https://doi.org/10.31871/WJRR.19.2.3
World Journal of Research and Review (WJRR)
ISSN: 2455-3956, Volume-19, Issue-2, August 2024 Pages 03-08

Tissue Budding Expression Associated with Histopathological Characteristics in Patients Undergoing Neoadjuvant Therapy for pT3N0M0/pT3N1M0 Adenocarcinoma of the Rectum Extraperitoneum

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Abstract— **Introduction: Malignant colorectal cancer is the third most common cancer in men and the second most common in women from the 5th decade onwards. The TNM system is often used in pre and post-operative staging, and the use of other hematimetric parameters for the prognosis of colorectal cancer are sometimes decisive in more accurate assessments. Tumor budding has been defined as the presence of isolated neoplastic cells or small groups of cells present in the stroma at the margin of the tumor, with studies showing its relationship with the prognosis of malignant disease, especially those related to poor survival and a high risk of recurrence. Objective: To investigate the prognostic value of tumor tissue budding expression in patients with extra-peritoneal rectal cancer pT3N0M0/pT3N1M0, undergoing radical curative surgery after neoadjuvant chemoradiotherapy, considering its influence on histopathological parameters and malignant disease-free survival time. Methodology: 46 results of the histopathological examination of patients undergoing neo-adjuvant therapy for the treatment of rectal-extraperitoneal adenocarcinoma were analyzed, taking into account the values of tissue budding, desmoplasia, angio-lymphatic invasion, peri-tumoral inflammatory infiltrate, presence of lymph node metastasis, tumor extension and neoplastic disease-free survival time. The study group consisted of 23 men and 23 women, regardless of race, with a mean overall age of 66.5 years. Results: Using Student's t-test, a statistical significance was defined for the relationship of positive budding with angio-lymphatic invasion (p=0.019), with ganglion resection count (p=0.001), tumor extension (p=0.028) and in neoplastic disease-free survival time (p=0.058). When associated according to gender, a statistically significant relationship was observed in male patients between angio-lymphatic invasion (p=0.006) and the presence of lymph node metastasis (p=0.009), while in females the relationship was present in terms of the number of resected lymph nodes (p=0.006) and with a greater trend in disease-free survival time (p=0.065). The Chi-Square test was applied to analyze the variables, desmoplasia (p=0,749), angio-lymphatic invasion (p=0,020) and peri-tumor inflammatory infiltrate (p=0,079). There was statistical significance in the relationship between angio-lymphatic invasion and the presence of lymph node metastasis (p=0.041). Considering p<0.05 as statistically significant. Conclusion: This study found that patients with extra-peritoneal pT3N0M0/pT3N1M0 adenocarcinoma of the rectum undergoing neo-adjuvant therapy associated with abdominal rectosigmoidectomy confirmed that the presence of tissue budding influences angio-lymphatic invasion, ganglion resection count, maximum lesion extension and malignant disease-free survival time, and the presence of metastatic ganglion involvement in males.**

Index Terms— **Tissue Budding, Colorectal Cancer, Desmoplasia, Angio-lymphatic Invasion, Peri-tumoral Inflammatory Infiltrate, Metastasis, Neoplastic Disease-free Survival.**

I. Introduction

Colorectal cancer is the third most frequently diagnosed cancer in men and the second in women. Neoadjuvant chemoradiotherapy accompanied by surgical resection is the standard therapeutic approach in extraperitoneal rectal cancer pT3N0M0/pT3N1M0, but there is no ideal marker for prognostic follow-up.[1,2] Long-term preoperative therapy with 5-FU (5-fluorouracil) and short-term preoperative radiotherapy (25 Gy, five applications) are considered the primary treatments of choice for patients with locally advanced extraperitoneal rectal cancer (T3/4 and/or with lymph node metastasis)[3], followed by intestinal resection with total excision of the mesorectum, which reduces the possibility of local recurrence by up to 90%. [4] The prognosis of colorectal cancer is influenced by numerous factors and even if standard therapy is followed, around 25% of early-stage patients will develop distant metastases.[5] In this condition, patient survival drops dramatically to less than 10% in five years. [6,7] The most important prognostic factor for overall survival rate is the TNM stage, lymph node invasion, vascular invasion, histopathological type and resection margin status. [8]

Tumor budding is referred to as the presence of isolated neoplastic cells or small groups of cells with fewer than five cells, present in the stroma at the margin of the tumor, with a direct relationship between the appearance of the tumor and the epithelial-mesenchymal transition process. In this

