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NEÜ YAYINEVİ

The Prognostic Importance of Tumor Budding in Intestinal-Type Gastric Adenocarcinoma

İntestinal Tip Mide Adenokarsinomlarında Tümör Tomurcuklanmasının Prognostik Önemi

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Öz

Amaç: Mide kanseri kansere bağlı ölümlerin önde gelen sebeplerinden biridir. Tümör tomurcuklanması birçok kanserde prognostik faktör olarak gösterilmiştir. Bu çalışmada intestinal tip mide adenokarsinomunda tümör tomurcuklanmasının prognostik önemini değerlendirmeyi amaçladık.
Hastalar ve Yöntem: Çalışmaya 2015-2021 yılları arasında Patoloji Kliniğinde intestinal tip mide adenokarsinom tanısı almış 152 olgu dahil edildi. Tümör tomurcuklanması düşük, orta, yüksek olarak gruplandı. Hematoksilen-Eosin boyalı preparatlar tümör diferansiyasyonu, lenfovasküler invazyon (LVİ), perinöral invazyon (PNİ), lenf nodu tutulumu, invazyon derinliği (pT) ve tümör tomurcuklanması açısından yeniden değerlendirildi.
Bulgular: Çalışmaya katılan olguların %30.9 (n=47)'unda tümör tomurcuklanması düşük, %37.5 (n=57)'inde orta, %31.4 (n=48)'ünde yüksek yoğunlukta idi. İstatistiksel olarak tümör tomurcuklanması arttıkça tümör boyutu artmakta (p<0,05), olguların takip süreleri kısalmakta, sağ kalım süresi (p<0,05) ve tümör diferansiasyonu (p<0,05) azalmakta idi. Tümör tomurcuklanması ile LVİ (p<0,05), PNİ (p<0,05), pT(p<0,05), lenf nodu tutulumu (p<0,05) ve olguların mortalitesi (p<0,05) arasında istatistiksel olarak anlamlı ilişki gözlendi. Tümör tomurcuklanması ile cinsiyet, yaş, tümör lokalizasyonu ve operasyon tipi arasında istatistiksel olarak anlamlı ilişki gözlenmedi (p>0,05).
Sonuç: Tümör tomurcuklanması kötü prognostik faktörlerle ilişkilidir. Tedavi seçiminde ve olguların takibinde önemli olabileceğinden tümör tomurcuklanma durumu patoloji raporlarına dahil edilebilir.

Anahtar Kelimeler: Adenokarsinom, intestinal, mide, tümör tomurcuklanması

Abstract

Aim: Gastric cancer is one of the leading causes of cancer-related deaths. Tumor budding has been shown to be a prognostic factor in many cancers. In this study, we aimed to evaluate the prognostic significance of tumor budding in intestinal-type gastric adenocarcinoma.
Patients and Method: A total of 152 cases diagnosed as intestinal type gastric adenocarcinoma in the Pathology Clinic between 2015 and 2021 were included in the study. Tumor budding was grouped as low, medium and high. Hematoxylin and eosin-stained slides were re-evaluated in terms of tumor differentiation, lymphovascular invasion (LVI), perineural invasion (PNI), lymph node involvement, depth of invasion (pT) and tumor budding.
Results: Tumor budding was low in 30.9% (n=47) of the subjects included in the study, moderate in 37.5% (n=57) and high in 31.4% (n=48). Statistically, as tumor budding increased, tumor size increased (p<0,05), follow-up times were shortened, survival time (p<0,05), and tumor differentiation (p<0,05) decreased. A statistically significant correlation was observed between tumor budding and LVI (p<0,05), PNI (p<0,05), pT(p<0,05), lymph node involvement(p<0,05), and mortality of the cases (p<0,05). No statistically significant correlation was observed between tumor budding and gender, age, tumor localization and operation type (p>0.05).
Conclusion: Tumor budding is associated with poor prognostic factors. As it may be important to guide the treatment modality and follow-up, tumor budding status may be mentioned in routine pathology reports.

Key words: Adenocarcinoma, intestinal, stomach, tumor budding

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INTRODUCTION

Gastric cancer is the fifth most common malignancy in the world and the third cause of cancer-related death worldwide in both genders (1). The prognosis is often poor, and in countries without screening programmes, most cases are diagnosed at an advanced stage. To study additional clinicopathological features is necessary to assess unfavorable prognostic parameters and identify individuals who may benefit from personalized treatment after resection. Better and more accurate prognostic indicators are needed for individual treatments (2).

