e C. Todos foram submetidos à lesão traumática hepática padronizada. No grupo A, a lesão foi tratada com o adesivo, no grupo B, com sutura convencional com fio absorvível, e no grupo C, não houve tratamento da lesão. Foram analisados o tempo de hemostasia, mortalidade, ocorrência de aderências e eventuais alterações histológicas.* **Resultados:** *os resultados mostraram que não houve diferença estatística em relação à mortalidade (p=0,5820). O grupo tratado com adesivo apresentou os menores tempos de hemostasia (p=0,0573 e odds ratio 13,5) e menor ocorrência de aderências (p=0,0119). Microscopicamente as alterações histológicas dos grupos A e B foram semelhantes, com a formação de granuloma de corpo estranho separando o material do adesivo e do fio de sutura do estroma hepático.* **Conclusão:** *o adesivo de colágeno associado ao fibrinogênio e trombina foi eficaz no tratamento do trauma hepático experimental, proporcionado menor ocorrência de aderências entre o fígado e as estruturas vizinhas.*

Descritores: *Ferimentos e Lesões. Fígado. Hemostáticos. Trombina. Adesivos Teciduais.*

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Received: 10/10/2015
Accepted for publication: 17/03/2016
Conflict of interest: none.
Source of funding: none.

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DOI: 10.1590/0100-69912016002008

Epidemiology and outcome of patients with postoperative abdominal fistula

Perfil epidemiológico, incidência e desfecho dos pacientes com fístula abdominal pós-operatória

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A B S T R A C T

Objective: to present the epidemiological profile, incidence and outcome of patients who developing postoperative abdominal fistula. **Methods**: This observational, cross-sectional, prospective study evaluated patients undergoing abdominal surgery. We studied the epidemiological profile, the incidence of postoperative fistulas and their characteristics, the outcome of this complication and the predictors of mortality. **Results**: The sample consisted of 1,148 patients. The incidence of fistula was 5.5%. There was predominance of biliary fistula (26%), followed by colonic fistulas (22%) and stomach (15%). The average time to onset of fistula was 6.3 days. For closure, the average was 25.6 days. The mortality rate of patients with fistula was 25.4%. Predictors of mortality in patients who developed fistula were age over 60 years, presence of comorbidities, fistula closure time more than 19 days, no spontaneous closure of the fistula, malnutrition, sepsis and need for admission to the Intensive Care Unit . **Conclusion**: abdominal postoperative fistulas are still relatively frequent and associated with significant morbidity and mortality.

Keywords: Epidemiology. Incidence. Fistula. Digestive System Fistula. Postoperative Complications.

INTRODUCTION

Digestive or gastrointestinal fistula is one of the most feared postoperative complications along with dehiscence and infection[1,2]. The topic is of great interest to the surgeon and in spite of numerous publications about it, a number of aspects related to digestive fistulas always deserves consideration.

Gastrointestinal or digestive fistula is an aberrant communication between the gut and any hollow viscus or the abdominal cavity (internal fistula), or with the skin surface (external fistula). Fistulas can be classified according to the anatomical location (gastric, pancreatic, duodenal, jejunal, ileal or colonic), output (high output > 500ml / 24h, and low output < 500ml / 24 h), origin (congenital or acquired) or as primary (due to intestinal disease processes), or secondary (surgery)[2,3]. Acquired fistulas can be inflammatory / infectious, neoplastic or traumatic[3].

Fistulas usually appear in the first week after surgery, with its highest peak around the fifth to the seventh days, which requires a strict postoperative evaluation, especially in patients with increased risk of developing such complications[4]. The main causes of death related to fistulas are malnutrition, electrolyte imbalance and sepsis. Another important factor that is associated with poor prognosis is the fistula high initial output.

At admission, about 35% to 40% of general surgery patients have some degree of malnutrition that may interfere with surgical outcomes[5], with increase in length of stay, with the need for reoperations and complications, which increase hospital costs and patients' suffering.

The mortality rate for most elective surgical procedures is less than 2%. However, in patients with gastrointestinal fistula mortality ranges from 6% to 48%, even after advances made in its treatment[6,7].

The treatment of a gastrointestinal fistula, especially the high output one, is a complex procedure that requires multi-professional work and dynamic and individualized approaches. Clinical and

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surgical measures do not compete, but join each other at different treatment stages in search of the fistula closure[7].

The topic is of great interest to the surgeon and always deserves consideration. This study aims to present the epidemiological profile, incidence, predictors of mortality and outcome of patients with abdominal postoperative fistula.

METHODS

This is an observational, cross-sectional, prospective study in a referral service in General Surgery. We evaluated patients undergoing abdominal surgery operated by the specialties of General Surgery, Coloproctology, Thoracic Surgery and Urology. We evaluated 1,615 patients in the period from April 1, 2013 to June 31, 2014. We excluded 467 patients who had surgery with access to the abdomen, resulting in a final sample of 1,148 patients.

From a form designed for this study, we collected epidemiological data of patients and operations that occurred in the period. We considered only the main procedure, since some patients underwent multiple surgeries.

We assessed the presence of preoperative risk factors and outcome among patients who developed postoperative fistula. Malignant disease, age equal to or over 60 years, hypertension, diabetes, inflammatory bowel disease and immune deficiency (defined as patients who were in chronic use of corticosteroids, prior use of chemotherapy or radiotherapy, patients with HIV, malnourished with albumin levels lower than 3g/dl, or transferrin below 150mg/dl).

After surgery, we searched for the occurrence of spontaneous closure and the need for surgery, whether for the fistula or for the peritonitis resulting from it. We also evaluated the occurrence of malnutrition and the need for prolonged use (more than seven days) of total parenteral nutrition, clinical outcomes with sepsis, need for admission to the Intensive Care Unit (ICU) and the death rate of patients who had postoperative fistula.

We described data as median, standard deviation (minimum and maximum) or absolute frequency (percentage). To study the association between categorical variables and death we used the Fisher test and computed the relative risk and 95% confidence interval. We used the median to determine the cutoffs for the number of days for the diagnosis and fistula closure.

This study was approved by the Ethics in Research Committee with Human Beings according to the opinion 645,873.

RESULTS

The study included 1,148 patients operated within 14 months. The mean age was 44.4 years, ranging from 14 to 94. There was a similar occurrence between genders, with predominance of emergency procedures. We recorded 63 cases of fistula, corresponding to an incidence of 5.5%, the most frequent complication in elective surgery, as described in Table 1.

The most frequent surgeries were for inflammatory and obstructive abdomen, or blunt, hemorrhagic or penetrating abdominal trauma. The procedures performed are listed in Figure 1.