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transition process, the epithelial cells lose intracellular and cell matrix contacts mediated by cadherin-E, resulting in invasion and, finally, dissemination of the metastatic cancer.[9,10,11] , defining its relationship with the prognosis of the disease in colorectal cancer, especially those related to poor survival and a high risk of recurrence.[12]

When a biopsy is performed in the diagnostic phase of the tumor lesion, the presence of budding is associated with a more advanced tumor and nodal stage, distal intratumoral dissemination, local recurrence, response to neoadjuvant chemoradiotherapy, disease-free survival and a positive predictor of cancer-related deaths[13,14] , and in cancer rectal was correlated with TNM stage, vascular invasion and tumour grade. [15] The rectal cancer associated with low budding was a predictor of complete pathological response after neo-adjuvant therapy in rectal cancer patients.[16]

Objective

To investigate the prognostic value of tumor budding expression in patients with pT3N0M0/pT3N1M0 extra-peritoneal rectal cancer who underwent radical curative surgery after neoadjuvant chemoradiotherapy, considering its influence on histopathological parameters and malignant disease-free survival time.

II. METHODOLOGY

A retrospective study was carried out on 46 pathology exams of patients with extraperitoneal pT3N0M0/pT3N1M0 adenocarcinoma of the rectum who underwent neo-adjuvant therapy followed by abdominal surgical resection between 2017 and 2018, after approval by the Ethics and Research Committee of the Federal University of São Paulo and the Vale do Paraíba Regional Hospital - Taubaté, Brazil. The group consisted of 43 men and 43 women, regardless of race. The overall average age was 66.5 years (Table 1). The inclusion criteria were: both sexes, no age limit, treated with complete neoadjuvant chemoradiotherapy followed by surgery, with histopathological examination and investigation of tissue budding, without treatment for another malignant disease and operated on an average of 6 to 8 weeks after the end of neoadjuvance and in postoperative stage I restricted to pT3N0M0 and pT3N1M0. Quantification of tumor budding is classified into three grades: 0-4 buds - low budding, 5-9 buds - intermediate budding and 10 or more buds - high budding.[12] Tumor budding will be analyzed in the previously paraffin-embedded tumor tissue. The identification of tumor budding will be determined by hematoxylin-eosin examination and quantified as present (low, intermediate and high) or absent. Disease-free survival was defined as the time from the date of surgery to the last visit.

Student's t-test and the Chi-Square test was applied to statistical analysis. The 95% confidence interval and p-values in the tests of 0.05 will be considered statistically significant.

General characteristics of the study group.

Sex	Male nº 23	Male	Female nº 23	Female
Budding	Positive Budding 10 – 43,5%	Negative Budding 13 – 56,5%	Positive Budding 11 – 47,8%	Negative Budding 12 – 52,2%
Maximum Age	78	84	77	81
Minimum Age	59	43	35	53
Average Age	69,7	67	66,4	62,8

Table 1: Distribution of the Presence of Tissue Budding According to Sex.

III. RESULT

The study group consisted of 46 patients/examinations, with the presence of tissue budding detected in 10(47%) men and 11(53%) women and its absence in 13(52%) men and 12(48%) women. All the patients had their exams studied according to the presence of tissue budding and histopathological exam data (Table 2) and with subsequent statistical and postoperative life span evaluation.

Distribution of histopathological findings in patients with extraperitoneal rectal tumor.

	Tissue Budding		Statistical Analysis p<0,05
Sex	Male	Female	
Positive	10-43.5%	11-47.8%	
Negative	13-56.5%	12-52.2%	
Desmoplasia			p=0,749
	Positive	Negative	
Absent	0	03-12.0%	
Discrete	11-52.4%	11-44.0%	
Moderate	09-42.9%	11-44.0%	

Intense	01-4.8%	0	
Angio-lymphatic Invasion			p=0,020
	Positive	Negative	
Absent	11-52.4%	21-84.0%	
Present	10-47.6%	04-16.0%	
Peritumoral Inflammatory Infiltrate			p=0,074
	Positive	Negative	
Absent	01-4.8%	04-16.0%	
Discrete	14-66.7%	09-36.0%	
Moderate	06-28.6%	12-48.0%	
Resected and metastatic lymph nodes			
	Positive	Negative	
Maximum Number	40	45	
Minimum Number	13	12	
Average Metastasis	1.2	0.6	

Table 2- Number distribution of histopathological findings.