Gastric adenocarcinomas are histologically, biologically and genetically heterogeneous, and there are several different classification systems. One of the most well-known classification is the Lauren system, which classifies gastric carcinomas as intestinal and diffuse types (3). Most intestinal type gastric cancers are located in the distal region and there are glands in various stages of differentiation. In diffuse type carcinomas, tumor cells show weak cohesions and gland formation is not observed (4).

Although the definition of tumor budding (TB) is not standardized, it is generally defined as single or clusters of less than 5 tumor cells separated from the main tumor, which can be evaluated on hematoxylin-eosin (H & E) stained sections or can be detected by keratin immunohistochemistry (5). The prognostic significance of TB has been most commonly defined in colorectal adenocarcinoma and has been investigated in different series including colorectal cancer as well as lung adenocarcinoma, esophageal carcinoma, ampullary carcinoma, pancreatic, and head and neck squamous cell carcinomas (5-14).

There are few studies evaluating TB in gastric adenocarcinomas (2, 15-17). In this study, it was aimed to investigate the prognostic importance of TB in intestinal-type gastric adenocarcinomas.

PATIENTS AND METHOD

Our study is a retrospective study performed for total of 152 patients with intestinal type gastric adenocarcinoma who underwent total and subtotal gastrectomy in the Pathology Clinic between 2015-2021. Gastric tumors of non-epithelial origin, gastric metastases, invasive tumors from extra-gastric neoplasms and tumors that received preoperative treatment, diffuse type and mixed type gastric adenocarcinoma with diffuse type components were not included in the study.

Hematoxylin-Eosin stained preparations registered in the archieves of our laboratories which were prepared with routine follow-up protocols after fixation in 10% buffered formalin solution were re-examined with light microscopy. The cases were re-evaluated in terms of age, gender, localization, tumor size, tumor differentiation, lymphovascular invasion (LVI), perineural invasion (PNI), depth of invasion (pT), lymph node involvement. The evaluation of pT and lymph node involvement was performed according to the AJCC TNM classification in all surgical resection materials (18). The death dates of the patients were obtained from the Death Notification System (obs.gov.tr). Interval between the time of operation obtained from the hospital system and the time of death obtained from the Death Notification System was evaluated as the overall survival of the patient after surgery. Time of death was determined however information about causes of death and gastric cancer recurrence time were not available which some patients did not undergo regular follow-up examinations in our hospital. Therefore, only overall survival could be determined, and disease-free survival could not be determined. Overall survival was assessed as alive/dead.

In the evaluation of TB, an isolated single cancer cell or cancer cell clusters consisting of less than 5 cells were considered as "budding focus". All tumor slides were scanned at x100 magnification and tumor budding was counted at x200 magnification by selecting the area with the maximum tumor budding density. Tumors were divided into 3 groups according to budding density; 0-4 buds – low (Fig. 1A), 5-9 buds – medium (Fig. 1B), 10 or more buds (Fig. 1C) were rated as high. All procedures followed were in accordance with the clinical research ethics committee (Decision number 21.09.2020, 96/08)

Statistical analysis

The analysis of the data obtained from the study was made with the SPSS 23.0 (Statistical Package for Social Science) statistical program. First of all, the mean and standard deviation (SD), minimum and maximum values of the quantitatively obtained data are given. The conformity of the data to the normal distribution was tested with the Kolmogorov-Smirnov test. The one-way analysis of variance (ANOVA) was used to determine whether there are any statistically significant differences between the means of three or more independent (unrelated) groups. Tukey's test, one of the Post Hoc tests, was used to find out which group or groups means caused the difference for the variables that differed. Pearson Chi-Square

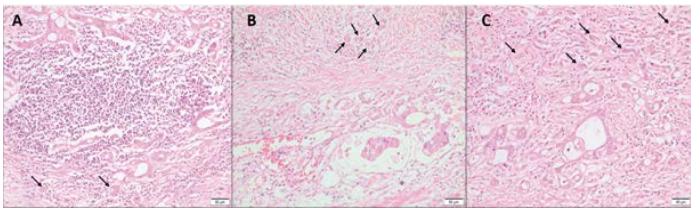

Figure 1. A: Low density tumor budding, **B:** Moderate density tumor budding, **C:** High density tumor budding (H&E x200). Black Arrow shows tumor budding