Among the 63 patients who developed postoperative fistula, 49% were elderly or had hypertension or diabetes, and 29% underwent surgery

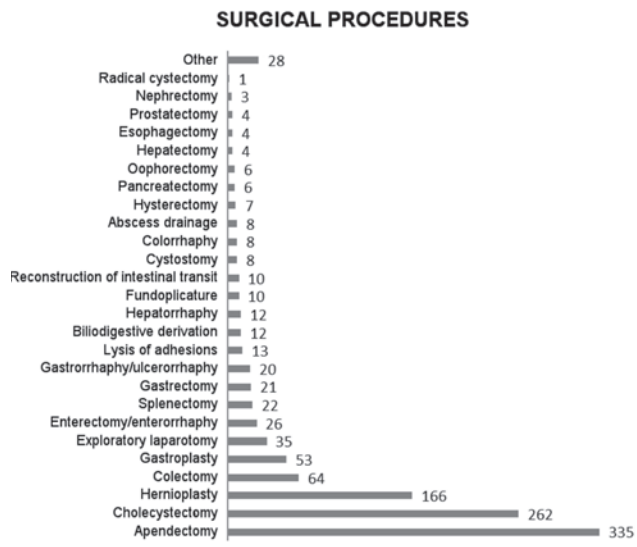

Figure 1. *Main operative procedure performed in patients enrolled in the study (n=1148).*

Table 1. *Sample distribution according to gender, age and surgery character (n=1148).*

	Without fistula n=1085	With fistula n=63	P <0,005
Age (years)*	44 (± 17,5)	51,9 (± 15)	<0,01
Gender			
Male	612 (56,4)	34 (53,4)	<0,70
Female	473 (43,6)	29 (46,6)	
Surgery character			
Elective	420 (38,7)	38 (60,3)	<0,001
Urgency	665 (61,3)	25 (39,7)	

** Data presented in mean ± standard deviation.*

Table 2. *Presence of risk factors among patients who developed postoperative fistula.*

Risk factors prior to surgery	n= 63 (%)
Malignant disease	20 (32)
Immunosuppression	26(41)
Hypertension/Diabetes/Advanced Age	31 (49)
Surgery in the presence of infection	18 (29)
Inflammatory bowel disease	5 (8)

Table 3. *Characteristics of postoperative fistulas found in the study.*

Characteristics of fistulas	n=63 (%)
Location	
Biliary	18 (28,6)
Colon	14 (22,2)
Stomach	10 (15,9)
Jejunoileal	9 (14,3)
Esophagus	4 (6,3)
Duodenum	2 (3,2)
Pancreas	2 (3,2)
Bladder	2 (3,2)
Rectum	2 (3,2)
Fistula debit	
High	13 (21)
Low	50 (79)
Drainage location	
Internal	12 (19)
External (enterocutaneous)	51 (81)
Path	
Long	56(89)
Short	7(11)

in the presence of infection. These data are shown in Table 2.

The diagnosis of postoperative fistula was performed on average 6.3 days after surgery, with a standard deviation 3.5 days (range 2 to 22). Among these patients, abdominal cavity drainage was used in 50 cases. There was a predominance of biliary fistulas, with 26%. Most fistulas were of low output, external type and had a long path (Table 3).

Regarding the diagnosis of postoperative digestive fistulas, we observed alterations in clinical signs, predominantly abdominal pain, abnormal abdominal examination, tachycardia, vomiting and fever. As diagnostic complement, we used abdomen CT, oral test with methylene blue, endoscopy, colonoscopy and fistulography. As for the drainage of the fistula content, the majority of patients had exteriorization. Table 4 shows the complementary methods for the diagnosis of postoperative fistulas and their forms of.

Table 4. *Diagnostic methods used in patients who developed postoperative fistula and forms of exteriorization.*

Diagnosis	n=63(%)
Diagnostic Tests	
Computerized Tomography	5 (8)
Methylene blue	7 (11)
Digestive Endoscopy	4 (6)
Fistulografy	7 (11)
Colonoscopy	1 (2)
Exteriorization	51 (81)
Through drain	33 (52)
Through surgical wound	7 (11)
Through drain and surgical wound	11 (17)

There was spontaneous fistula closure in 19 patients (30%). The average time for fistula closure was 25.6±19.3 days (range 8-89). Reoperation for the treatment of fistulas was necessary in 32 patients (47.6%) and surgery for the treatment of peritonitis was performed in 35 cases (56%). We observed malnutrition in 32 patients (51%), of whom 11 (18%) required parenteral nutrition for more than seven days. Sepsis ensued in 46 patients (73%). In 32 cases (51%), their conditions required ICU admission. There were 16 deaths, with a mortality rate of 25.4%.

Factors that were associated with mortality among patients who developed fistula were age over 60 years, presence of comorbidities, no spontaneous closure of the fistula or closure after 19 days, malnutrition, sepsis and need for ICU (Table 5).

DISCUSSION

Among 1,148 patients, there were 63 cases of fistulas, corresponding to 5.5%. This rate is within the standard of other studies[8]. The mean age was 44.4 years, with no difference between genders. Visschers *et al*.[9] reported an average age of 59 years, 56% being male. Bradley *et al*.[8] found that 20% of patients were older than 55 years, with a 79% prevalence of men[8,9].

There was a predominance of emergency surgery, but the incidence of fistula was observed with the highest proportion in elective procedures (60.3%), corroborating the study of Torres *et al*.[5], who found that 69.2% of fistulas took place in elective surgeries. Urgent surgery is a risk factor related to preoperative preparation, since the time for them tends to be shorter, acting as a prognostic factor[5-9]. There is thus an increased risk of complications, which was not observed in this study.

The most frequent fistulas were biliary and colonic, followed by gastric, this sequence being different from the one a study of 188 patients, which showed predominance of jejunoileal (28.7%), biliopancreatic (24, 9%) and colonic (23.9%) fistulas. The characteristics shown by most fistulas are favorable prognosis. The anatomical location is important and is assessed as a risk factor for worse prognosis[10].

The average time for the fistula closure was 6.3 days (range 2-22). In an Israeli study with 389 patients, Bala *et al*. showed that the average time for spontaneous closure was eight days, ranging from five to 19[10].

Most fistulas were of low output, which means lower loss of a complete solution that is rich in protein, electrolytes and complexes that could lead to electrolyte disturbances[11].

Total parenteral nutrition (TPN) extending longer than seven days was assessed in this study and showed no statistical significance. However, some studies have shown that the prolonged use of TPN leads to worse prognosis, further reducing protein rate and increasing catabolism. Malnutrition can be both a cause and a result of this anatomical and metabolic instability[10-12].

Patients without complications remain hospitalized on average 14.24 days less than patients with complications[11].

The spontaneous closure of the fistula occurred in 30% of cases, on average after 25.6 days, shorter than that observed by Pepe *et al*. in 2014, a mean time of spontaneous closure of 36.4 days[4]. As for fistula management, 47.6% required surgical reintervention in this study, also a lower rate than that observed by Pepe *et al*., which was 69%[4].

In our study, 16 patients died (25.4%), a high mortality rate, but within a range of 6% to 33% observed in a meta-analysis published in 2012[13].

The treatment of digestive fistulas advanced considerably in recent decades, but is still a thorny issue for the surgeon. Early diagnosis and prompt institution of treatment, infection control, fistula path orientation and electrolyte and nutritional support measures are capable of reducing complications and mortality[13-16].

In conclusion, the incidence of postoperative fistula was 5.5%, more than 50% being after elective surgeries and 26% of biliary type. Most were of low output and had a long path. Mortality was 25.4%.