Considering the distribution of patients according to the post-operative anatomopathological stage and the presence of tissue budding, it was observed that in the present detection, the pT3N0M0 stage was found in 11 (52%) cases, while the pT3N1M0 stage was found in 10 (48%) cases. In the absence of tissue budding in the samples, the pT3N0M0 stage was observed in 21 (84%) cases, while the pT3N1M0 stage was observed in 4 (16%) cases. Survival time free of neoplastic disease up to the conclusion of this study was calculated depending on the presence or absence of tissue budding. Thus, the maximum time in cases of presence was 7.2 years, with a minimum of 2.7 years and a mean time of 4.9 years, while in cases of absence, the maximum time was 8.7 years, with a minimum of 2.3 years and a mean of 5.5 years.

Using Student's t-test, statistical significance was found for the relationship between positive budding and angio-lymphatic invasion (p=0.019), ganglion resection count (p=0.001), tumor extension in centimeters (p=0.028) and neoplastic disease-free survival time (p=0.058). When associated according to gender, a statistically significant relationship was observed in male patients between angio-lymphatic invasion (p=0.006) and the presence of lymph node metastasis (p=0.009), while in females the relationship was present in terms of the number of resected nodes (p=0.006), with a greater tendency towards disease-free survival time (p=0.006). There was statistical significance in the relationship between angio-lymphatic invasion and the presence of lymph node metastasis (p=0.041). The Chi-Square test was applied to analyze the variables, desmoplasia (p=0,749), angio-lymphatic invasion (p=0,020) and peri-tumor inflammatory infiltrate (p=0,079), as shown in Table 3.

Relationship of values with statistical significance.

Histopathological examination	General		Male	Female
	T´Student	Chi-Square		
Desmoplasia	0,775	0,749	0,977	0,719
Angio-lymphatic invasion	0,019 *	0,020*	0,006*	0,559
Peritumoral Inflammatory Infiltrate	0,677	0,079	0,130	0,505
Metastatic nodes	0,271		0,009*	0,702
Resected nodes	0,015*		1,055	0,006*
Extent of tumor lesion	0,028*		0,212	0,082
Survival time	0,058*		0,987	0,065

Table 3 - Statistical analysis of the relationship between patients with detected and undetected tissue budding in the samples studied.

IV. DISCUSSION

The study involved patients with extraperitoneal adenocarcinoma of the rectum at stages pT3N0M0 and pT3N1M0 who underwent neoadjuvant therapy and surgery, with complete pathological examination and quantification of tumor tissue budding. In this way, 46 patients were selected from 2017 to 2018, and evaluated as to whether the presence of tumor tissue budding interferes with histopathological examinations and malignant neoplastic disease-free survival time.

The stage of malignant colorectal disease determined by the TNM system is one of the factors in assessing the prognosis of the disease, which, together with the determination of lymph node invasion, vascular invasion, histopathological type and the state of the resection margin, are the main parameters considered when determining the treatment plan for each patient. Preoperative neoadjuvant therapy has been recommended as the standard treatment for locally advanced disease of the middle and lower third of the rectum, significantly reducing the risk of local recurrence.[17] Other histopathological parameters are being explored as

possible prognostic biomarkers for colorectal cancer, such as tumor budding, poorly differentiated clusters, extramural vascular invasion (vein), perineural invasion, tumor deposits, mucin pools and extranodal extension of nodal metastasis, but some of them have not yet been fully investigated.[18]

Colorectal cancer is the third most common cause of death from malignancy. [19] The standard treatment strategy for locally advanced rectal cancer is neoadjuvant chemoradiotherapy followed by surgical resection with total mesorectal excision.[20] Neoadjuvant therapy can regress the T and N stages to the point of complete regression of the lesion, which has been the subject of much discussion regarding the possibility of expetant therapy in the face of a complete clinical response. [21]

The study group was made up of 46 abdominal rectosigmoidectomy specimens, divided into equal groups according to gender, with tissue budding being present in 10(43.5%) of the men and 11(47.8%) of the women, and being undetected in 13(56.5%) of the men and 12(52.2%) of the women. Tumor budding is a well-established indicator of aggressive tumor biology in colorectal cancer, a relevant factor for patient management in endoscopically resected pT1 colorectal cancer, stage II tumors and preoperative biopsies.[2] Defined as an isolated cancer cell or a cluster composed of fewer than 5 cells in the invasive frontal region. The number of buddings is counted in a single field. Depending on the number of buddings, the grade of budding is defined as low (0–4) or high (>5) [23], and is associated with lymphovascular invasion, distant metastases and poor prognosis. [24]

Lympho-vascular invasion was defined as the presence of tumour cells within the endothelial-lined spaces. Colorectal cancer metastasizes by five means: direct extension, lymphatic spread, portal venous spread to liver, peritoneal dissemination, and vascular spread to distant organs including lung, bone, and brain.[25] Angio-lymphatic invasion was observed in 10 (47.6%) samples associated with tissue budding and in 04 (16%) of those not associated with tissue budding. The angio-lymphatic invasion was quantified in this study, with a statistically significant difference when the Student's t test (p=0.019) and the Chi Square test (p=0.020) were applied and in female patients (p=0.0006) whose histological analysis showed presence of tumor growth.