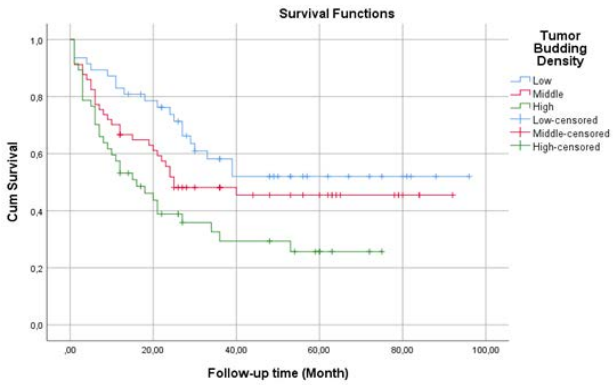

Figure 2. Tumor Budding Density Kaplan- Meier Curve

tests were used to determine whether there was a relationship between the variables through cross tables. Median survival time of patients after diagnosis was estimated by Kaplan-Meier survival analysis (Log Rank). Statistical significance was determined with a value of $p<0.05$.

RESULTS

152 cases were enrolled in the study. The median age at the time of the surgery was 67.06±10.97. Tumor size was 51.68 ±28.22 mm and mean follow-up time was 29.87 ± 25.46 months (Table 1).

71.1% (n=108) of the patients in the study were male and 28.9% (n=44) were female. TB was low density in 30.9% (n=47), medium-density in 37.5% (n=57), and high-density in 31.4% (n=48) of the patients. 5.2% (n=8) of the cases were evaluated as well differentiated, 55.9% (n=85) as moderately differentiated, 38.8% (n=59) as poorly differentiated. LVI was observed in 76.3% (n=116) and not observed in 23.6% (n=36). Lymph node involvement was observed in 69.7% (n=106). 30.2% (n=46) had no lymph node involvement. PNI was seen in 61.8% (n=94), not seen in 38.1% (n=58) of all patients. pT1 represented 12.5% (n=19), pT2 14.4% (n=22), pT3 27.6% (n=42), and pT4 represented 45.3% (n=69) of the cases. 15.7% (n=24) was located in the upper 1/3 of the stomach, 27.6% (n=42) in the middle 1/3, and 56.5% (n=86) was located in the lower 1/3. 33.5% (n= 51) of the cases had subtotal resection and 66.4% (n= 101) had total resection. While 54.6% (n=83) of the cases were dead after the follow-up, 45.4% (n=69) were alive.

As the TB density increases, the tumor size increases ($p<0.05$), the follow-up period of the cases is shortened, and the survival time decreases ($p<0.05$). TB density increment is statistically associated with tumor undifferentiation significantly ($p<0.05$). In addition, TB is significantly associated with LVI ($p<0.05$), PNI ($p<0.05$), pT ($p<0.05$), lymph node involvement ($p<0.05$) and mortality ($p<0.05$). No statistically significant correlation was observed between tumor budding and gender, age, tumor localization and operation type ($p>0.05$) (Table 2).

In the present study, the interval from the date of diagnosis to the date of death of the patients was as identified as overall survival. The death of the patient was expressed as a failure. Surviving patients were defined as censored. When the follow-up period was ended, 54.6% was failure and 45.4% was cencored. It was tested with the Log-Rank test that the follow-up period of the patients was different according to the TB density ($p<0.05$). The median survival time relative to TB density was 59,486 months at low

Table 1. Descriptive Statistics

Variables	Mininum	Maximum	Mean	Std. Deviation
Tumor Size (mm)	10	160	51,68	28,229
Age	27	99	67,06	10,973
Follow-up time (Month)	1,00	96,00	29,8421	25,46602