Table 5. *Factors associated with mortality in patients with fistula.*

Variable	Survival (%)	Death (%)	p	RR (IC)
Age > 60				
Yes	10 (15,8)	9 (14,2)	0,008	1,6 (1,02- 2,50)
No	37 (58,7)	7 (11,3)		
Male				
Yes	28 (44,4)	6 (9,5)	0,13	1,26 (0,92-1,7)
No	19 (30,1)	10 (15,8)		
Emergency surgery				
Yes	18 (28,5)	7 (11,3)	0,70	1,06 (0,78- 1,43)
No	29 (46)	9 (14,2)		
Malignant disease				
Yes	13 (20,6)	7 (11,3)	0,23	0,82 (0,57-1,17)
No	34 (53,9)	9 (14,2)		
Immunosuppression				
Yes	17 (26,9)	9 (14,2)	0,16	1,83(0,78-4,28)
No	30 (47,6)	7 (11,3)		
Hypertension/Diabetes				
Yes	18 (28,5)	13 (20,6)	0,003	4,47 (1,41- 14,1)
No	29 (46)	3 (4,7)		
Infection in surgery				
Yes	11 (17,4)	7 (11,3)	0,12	1,94 (0,85-4,42)
No	36 (57,1)	9 (14,2)		
TPN for more than seven days				
Yes	8 (12,6)	3 (4,7)	0,87	1,09 (0,37- 3,19)
No	39 (61,9)	13 (20,6)		
Surgery for fistula				
Yes	22 (35)	10 (15,8)	0,28	1,61 (0,67-3,91)
No	25 (39,6)	6 (9,5)		
Surgery for peritonitis				
Yes	23 (36,5)	12 (19)	0,070	2,4 (0,87- 6,63)
No	24 (38,0)	4 (6,3)		
Sepsis				
Yes	30 (47,6)	16 (25,4)	0,005	0,65 (0,52-0,85)
No	17 (26,9)	0 (0)		
Malnutrition				
Yes	20 (31,7)	12 (19)	0,02	2,90 (1,05-8,04)
No	27 (42,8)	4 (6,3)		
Admission to ICU				
Yes	18 (28,5)	14 (22,2)	0,001	6,78 (1,68- 27,4)
No	29 (46)	2 (3,17)		
Spontaneous closure				
Not closed	29 (46)	15 (23,8)	0,01	1,44 (1,14- 1,82)
Closed	18 (28,5)	1 (1,5)		
High debit fistula				
Yes	7 (11,1)	4 (6,3)	0,36	1,57 (0,62-3,98)
No	40 (63,4)	12 (19)		
Days to diagnosis				
Less than 6 days	29 (46)	9 (14,2)	0,70	1,06 (0,78-1,4)
More than 6 days	18 (28,5)	7 (11,1)		
Days to closure				
Less than 19 days	22 (35)	1 (1,5)	0,004	1,53 (1,19-1,97)
More than 19 days	25 (39,6)	15 (23,8)		

** Confidence interval 95%. TPN: Total Parenteral Nutrition.*

R E S U M O

Objetivo: *apresentar o perfil epidemiológico, incidência e desfecho em pacientes que evoluíram com fístula abdominal pós-operatória.* ***Métodos:*** *trata-se de um estudo prospectivo transversal observacional que avaliou pacientes submetidos à cirurgia abdominal. Foram estudados o perfil epidemiológico, a incidência das fístulas pós-operatórias e suas características, desfecho desta complicação e fatores preditivos de mortalidade. Resultados: a amostra constou de 1148 pacientes. A incidência de fístula foi 5,5%. Houve predominância de fístulas biliares (26%), seguidas de fístulas colônicas (22%) e gástricas (15%). O tempo médio para o surgimento da fístula foi 6,3 dias. Para o fechamento, a média foi 25,6 dias. A taxa de mortalidade dos pacientes com fístula foi 25,4%. Os fatores preditivos de mortalidade nos casos que desenvolveram fístula foram idade maior do que 60 anos, presença de comorbidades, tempo de fechamento da fístula superior a 19 dias, não fechamento espontâneo da fístula, desnutrição, sepse e necessidade de admissão em Unidade de Terapia Intensiva.* ***Conclusão:*** *as fístulas pós-operatórias abdominais ainda são relativamente frequentes e associadas à morbidade e mortalidade significativas.*

Descritores: *Epidemiologia. Incidência. Fístula. Fístula do Sistema Digestório. Complicações Pós-Operatórias.*

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Received: 09/12/2015
Accepted for publication: 28/03/2016
Conflict of interest: none.
Source of funding: none.

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DOI: 10.1590/0100-69912016002009

Analysis of electrocautery generated smoke by chromatographic-mass spectrometry

Análise, mediante cromatografia/espectrometria de massas, da fumaça gerada por eletrocautério

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A B S T R A C T

Objective: to analyze the chemical components of the smoke from electrocautery from coagulating muscle and liver tissues of pigs. **Methods**: we collected smoke produced by electrocautery applied to porcine tissue in previously evacuated bottles, with qualitative and quantitative analysis of the compounds present through the hyphenated technique gas chromatography / mass spectrometry. **Results**: there was a majority of decanal aldehyde in the fumes from the subcutaneous, muscle and liver tissues. Fumes of subcutaneous and muscular tissues also showed the presence of hexanal and phenol. In the fumes of subcutaneous and liver tissues we also found toluene and limonene and, finally, nonanal smoke was present in the muscle and liver tissues. **Conclusion**: there is increasing evidence showing that smoke from electrocautery used in subcutaneous, muscle and liver tissue is harmful to human health. Thus, there is need to reduce exposure to it or wear masks with filters capable of retaining these particles.

Keywords: Smoke. Subcutaneous Tissue. Mass Spectrometry. Chromatography, Gas. Aldehydes.

INTRODUCTION

Surgical incision, dissection, coagulation and vaporization with electrocautery are widely used and recognized as a major advance in surgical technique. However, these techniques intentionally destroy tissue, creating vapors, popularly known as cautery or surgical smoke (SS)[1]. This smoke, with characteristic odor and made up of particles with micro and / or submicron size, diffuses in the environment and is inhaled by professional medical staff present in operating rooms. It is produced when the heat reaches the cells, ruptures their membranes and vaporizes its constituents, dispersing them and generating other substances during tissue combustion[2].

In vitro experiments have demonstrated the smoke constituents from the use of cautery on subcutaneous and prostate tissues, in breast lifting procedures, laparotomy and TURP[3,4]. It is known today that many of these components are toxic, mutagenic, such like the cigarette smoke, the smoke generated by a gram of tissue destroyed equals the one of six cigarettes without filter[5].

The constituents present in greater quantities in the smoke of subcutaneous tissue are hydrocarbons and nitrogen compounds, the hydrogen cyanide, formaldehyde, and benzene being the most toxic[5]. The number, proportion, the amount and nature of the substances present in the smoke depend on the tissue, on its condition and on the area under treatment with electrocautery, on the duration of the procedure, on the electric power and on the technique used (incision, coagulation, vaporization or dissection)[6].

Although there is a reasonable number of studies that analyze these constituents, the size and shape of these particles in the smoke, the interference in the surgical field visualization[7] and the use of smoke suction[8], those analyzes were performed only on the subcutaneous tissue. However, electrocautery is widely used in other tissues such as muscle and liver, producing a lot of smoke. Thus, this study aims to comparatively demonstrate which compounds are

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Table 1. *Compounds present in the ambient air sample.*

Substance	% (area)	Elution time (min)	Quality
carbon dioxide	12.35	1.464	4
ethylene oxide	12.35	1.464	3
acetonitrile	12.71	1.719	7
ethylamine	12.71	1.719	5
trimethylphosphine oxide	30.75	2.956	9
dimetilsilanodiol	30.75	2.956	9
2 chlorine 2 nitro propane	30.75	2.956	4
hexametilciclotrisiloxano	13.48	5.925	91

present in the smoke from three electrocauterized tissue, subcutaneous, muscle and liver, from pigs.