In the presence of a mature desmoplastic reaction, there is greater lymphatic invasion, while in immature tumor stromal fibrosis there is greater growth of the infiltrating tumor. Desmoplastic maturation reactions have been associated with the infiltrative growth pattern of the tumor and local invasion, such as lymphatic invasion and the formation of tumor buds.[26] Tissue desmoplasia was observed in the samples positive for tissue budding in a mild form in 11 (52.4%), in a moderate form in 09 (42.9%) and in an intense form in 01 (4.8%), while in the negative samples we found 11 (44.0%) in a mild form, 11 (44.0%) in a moderate form and it was absent in 03 (12%) of the samples. Tissue desmoplasia was quantified in this study and no statistically significant difference was identified using the Student t test (p=0.775) and the Chi-square test (p=0.749) in the global analysis or according to sex.

It is known that inflammation around the tumor can change biological conditions by producing an increase in vascular permeability, angiogenesis, cell proliferation and cell mobilization, therefore stimulating the inflammatory process, both tumor proliferation and possible metastasis.[27] Peritumoral inflammatory infiltrate was identified in samples positive for tissue budding in a discrete form in 14 (66.7%), moderately in 06 (28.6%) and absent in 01 (4.8%), while in negative samples we found 09 (36.0%) discreetly, 12 (48.0%) moderately and absent in 04 (16%) of the samples. Peritumoral inflammatory infiltrate was quantified in this study and no statistically significant difference was identified Student t test (p=0.677) and Chi Square test (p=0.079) in the overall analysis or according to gender. Inflammatory activity, as a local peri-tumor immunological response, may be a prognostic factor, as the smaller the inflammatory infiltrate, the greater the risk of regional or distant dissemination. However, a correlation was found between the highest degree of malignancy and the greatest inflammatory intensity, suggesting a positive relationship between the intensity of the inflammatory response and the degree of tumor differentiation, without necessarily having an influence on the patients' prognosis.[28]

Some points of the histopathological examination are associated with a higher rate of tumor recurrence or the involvement of lymph node metastases, where the most important are the degree of differentiation with marked dedifferentiation, the presence of tumor budding, the depth of tumor invasion into the intestinal wall and from the submucosal layer or lymphovascular invasion.[29] According to the literature, adequate surgical resection should involve the removal of at least 12 lymph nodes, considering that a number between 12 and 15 negative lymph nodes does not predict involvement of regional metastatic lymph nodes.[30,31] In the histopathological examinations of the present study, an average resection of 24 nodes was found, with a maximum of 40 in the group with present tissue budding and 45 in the group with undetected tissue budding. The minimum number of resected nodes was 13 in the group with tissue budding present and 12 in the group where tissue budding was not detected. The number of resected nodes also presented a statistically significant difference (p=0.015), when we compared the group with the presence of tissue budding compared to the group with undetected budding, as well as in females where changes occurred with a statistical difference significant (p=0.0006). The presence of intratumoral budding identified from the biopsy of the preoperative rectal lesion is related to a high possibility of being associated with the presence of lymph node metastases in the pre-treatment period, which may modify the clinical evolution.[32]

Considering the observation period of this study, the survival time free from neoplastic disease was a maximum of 7.2 years, with a minimum of 2.7 years and an average time of 4.9 years in the tissue budding group present, while in the in cases of absence, the maximum time was 8.7 years, with a minimum of 2.3 years and an average of 5.5 years. It is noteworthy that not all patients who died were due to malignant disease, as there were cases of COVID, heart and coronary failure, chronic and acute renal failure. There were 5 (23.8%) deaths due to malignant peritoneal neoplasia in the

first 2 years in patients with tissue budding present and 2 (8%) deaths in cases in which tissue budding was not detected, being a statistically significant result (p=0.058) The presence of tissue budding is a prognostic factor related to death from cancer in univariate and multivariate analyses, with mortality rate significant.[33] Studies demonstrate that the cumulative survival rate of patients with tumor budding was significantly lower than those no tumor budding.[34]

V. CONCLUSION

In our study, the relationship between tumor budding and survival is very strong, as well as in the presence of angio-lymphatic invasion, peri-tumoral inflammation and the overall count of metastatic ganglions, which demonstrates a potential for greater tumor aggressiveness in these histopathological conditions.

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