Table 2. Relationship Between Tumor Budding and Pathological Data

Parameters	Low density tumor budding (n=47) (%)	Middle density tumor budding (n=57) (%)	High density tumor budding (n=48) (%)	p-value
Gender				
Female	14 (29,8)	17 (29,8)	13 (27,1)	0,942
Male	33 (70,2)	40 (70,2)	35 (72,9)	
Age				
Mean ± Std. Deviation,	68,30±8,68	67,28±11,09	65,58±12,75	0,478
min-max t	45-86	40-99	25-160	
Tumor Size (mm)				
Mean ± Std. Deviation	44,53±23,96	48,77±25,18	62,13±32,70	0,005**
min-max t	10-110	10-110	25-160	
Follow-up time (Month)	36,87±25,50	31,24±27,02	21,29±21,22	0,009**
Mean ± Std. Deviation,				
min-max	1-96	1-92	27-99	
Location				
Middle 1/3	13 (27,7)	17 (29,8)	12 (25)	0,139
Lower 1/3	26 (55,3)	27 (47,4)	33 (68,8)	
Upper1/3	8 (17,0)	13 (22,8)	3 (6,3)	
Differentiation				
Well	5 (10,6)	2 (3,5)	1 (2,1)	0,000*
Moderate	33 (70,2)	37 (64,9)	15 (31,3)	
Poor	9 (19,2)	18 (31,6)	32 (66,7)	
Lenfovascular invasion				
No	19 (40,4)	11 (19,3)	6 (12,5)	0,004*
Yes	28 (59,6)	46 (80,7)	42 (87,5)	
Lymph node involvement				
0	24 (51,1)	15 (26,3)	7 (14,6)	0,000*
1-2 number (pN1)	7 (14,9)	9 (15,8)	9 (18,8)	
3-6 number (pN2)	5 (10,6)	15 (26,3)	5 (10,4)	
7 and more (PN3)	11 (23,4)	18 (31,6)	27 (56,3)	
Perineural invasion				
No	30 (63,8)	17 (29,8)	11 (22,9)	0,000*
Yes	17 (36,2)	40 (70,2)	37 (77,1)	
Resection Type				
Subtotal	22 (46,8)	17 (29,8)	12 (25)	0,060
Total	25 (53,2)	40 (70,2)	36 (75)	
Depth of invasion (pT)				
pT1	13 (27,7)	6 (10,5)	0 (0)	0,000*
pT 2	13(27,7)	6 (10,5)	3 (6,3)	
pT 3	12 (25,5)	19 (33,3)	11 (22,9)	
pT 4	9 (19,1)	26 (45,6)	34 (70,8)	
Survival				
Dead	20 (42,6)	30 (52,6)	33 (68,8)	0,0350*
Alive	27 (57,4)	27 (47,4)	15 (31,3)	

* According to the chi-square test, p< 0.05 was considered as significant. (Tested for relationship)
t One-Way Anova Test to compare the difference between three independent groups.
** In the One-Way Analysis of Variance (Anova) Test, p< 0.05 was evaluated as significant. (There is difference between groups)

density; medium density 48,714 months; at high density 29,013 months and standard errors of 6,062, respectively; 5,448; 4,404 (Table 3).

Figure 2 shows that patients with low TB density have a higher survival time and patients with high budding density have a lower survival time. It can be concluded that the survival time of the patients will be less as the tumor budding density increases.

DISCUSSION

The TNM classification system, based on tumor invasion depth (pT), lymph node metastasis status, and presence/absence of distant metastases, is the most commonly used method in evaluating the prognosis of patients with gastric cancer. However, evaluation of additional histological patterns can increase the prognostic impact of the TNM

Table 3. Tumor Budding Density Kaplan-Meier Results

	Median Survival Time (months)		95% Confidence Interval		Log-Rank (Chi-Square)	p-value
Tumor Budding	Estimate	Std. Error	Lower Bound	Upper Bound		
Low	59,486	6,062	47,604	71,369	9,442	0,009*
Medium	48,714	5,448	38,037	59,391		
High	29,013	4,409	20,372	37,654		
General	48,193	3,491	41,351	55,036		

p*<0,05

classification system and help identify patients who will benefit from treatment and identify individuals who may benefit from personalized treatment.

TB is accepted as the first step in cancer metastasis which TB cells are thought to migrate from the extracellular matrix, invade lymphatic vessel structures, and form metastatic tumor colonies in lymph nodes and distant sites. The initiation and biological importance of TB is based on and related to the epithelial-mesenchymal transition (EMT) process (19). The migration and invasion capacity of TB cells is high (20).