METHODS

The tissue used for the research was from a pig of the Large White breed, which is closest to human tissue[5]. The animal had its used approved for teaching and research by the Ethics Committee on Animal Use of the Biology Institute of the Universidade Estadual de Campinas.

The collection was performed at the Experimental Surgery Center of the Universidade Estadual de Campinas, with fresh tissues, using a monopolar electrocautery with 30w power, long enough to produce smoke.

The samples were collected in four vials, previously evacuated and hermetically sealed. One of the vials was used to collect air in the operating room prior to cautery use, serving as a control. In the three other vials we collected smoke from the cautery use, in pure coagulation mode, at the site of its production in the subcutaneous, muscle and liver tissues.

These previously evacuated vials are made from Pyrex glass, provided with a teflon high vacuum tap with a tap screw cap containing silicone septa for the introduction of the needle containing the gas absorbing fiber, and then introduced into the gas chromatograph using helium as the carrier gas. Before the introduction of the samples we performed the chromatogram / mass spectra of the reference (only the fiber) to verify that the peaks relating to the chromatograph eluted samples were not due to the reference. The chromatogram / mass spectra were compared with the chromatogram / mass spectra of the samples library existing in the equipment to identify the substances responsible for chromatographic peaks present in the samples collected.

The equipment used in the analysis was the gas chromatograph (Agilent 7890A model) coupled to the mass spectrometer (Agilent, 5975C model).

Table 2. *Compounds present in subcutaneous tissue sample.*

Substance	% (area)	Elution time (min)	Quality
toluene	9.93	4.395	91
hexanal	0.85	5.251	90
1.3 dimethyl benzene	0.32	7.597	93
o-xylene	0.32	7.597	93
p-xylene	0.32	7.597	93
phenol	6.82	13.445	94
limonene	0.64	15.992	90
dodecane	0.55	27.525	90
decanal	0.54	27.921	90

Table 3. *Compounds present in the muscle sample.*

Substance	% (area)	Elution time (min)	Quality
hexanal	2.97	5.238	91
tetrachloroethylene	0.77	5.501	97
heptanal	2.57	9.064	95
phenol	2.48	13.479	95
octanal	6.10	14.581	90
nonanal	13.17	21.092	91
decanal	17.84	27.900	91

The technique for sampling was Solid Phase Micro Extraction (SPME) using a needle with SUPELCO, gas-absorbing triple fiber: 50/30mm DVB/CAR/PDMS (polydimethylsiloxane), heated at 100ºC for 40 minutes to release the adsorbed compounds.

We tabulated and presented data in a qualitative way, with no statistical study.

RESULTS

The results of each sample components analysis are shown in Tables 1, 2, 3 and 4, being, respectively, the ambient air control sample, subcutaneous, muscle, and liver tissues. Tables indicate chemicals, the percentage area of the chromatographic peak for each compound, its elution time (in minutes), and quality. This last parameter refers to the degree of similarity between the detected substance and the existing compounds in the mass spectrometer database.

We found decanal in all three tissues; common substances in the smokes from subcutaneous and muscle tissues were hexanal and phenol; common compounds in the smokes from subcutaneous and liver tissues were toluene and limonene; and the common compound in the smokes from muscle and liver tissues was nonanal.

DISCUSSION

The aromatic hydrocarbon toluene has been widely found in subcutaneous tissue smoke[3]. However, there was no evidence in the literature of its presence in the smoke from liver tissue. Aldehydes have also been widely cited in the literature as present in subcutaneous tissue smoke and, as shown in this study, are not restricted to it, being also present in the smoke from muscle and liver, in the forms of hexanal, nonanal and decanal. The presence of d-limonene has not been reported in other studies in subcutaneous tissue smoke.

Wenig *et al*.[9] evaluated cautery smoke exposure in rats and noticed that they were stunned during the exposure period, returning to normal after an exposure-free period. Furthermore, when analyzing the rats' lungs, they observed vessels hypertrophy, cellular congestion and emphysematous changes. They supported the idea that these changes were from exposure to benzene, formaldehyde and acrolein, substances present in the subcutaneous tissue, muscle and liver smoke.

The presence of volatile organic compounds within the smoke, as mentioned by

Table 4. *Compounds present in the liver sample.*

Substance	% (area)	Elution time (min)	Quality
toluene	21.75	2.906	95
d-limonene	2.68	15.540	94
nonanal	3.04	20.877	86
decanal	1.43	27.822	86

Moot *et al*.[10], although in low concentrations, can chronically inflict the same health hazards of passive smoking. Furthermore, two compounds identified by this group, hydrogen cyanide and butadiene, are implicated as cardiotoxic and carcinogenic, respectively. They also showed that benzene, butadiene and decene are carcinogenic substances[10].

El Ghawabi *et al*.[11] and Chandra *et al*.[12] showed that chronic exposure to low concentrations of hydrocarbons – hexanal, heptanal, octanal, nonanal and decanal – cause headache, weakness, touch and smell changes, lacrimation, salivation, abdominal colic pain and nervous instability. Moreover, Blanc *et al*.[13] showed that hydrocarbons can lead to deficiency of vitamin B12 and folate and increase in thyroid stimulating hormone (TSH), leading to goiter. Laugesen *et al*.[14], in a review study, stated that in cigarette smoke, the butadiene amounted to 45% of the cancer risk, hydrocarbons corresponded to 89% risk of cardiovascular disease, and acrolein (aldehyde, like the others found in all three tissues) corresponded to 97% risk of lung disease.

There is growing body of evidence that the smoke produced by electrocautery used in biological tissues, be them subcutaneous, muscle or liver, is harmful to the human health. The need to reduce such exposure is evident, whether by suction of this smoke by means of suitable devices or by using surgical instruments that do not generate heat, like some kinds of laser.

ACKNOWLEDGEMENTS

We thank Dr. José Henrique Silveira Virgili for the contribution during the experimental surgery, and the staff of the Experimental Surgery Center.

R E S U M O

Objetivo: *analisar quimicamente os componentes da fumaça do eletrocautério, provenientes da coagulação de tecidos, muscular e hepático de suíno.* **Métodos:** *coleta de fumaça produzida por eletrocauterização de tecido porcino em frascos previamente evacuados com análise qualitativa e quantitativa dos compostos presentes, através de técnica hifenada, cromatografia a gás/espectrometria de massas.* **Resultados:** *houve presença majoritária do aldeído decanal nas fumaças provenientes dos tecidos subcutâneo, muscular e hepático. Fumaças dos tecidos subcutâneo e muscular mostraram também a presença de hexanal e fenol. Nas fumaças dos tecidos subcutâneo e hepático foram encontrados ainda tolueno e limoneno e, por fim, nonanal estava presente nas fumaças dos tecidos muscular e hepático.* **Conclusão:** *há número crescente de evidências mostrando que fumaça proveniente de eletrocauterização de tecidos subcutâneo, muscular e hepático é nociva à saúde de seres humanos. Portanto, há necessidade de reduzir a exposição a ela ou usar máscara com filtro capaz de reter essas partículas.*

Descritores: *Fumaça. Tecido Subcutâneo. Espectrometria de Massas. Cromatografia Gasosa. Aldeídos.*

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Received: 29/11/2015
Accepted for publication: 28/03/2016
Conflict of interest: none.
Source of funding: none.

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DOI: 10.1590/0100-69912016002010

Preliminary analysis of hybrid laparoscopic procedure for resection of gastric submucosal tumors

Avaliação preliminar do procedimento videolaparoscópico híbrido para ressecção de tumores gástricos submucosos

Pedro Henrique Lambach Caron[1]; Mariana Ismael Dias Martins[1]; Pedro Luiz Bertevello[1].

ABSTRACT

Objective: *to evaluate the feasibility, safety and benefits of minimally invasive surgery for resection of gastric submucosal tumor (GSMT).* ***Methods:*** *we conducted a retrospective study of medical records of patients undergoing endoscopy-assisted laparoscopic resection of gastric submucosal tumors (prospectively collected) from 2011 to 2014. We evaluated clinical data, surgical approach, clinicopathological characteristics of the GSMT (size, location, histopathological and immunohistochemical exams), outcome and patients follow-up.* ***Results:*** *we evaluated six patients, 50% male, mean age 52±18 years and common symptoms of heartburn and gastric fullness. All patients underwent hybrid procedure without anatomical impairment of the organ. The average length of stay was 3.5 days and the average size of the tumors was 2.0±0.8cm, five of them (83%) in the proximal third of the stomach. The surgical specimens pathological and immunohistochemistry examination revealed one case of ectopic pancreas (17%), one grade 2 neuroendocrine tumor (17%), one lipoma (17%), one GIST (17%) and two leiomyomas (32%). There were no episodes of tumor rupture or intraoperative complications and no conversion to open surgery. During the postoperative follow-up period, none of the patients had recurrence, metastasis, fistula or stenosis.* ***Conclusion:*** *the results showed that endoscopy-assisted laparoscopic resection is feasible and safe for patients with GSMT. Endoscopy proved to be essential in the location of lesions and as intraoperative support, especially when attempting to preserve the pylorus and cardia during surgery.*

Keywords: *Surgical Procedures, Operative. Video-Assisted Surgery. Leimyoma.*

INTRODUCTION

Gastric subepithelial lesions are common findings during routine upper endoscopy, usually identified as a mass, lump or imprint covered by normal mucosa. The actual incidence of these lesions is difficult to estimate, being found in up to 0.4% of the population[1,2]. Macroscopically, they exhibit a broad spectrum; they can be benign or malignant, the gastrointestinal stromal tumors (GISTs) being the most common type. Currently, is indicated for diagnosis of submucosal gastric tumors[1,2].

Local excision with adequate surgical margins is indicated in most cases of gastric submucosal tumors (GSMT)[2]. Depending on their characteristics, they can be resected endoscopically, laparoscopically[3], or through a hybrid procedure. The laparoscopic resection technique was first applied by Ohgami et al., in 1999[4]. However, the use of the laparoscopic method can show methodological limitations for resection, for example, the size of the lesion[1-3]. Intraoperative upper endoscopy helps locating the tumor and evaluates safely surgical resection. Thus, laparoscopic resection assisted by endoscopy is indicated for the removal of GSMT (leiomyomas, lipomas and schwannomas), broad-base polyps, gastric epithelial tumoral degeneration (moderate or severe atypical hyperplasia), ectopic pancreas, low malignant potential lesions (carcinoid tumor and GIST), and some cases of early gastric carcinoma[3,5].

Given the above and the limited literature, the aim of this study was to evaluate the feasibility, safety and advantages of applying the hybrid method for resection of gastric submucosal tumors in a specialized service.

METHODS

We conducted a descriptive study, in which we analyzed the hospital records of six patients un-

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dergoing endoscopy-assisted laparoscopic resection for gastric submucosal tumors, from March 2011 to May 2014, by the Gastrosurgery team (ProGastro) in the Real e Benemérita Associação Portuguesa de Beneficência de São Paulo.

All patients underwent upper endoscopy with biopsy, total abdominal ultrasound and/or CT scan, laboratory tests (including tumor markers) and endoscopic ultrasonography in selected cases.

We analyzed demographic and preoperative clinical characteristics (gender, age and duration of the operative procedure), and postoperative data included possible surgical complications and length of stay. The clinicopathological characteristics of resected GSMT included size, location, histopathology and immunohistochemistry (Table 1).

Regarding the surgical procedure, patients were placed in the supine position under general anesthesia, according to the American Technique (AT)[6], with the surgeon positioned to the right of the patient. Video monitors were placed laterally to the patient's shoulders. The abdominal cavity is accessed with a Veress needle for establishing the pneumoperitoneum and passage of the optics trocar (10 mm) in the midline, about 10 to 12 cm from the xiphoid process. We inserted four additional trocars (three 5mm and one 12mm in diameter) as diagrammed in Figure 1 under direct vision of a rigid 30° laparoscope.

Intraoperative upper endoscopy was performed to define the location of the lesion, determine the most appropriate technique for resection, evaluate the resection margins and the integrity of the suture lines after resection. The injuries were not directly manipulated with laparoscopic instruments to prevent tumor rupture.

Once the patient is anesthetized, the endoscope is introduced through the oropharynx. The lining of the esophagus and stomach was evaluated with care not to overly inflate the stomach. The location of the GSMT was confirmed, all liquids and gas taken from the gastric chamber and the endoscope retracted through the cardia, staying in the esophagus.

Tumors in the anterior gastric wall were resected after endoscopic location of the cardinal points of the lesion to define the resection safety

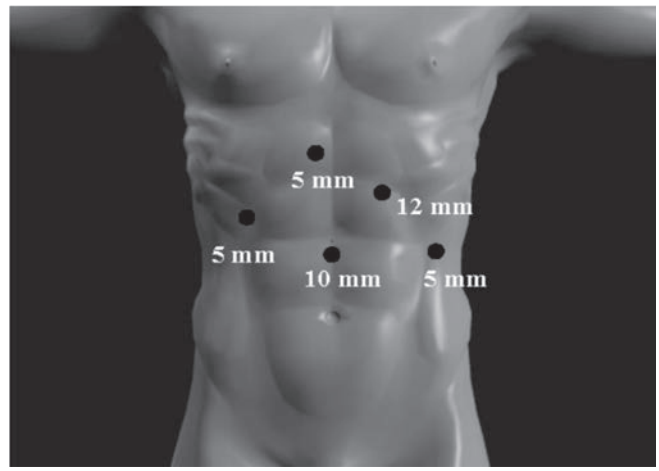

Figure 1. *Insertion of the trocars for realization of the laparoscopic resection of gastric submucosal tumors after instilation of the pneumoperitoneum.*

margin. Tumors were resected with ultrasonic coagulation shears. The gastrotomy was closed in two planes of continuous, multifilament, absorbable suture, followed by a novel endoscopic evaluation to ensure complete resection of the lesion, to perform a hyperinflation maneuver to rule extravasation and to review hemostasis of the suture line. The resected specimens were packed in an endoscopic recovery bag and removed from the abdominal cavity. Tumors of the posterior gastric wall were resected after the release of the gastric curvature that was nearest to the tumor for proper exposure, using the ultrasonic coagulating shears. Then the posterior wall was exposed, and the tumor, removed through a technique similar to that described for the previous lesions.