TB is a promising prognostic factor in many cancers. Multiple studies on colorectal cancer have shown that high TB is associated with a higher tumor-lymph node-metastasis (TNM) stage, lymph node metastases, distant metastases, and poor outcomes. The prognostic significance of TB has been most commonly described in colorectal adenocarcinoma (5-9). In addition to colorectal tumors, TB density relation has also been investigated in lung adenocarcinoma, esophageal carcinoma, ampullary carcinoma, pancreatic and head and neck squamous cell carcinomas. Increased TB has been found to be associated with higher TNM stage, tumor grade, lymphovascular invasion, lymph node involvement, and distant metastasis (5-14). In our study, a statistically significant correlation was observed between poor prognostic factors such as pT, lymph node involvement, tumor differentiation, LVI, PNI, and shorter survival and higher TB.

The importance and prognostic effect of TB in gastric cancer was unclear and several researches have been performed in this field (2, 15-17). There is no generally accepted scoring system for the assessment of TB. The 'International Tumor Budding Consensus Conference (ITBCC)' made recommendations for reporting TB in cases with colorectal cancer (21). TB was defined as single tumor cells or clusters of less than five cells and it is recommended to use the triple grading system (0-4 buds: low; 5-9 buds: medium; 10 or more buds: high) and the 'hot spot' technique which the TB is most intense at 0.785 mm2 (x20 objective) in evaluating TB (21). In the future, a scoring system should be developed for the assessment of cancers of the gastrointestinal tract excluding colon tumors, and standardization should be ensured to identify and evaluate TB.

Evaluation of TB in diffuse and mixed-type gastric adenocarsinomas may not be practical or meaningful because diffuse type gastric carcinomas show loss of cohesion and high level of budding which may be a disadvantage through assessment. Diffuse gastric carcinomas and mixed adenocarcinomas with diffuse components were not included in our study. Niko Kemi et al. reported that there was no statistically significant relationship between TB and overall survival (OS) in diffuse type gastric carcinomas (22) so the authors did not recommend to evaluate TB in diffuse-type gastric adenocarcinomas.

Gabbert et al. found a relationship between TB and decrease in survival (15). Tanaka et al. found a relationship between high TB and increased T-stage, N stage, lymphatic involvement and metastasis in univariate analyzes (23). Gulluoglu et al. found a relationship between TB and lymph node metastasis, and the presence of TB was found to be important in predicting lymph node metastasis in their studies (16). However, their study included only cases of early gastric cancer with all histological subtypes.

Che et al. (2) observed that on univariate analysis, patients with high TB predicted poor overall survival compared to patients with low TB, and reported that TB and single cell invasion were independent risk factors for gastric adenocarcinoma in multivariate analysis. In a previous study, the patients with high TB showed a poor prognosis, and a significant relationship was found between overall and disease-free survival and TB (24). In the present study, a statistically significant relationship was observed between overall survival and TB, and lower survival was found in those with higher TB. The 'Death

Notification System' was used to evaluate overall survival and patients were evaluated as alive/dead. There was insufficient information available to state the cause of death and gastric cancer reccurrence time. Therefore disease-free survival could not be determined. Data about the regular follow-up examinations were not available which was limiting factor for our study. The relationship between overall survival, disease-free survival and TB in gastric cancers should be investigated in large case series supported by clinical data.

In a previous study high TB was significantly correlated with higher TNM stage, larger tumor size and lymph node metastasis (25). Olsen et al. found a correlation between higher TB and poor prognosis such as higher T stage, N stage, and recurrence (17). In a meta-analysis, high TB was associated with tumor stage, tumor differentiation, LVI, and lymph node metastasis in intestinal-type gastric adenocarcinomas, and was also associated with shorter survival (26). In another study, TB score was associated with gender, Laurén phenotype, pT, pN, and M categories, histological grade, lymphatic invasion, perineural invasion, and HER2-, MET, and MSI status. In addition, significant differences were found in overall survival (OS) and tumor-specific survival (TSS) between TB groups (27).

In all these studies, TB in gastric cancer seems to be associated with unfavorable prognostic parameters however the number of cases is limited and the methods are varied. In our study, similar results were obtained with previous studies, and a statistically significant correlation was observed between poor prognostic factors such as pT, lymph node involvement, differentiation, LVI, PNI, and shorter survival and higher TB.

In conclusion, TB is an easily applicable and inexpensive method. It is associated with poor prognostic parameters in intestinal type gastric adenocarcinomas. It is thought that it can be a guide in estimating the aggressiveness of gastric cancer, predicting the prognosis, Routine reporting about the TB density may provide valuable information to guide clinical management.

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