We used the transgastric technique in one case, with gastrotomy of the anterior wall and resection of the posterior wall lesion with electrocautery and ultrasonic coagulating shears. We then closed the anterior wall gastrotomy with absorbable, multifilament suture, in a continuous fashion.

One of the patients who had a tumor in the gastric greater curvature, after release of the greater omentum, had his lesion resected with laparoscopic stapler.

On the first day after surgery we instituted a fractionated liquid diet with progression till acceptance of regular diet, when the patient was discharged. Routine outpatient visits were set to 10 and 30 days postoperatively. Upper endoscopy was per-

formed about six months and one year after surgery, and repeated annually for two years. Follow up with imaging tests such as computed tomography of the chest or abdomen, magnetic resonance imaging of the chest or abdomen and pet Scan® were indicated should we find any abnormality. The follow-up ranged from six months to one year.

Descriptive statistics were performed and the study was approved by the Ethics in Research Committee of the Hospital Beneficência Portuguesa de São Paulo, opinion 1035/2014, with informed consent of the participants.

Table 1. *Preoperative clinicopathological characteristics of gastric submucosal tumors submitted to upper digestive endoscopy and/or endoscopic ultrasound with fine needle aspiration (FNA) biopsy.*

Gender/age (years)	PREOPERATIVE ENDOSCOPY	LESION LOCATION	ENDOSCOPIC ULTRASOUND	BIOPSY
Female/61	Subepithelial tumor with stromal appearance	Gastric Fundus	No	No
Female/31	Gastritis, subepithelial lesion, 2cm	Subcardia	Mobile lesion on Cardia muscular layer (Leiomyoma? GIST?). Hypoechoic lesion, regular and heterogeneous, 1,.7x1.5 cm.	Leiomyoma (mesenchymal neoplasia). Mitotic activity not detected necrosis not detected, mild pleomorphism
Male/50	Elevated, lobulated lesion, covered with intact mucosa, measuring 3cm, which protrudes from the cardia to the small curvature (GIST?).	Cardia	Subepithelial lesion on muscular layer, compatible with leiomyoma	Mesenchymal neoplasm of low degree (Leiomyoma). Irregular structures: 3.5 x 2.7x1 cm and 5.3 x 0.3 x 3 cm
Female/85	Elevated lesion in greater curvatuve, of ± 2 cm	Greater curvature	No	No
Male/31	Elevated lesion of gastric body of 1.3 cm, proximal body in lesser curvature, no biopsy	Posterior gastric wall	Posterior gastric wall lesion of 5 cm far from the GET *, smooth surface, hiperemy, with discreet apical depression, firm, with irregular submucose vessels of 1.3 cm.	Biopsy of the lesion in GET, histological grade 2 neuroendocrine neoplasia/tumor in cardia pattern mucosa
Male/58	smooth subepithelial lesion of about 12 mm on the posterior face of the medial antrum. Acanthosis glycogen of the esophagus.	Gastric antrum	Submucosal lesion 1, 3 cm	No

GET: Gastro-esophageal transition..*

RESULTS

We analyzed the data of the six patients who underwent endoscopy-assisted laparoscopic resection of gastric submucosal tumors from March 2011 to May 2014. Of these patients, three were male and three female. The mean age was 52 ± 18 years (range 31-85). The most common symptoms among patients were heartburn and gastric fullness. All patients underwent preoperative upper endoscopy with macroscopic biopsy. Among these, four patients underwent endoscopic ultrasound with fine needle aspiration biopsy (Table 1).

The mean duration of surgery was 181 ± 41 minutes (range 145-250). In addition to the laparoscopic wedge resection, one patient also underwent laparoscopic inguinal hernia repair right, this procedure lasting longer (250 minutes).

The average length of stay was 3.5 days, ranging from three to four.

The average tumor size was 2.0 ± 0.8 cm (varying from 1.0 to 3.4) and five of them (83.33%) were located in the proximal third of the stomach. The location of the tumors was as follows: two in the cardia, two in the fundus, one in the body and one in the antrum. The cardia tumors were located in the gastric anterior wall, one being near the small gastric curve. Of the gastric fundus lesions, one was located in the greater curvature. The high gastric body tumor was located in the posterior wall and near the gastric lesser curvature. The antral tumor was located in the posterior wall.

The preoperative histopathologic study obtained the following results: one endoscopic lesion suggestive of GIST (1.7x1.5 cm) and FNA suggesting leiomyoma; one 3.5x2.7 cm lesion suggestive

Tabela 2. *Características clínico-patológicas dos tumores submucosos gástricos ressecados (tamanho, localização, exame anatomopatológico e imuno-histoquímico)*

Paciente	Tamanho	Localização	Anatomopatológico	Imuno-histoquímica
Female, 61 years	1,0cm	Gastric Fundus	Ectopic Pancreas Absence of neoplasia	———
Female, 31 years	1,5cm	Cardia/ anterior wall	Leiomyoma, absence of tumor necrosis, cellular atypia or mitosis	Leiomyoma, Vimentin +/ Actin+/ Desmin+/Ki 67 + in less than 1%/ HHF-35 +
Male, 50 years	3,0cm	Cardia/anterior wall/ lesser curvature	Leiomyoma, absence of tumor necrosis, cellular atypia or mitosis	Leiomyoma, Actin diffusely +/ difuse Desmin / Ki 67 + in less than 1%
Female, 85 years	3,4cm	Gastric fundus/ greater curvature	Stromal tumor (GIST), necrosis (3%), Mitotic activity: 04/10 CGA	GIST CD-117 + / c-kit + / CD-34 +
Male, 31 years	1,3cm	High body/ posterior wall/ lesser curvature	Grade 2 neuroendocrine tumor (NET G2) Mitotic activity: 01/10 CGA	NETG2, CK 8/CK 18 +/ chromogranin +/ synaptophysin+/ CD 56 +/ Ki 67 + (3-5% of neoplastic cells)/ somatostatin -/ glucagon -/ serotonin -/ insulin -/ gastrin -
Male, 58 years	2,0cm	Antrum/posterior wall	Lipoma	———

of leiomyoma; one case of a 1.3cm lesion with FNA suspecting neuroendocrine tumor; one 1.2 cm lesion with inconclusive FNA. In two cases ewe have not performed FNA: in one of these, the endoscopic appearance was of a 1.2 cm stromal tumor; and in the other, with a 2 cm gastric fundus lesion, there were technical problems preventing biopsy by eco-endoscopy.

In the post-resection pathology study, we obtained the following results: one ectopic pancreas, two leiomyomas, one GIST, one grade 2 neuroendocrine tumor and one lipoma. The latter was operated on due to the preoperative endoscopic suspicion of GIST. One leiomyoma presented the following immunohistochemical analysis: vimentin (+); Actin (+); Desmin (+); Ki-67 (+) in less than 1% and HHF-35 (+). The other leiomyoma resulted in: Actin diffusely (+); Desmin diffuse and Ki-67 (+) less than 1%. The GIST immunohistochemical analysis demonstrated: CD-117 (+); c-kit (+) and CD-34 (+). The grade 2 neuroendocrine tumor (NET G2) showed: CK 8 / CK 18 (+); chromogranin A (+); synaptophysin (+); CD 56 (+); Ki-67 (+) (3-5% of neoplastic cells); Somatostatin (-); glucagon (-); Serotonin (-); insulin (-), and gastrin (-) (Table 2).

There were no episodes of tumor rupture, intraoperative complications and no conversion to open surgery. None of patients showed immediate or late postoperative complications.

DISCUSSION

The laparoscopic and endoscopic surgical techniques have evolved and generated good results in the treatment of benign and malignant gastrointestinal diseases. To date, only case reports have been published on the application of a laparoendoscopic approach GSMT resection [7]. Hence, the relevance of this study.

GSMT are rare lesions that are being more frequently diagnosed because of the ease and awareness of the importance of routine endoscopic examination. They are usually observed as a mass, lump, or imprint covered by normal mucosa. The actual incidence rate of these lesions is difficult to estimate and can be found in up to 0.4% of the population[1,8,9].

With respect to the location, in 83.3% of GSMT were in the proximal stomach, corroborating the literature, which states that two-thirds of these tumors are located in this region[10]. In 59.1% of patients, the tumors were located at the fundus and the resection next to the cardia could result in symptoms of gastroesophageal reflux[9,11,12].

Another point of discussion taken into consideration is that tumors located in the middle third of the stomach can easily be treated by laparoscopy, while tumors in the proximal and distal thirds of the stomach, near the cardia and pylorus, are at increased risk of post-resection stenosis. Thus, some authors recommend open surgery for such locations. Recently, to prevent deformity or stenosis of these areas, several minimally invasive techniques with organ preservation are being developed[1,7,11,13].

As for length of stay, the study of De Vogelaere et al. [14] reported seven days for the laparoscopy group was compared with 14 days for the open group[4]. In our study, the length of stay was on average 3.5 days, below the average of studies found[7,14,15]. Other works also demonstrate lower incidence of post-operative pain, smaller wound size, early return of bowel function with return to normal diet and shorter postoperative hospital stay[15,16].

In our series, the approach to a stromal tumor (GIST) followed the main strategy to achieve an anatomical or non-anatomic resection, with tumor-free margins. This unique feature has allowed for a wider role of minimally invasive techniques, especially laparoscopic wedge gastrectomy, and was consistent with other studies regarding GIST treatment[2,4,8-11,14,17]. Concerning our patients' age, several studies describe that most patients with gastric GIST are in their sixth or seventh decade of life, with only 10% of patients are under 40[12,14,15]. According to the classification published by Fletcher *et al*.[18], the GIST from this study was considered as low risk and did not require adjuvant therapy. The guidelines of the National Comprehensive Cancer Network[19] before 2007 did not recommend

laparoscopic surgery for GIST resection, except for tumors smaller than 2 cm in diameter and with a low rupture risk[9]. Although the tumor evaluated in this study was greater than 2cm in diameter, we opted for the laparoscopic resection and confirmed the non-infringement of the capsule, which allows us to strengthen the laparoscopic indication regardless of tumor size. This result indicates that the performance of laparoscopic and endoscopic techniques by qualified operators, without contact with the tumor during surgery and the use of a sample recovery bags, is essential for good surgical results. Thus, as in the study Novitsky *et al*.[20], we preserved the surgical margin of 1 to 2 cm. Our results converge with the ones of these authors, who demonstrated to offer curative approach for all GIST, even if they are at higher risk and with a diameter greater than 5cm.

In conclusion, this study demonstrated the viability and satisfactory surgical outcomes of endoscopy-assisted laparoscopic resection of gastric benign lesions and GIST. Endoscopy was important to locate the tumor and evaluate the resection. The hybrid proposed technique proved therefore to be an alternative procedure for gastric wedge resection, with minimal gastric deformity.

R E S U M O

Objetivo: *avaliar a viabilidade, segurança e vantagens da cirurgia minimamente invasiva para ressecção de tumores submucosos gástricos (TUSG).* ***Métodos:*** *estudo retrospectivo dos prontuários de pacientes submetidos à ressecção videolaparoscópica assistida por endoscopia digestiva alta para tumores submucosos gástricos (coletados prospectivamente) de 2011 a 2014. Os fatores avaliados foram dados clínicos, abordagem cirúrgica, características clinicopatológicas dos TUSG (tamanho, localização, exame anatomopatológico e imuno-histoquímico), resultados e acompanhamento dos pacientes.* ***Resultados:*** *foram avaliados seis pacientes, 50% do sexo masculino, com média de idade 52±18 anos e sintomas comuns de pirose e plenitude gástrica. Todos os pacientes foram submetidos ao procedimento híbrido e sem comprometimento anatômico do órgão. O tempo médio de internação foi 3,5 dias e o tamanho médio dos tumores foi 2,0±0,8cm, cinco deles (83%) no terço proximal do estômago. Os exames anatomopatológicos e imuno-histoquímicos das peças cirúrgicas demonstraram um caso de pâncreas ectópico (17%), um tumor neuroendócrino grau 2 (17%), um lipoma (17%), um GIST (17%) e dois leiomiomas (32%). Não houve episódios de ruptura do tumor nem complicações intraoperatórias e nenhuma conversão para cirurgia aberta. Durante o período de acompanhamento pós-operatório nenhum dos pacientes apresentou recidiva, metástase, fístula ou estenose.* ***Conclusão:*** *os resultados obtidos mostraram que a ressecção laparoscópica assistida por endoscopia é viável e segura para pacientes com TUSG. A endoscopia mostrou-se fundamental na localização das lesões e suporte intraoperatório, principalmente na tentativa de preservar a cárdia e o piloro durante a cirurgia.*

Descritores: *Procedimentos Cirurgicos Operatórios. Cirurgia Videoassistida. Leiomioma.*

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Recebido em: 25/10/2015
Aceito para publicação em: 18/03/2016
Conflito de interesse: nenhum.
Fonte de financiamento: nenhuma.

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DOI: 10.1590/0100-69912016002011

Duodenum inclusion in alimentary transit for preventing or correcting nutritional deficiencies resulting from Roux-en-y gastric bypass in obesity treatment

Inclusão do duodeno no trânsito alimentar para prevenção ou correção de deficiências nutricionais resultantes da derivação gástrica em y de Roux no tratamento da obesidade

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ABSTRACT

Nutritional and metabolic complications can develop after Roux-en-Y gastric bypass (RYGB) when there is an exaggerated response to the anatomical and functional changes or when there is inadequate nutritional supplementation. Severe malnutrition is rare, but deficiencies of vitamin B12, iron, calcium and thiamin, metabolic bone disease and gallstones are common after RYGB. Shortage of vitamin B12, iron, calcium and also cholelithiasis are caused at least partially by excluding the duodenum and proximal jejunum from food transit. We designed a new procedure, with the maintenance of the duodenum and proximal jejunum in the gastrointestinal transit through interposition of jejunal loop, as a primary operation to prevent such deficiencies or as corrective surgery for severe malnutrition after RYGB with failure in responding to conservative treatment.

Keywords: Obesity, Morbid. Gastric Bypass. Anastomosis, Roux-en-Y. Malnutrition. Second-Look Surgery.

INTRODUCTION

The Roux-en-Y gastric bypass (RYGB) is the most universally accepted technique for the surgical treatment of morbid obesity, with significant weight loss and frequent resolution of comorbidities in most patients.

The anatomical changes that are imposed by RYGB lead to dramatic reduction in the amount of nutrients available to patients. Nutritional and metabolic complications can develop when there is an exaggerated response to the anatomical and functional changes or when there is inadequate nutritional supplementation.

In RYGB the stomach volume is reduced; the surgeon creates a proximal gastric pouch with a capacity of 30 to 50 ml, anastomosed to a 70 to 100 cm jejunal loop, with transit reconstruction in a Roux-en-Y fashion with a jejunojejunostomy 50-70cm distal to the angle of Treitz. The exclusion of the distal stomach, duodenum and proximal jejunum causes a marked reduction in the absorptive capacity of nutrients, electrolytes and bile salts[1] and may also hamper the entero-hepatic circulation of bile salts.

Complications like severe malnutrition (4.7%)[2], hypoalbuminemia (5.3%)[3], malabsorption of fats, fat-soluble vitamins and folate deficiency are uncommon; deficiency of Vitamin B12 (over 30%)[1], iron (20 to 49%)[1], Calcium (16.7%)[4] and thiamine, metabolic bone disease and cholelithiasis (50%)[1] are common after RYGB, though. The pathophysiology of these deficiencies in RYGB is related to reduced nutrient intake, decreased acidity, pepsin and intrinsic factor due to the stomach reduction and the exclusion of the stomach acid medium and of the absorptive surface of the duodenum and proximal jejunum.

Of these aforementioned RYGB-related deficiencies, the ones of B12 vitamin, iron and calcium, and also cholelithiasis, are caused, at least in part, by the duodenum and proximal jejunum exclusion.

On the other hand, there are studies that show excellent results in the treatment of serious nutritional deficiencies after extensive gastrectomy by converting the Billroth II reconstruction to a Henley, with reintroduction of the duodenum in the food transit through the interposition of a jejunal loop between the gastric stump and the duodenum[5] .

The interest in preventing or minimizing such postoperative complications justifies the attempt to modify the RYGB technique by maintaining the duodenum and proximal jejunum in the food transit.

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SURGICAL PROCEDURE

The surgical procedure is done according to the technique schematics devised by the author's graphics (Figures 1 and 2).

The primary operation follows the technical steps of RYGB (Figure 1a) except for the reconstruction of the food transit: after gastrojejunostomy, the jejunal loop brought to the supramesocolic area and sectioned at approximately 25 to 30 cm from the anastomosis (Figure 1b) is latero-laterally anastomosed to the second portion of the duodenum, being complemented with the inframesocolic, termino-terminal, or preferably latero-lateral, jejunojejunal anastomosis (Figure 1c).

The 20 to 30 cm jejunal loop interposed between the stomach and duodenum is as effective as Roux-en-Y bypass to promote the reduction of entero-gastric reflux[6].

The inclusion of the duodenum in the food transit can also be used as a corrective surgery in patients with severe protein-calorie malnutrition unsolved by exhaustive conservative treatment attempts, and can be achieved through two techniques (Figure 2).

The first technique involves two sections and two anastomoses (Figures 2b and 2c) and the second, only one section and one anastomosis (Figures 2b1 and 2c1), but the former, with a short interposed jejunal loop, should be the choice when the RYGB original alimentary loop (Figure 2a) is long, favoring angulation.

The results of clinical evolution and nutritional and metabolic aspects of patients undergoing this technique as a primary or corrective surgery for severe protein-calorie malnutrition with conservative treatment failure were satisfactory and, in due course, will be published.

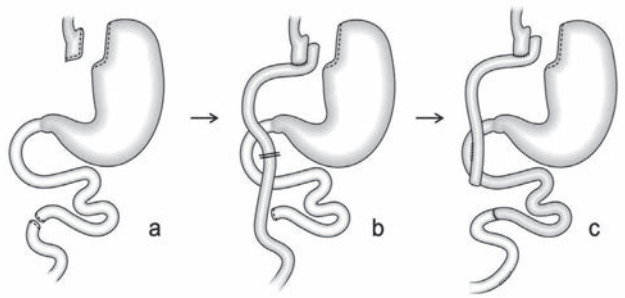

Figure 1. *Roux-en-Y gastric bypass modified by the inclusion of the duodenum and proximal jejunum in the food transit as the primary surgery. a- making of the gastric pouch and section of the jejunum 70 cm distal to the angle of Treitz; b- gastro-jejunal anastomosis and section of the alimentary loop 20 to 30 cm distal to the gastrojejunostomy; c- transit reconstruction with two anastomoses, one latero-lateral jejunoduodenostomy and one termino-terminal jejunojejunostomy.*

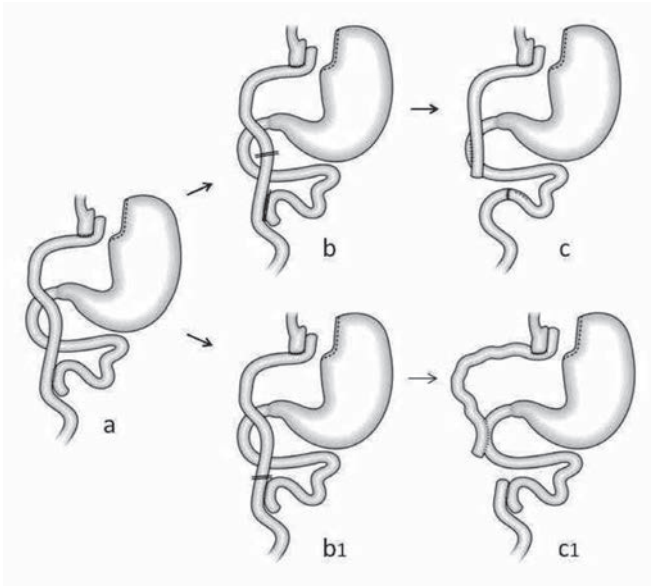

Figure 2. *Inclusion of the duodenum and proximal jejunum in the food transit with interposition of jejunal loop as a corrective surgery after Roux-en-Y gastric bypass. a- Roux-en-Y gastric bypass; b- Stapler section of the alimentary loop 20 to 30 cm distal to the gastrojejunostomy and of the biliopancreatic loop next to the jejunojejunal anastomosis; c- transit reconstruction with jejunoduodenal and jejunojejunal anastomoses; b1- Stapler section of the alimentary loop next to the jejunojejunal anastomosis; c1- transit reconstruction with jejunoduodenal anastomosis.*

R E S U M O

Complicações nutricionais e metabólicas podem se desenvolver após a derivação gástrica em Y de Roux (DGYR) quando há uma resposta exagerada às mudanças anatômicas e funcionais ou quando há suplementação nutricional inadequada. A desnutrição grave é rara, mas deficiências de vitamina B12, ferro, cálcio e tiamina, doença óssea metabólica e cálculos biliares são comuns após a DGYR. Dessas deficiências mencionadas, a de vitamina B12, de ferro, de cálcio e também a colelitíase, são causadas, ao menos parcialmente, pela exclusão do duodeno e jejuno proximal. Um novo procedimento com a manutenção do duodeno e do jejuno proximal no trânsito gastrointestinal, mediante interposição de alça jejunal, foi idealizado como operação primária para prevenir essas deficiências ou como cirurgia corretiva de desnutrição grave após DGYR com falha na resposta a exaustivas tentativas de tratamento conservador.

Descritores: *Obesidade Mórbida. Derivação Gástrica. Anastomose em-Y de Roux. Desnutrição. Cirurgia de Revisão.*

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Received in: 07/11/2015
Accepted for publication: 08/03/2016
Conflict of interest: none.
Source of funding: none.

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