Turkish Journal of Clinics and Laboratory

To cite this article: Zengin M, Novel prognostic markers associated with poor survival in stage III rectal cancers: Invasive growth pattern and Tumor necrosis. Turk J Clin Lab 2019; 11: 369-376.

Original Article

Novel prognostic markers associated with poor survival in stage III rectal cancers: Invasive growth pattern and Tumor necrosis

Evre III rektal kanserlerde kötü sağkalım ile ilişkili yeni prognostik belirteçler: İnvasiv büyüme paterni ve Tümör nekrozu

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Abstract

Aim: Rectal carcinomas (RC) are one of the most common cancers in the Western World. TNM system is the most significant predictive indicator in these tumors but patients characterized by the same stage often have prominent distinct survival. In this research, we analyzed the survival effect of Invasive growth pattern (IGP) and Tumor necrosis (TN) in stage III RC.

Material and Methods: A hundred forty-five patients operated for stage III RC during 1999-2012 at Kırıkkale University were included in this research. These parameters were scored on hematoxylin and eosin stained sections. The relationship between the results and the clinicopathological characteristics was analyzed.

Results: These parameters were significantly upregulated in RCs which classified as higher tumor size (IGP: p<0.001; TN: p=0.033), higher pT (IGP: p=0.016; TN: p=0.047), angiolymphatic invasion (IGP: p=0.025), high number of metastatic lymph nodes (IGP: p<0.001; TN: p=0.001), advanced stage (IGP: p<0.001; TN: p=0.018), and advanced grade (IGP: p<0.001). In univariate analysis, patients with these two parameters had worse 5-year survivals ([IGP= RFS: 50%, p=0.001; OS=55%, p=0.003], [TN= RFS: 53%, p=0.005; OS: 58%, p=0.017]). Multivariate analyzes confirmed that these two parameters are independent worse survival parameters for RFS (IGP=Hazard ratio [HR]: 1.58 [1.05-2.66], p=0.005; TN=1.44 [1.07–2.34], p=0.013) and OS (IGP=HR: 1.55 [1.11–3.18], p=0.008; TN= 1.38 [1.09–2.28], p=0.024). In addition, IGP was found to be more successful than TN.

Conclusion: Our data suggest that IGP and TN provide valuable prognostic information for RC, and adding these parameters to the current risk classification may contribute to better patient selection.

Keywords: Invasive growth pattern; tumor necrosis; rectal cancer; prognostic markers; stage III

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Recevied: 31.05.2019 accepted : 22.08.2019
Doi: 10.18663/tjcl.572566

Öz

Amaç: Rektal kanserler (RK) batı dünyasında en sık görülen kanserlerden biridir. RK için en önemli prediktif gösterge TNM sistemidir. Bununla birlikte, aynı tümör evresi ile karakterize edilen hastalar sıklıkla belirgin farklı sağ kalımlara sahiptir. Bu araştırmada, stage III RK'larda İnvasiv büyüme paterni (İBP) ve Tümör nekrozu (TN)'nun hayatta kalmaya etkisini analiz ettik.

Gereç ve Yöntemler: 1999-2012 yılları arasında Kırıkkale Üniversite'sinde stage III RK nedeniyle opere olan yüzkırk beş hasta bu çalışmaya dahil edildi. Bu parametreler hematoksilen ve eozin boyalı kesitlerde skorlandı. Sonuçlar ve klinikopatolojik özellikler arasındaki ilişki analiz edildi.

Bulgular: Bu parametreler, büyük boyutlu tümör (İBP: p<0.001; TN: p=0.033), ileri PT (İBP: p=0.016; TN: p=0.047), angiolymphatic invazyon (İBP: p = 0.025), lenf nodu metastazı sayısı (İBP: p<0.001; TN: p=0.001), ileri evre (İBP: p<0.001; TN: p=0.018) ve ileri grade (İBP: p < 0.001) bulgusu gösteren RK'larda anlamlı olarak artırıyordu. Tek değişkenli analizde, bu iki parametreye sahip hastalar 5 yıllık kötü sağkalıma sahipti ([İBP= RFS: 50%, p=0.001; OS=55%, p=0.003], [TN= RFS: 53%, p=0.005; OS: 58%, p=0.017]). Çok değişkenli analizler, bu iki parametrenin RFS (İBP=Hazard ratio [HR]: 1.58 [1.05-2.66], p=0.005; TN=1.44 [1.07–2.34], p=0.013) ve OS (İBP=HR: 1.55 [1.11–3.18], p=0.008; TN= 1.38 [1.09–2.28], p=0.024) için bağımsız bir kötü hayatta kalma parametresi olduğunu doğruladı. Ayrıca, IGP'nin TN'den daha yararlı olduğu bulundu.

Sonuç: Verilerimiz İBP ve TN'nin RK için değerli prognostik bilgiler sağladığını ve mevcut risk sınıflamasına bu parametrelerin eklenmesinin daha iyi hasta seçimine katkıda bulunabileceğini göstermektedir.

Anahtar kelimeler: invazif büyüme paterni; tümör nekrozu; rektal kanser; prognostik belirteçler; evre III

Introduction

In the Western World, rectal cancers (RC) are one of the most frequent tumors. Prognosis is mainly affected by the spreading of the tumor at the diagnosis time. In the late stage of patients, especially stage III, there is generally a poor survival with an approximately 50-60% of 5-year overall survival after surgery and using of adjuvant chemo-radiotherapy is certain [1]. However, the same stage patients of cancer have often prominent distinct prognosis. In these cases, the TNM system does not consider other evidence that allows risk stratification such as Invasive growth pattern (IGP) and Tumor necrosis (TN). This is a particular clinical challenge and recent markers are needed to determined high-risk cases in RC [2].

Various histological features, including tumor differentiation, nodal metastases, and perineural or angiolymphatic invasion have been described as predictors of tumor recurrence. Since 1987, many studies described the presence of the IGP as a survival marker in RC with pushing cancers having a good prognosis than infiltrative cancers [3,4]. In addition, the IGP of RC's with liver metastases were shown to predict prognosis after liver resection [5]. However, it is not known whether the IGP is a response to local tumor-stromal interaction, genetically determined feature of the tumor, or a combination of the two. Moreover, TN has been considered as an independent survival marker in RC [6, 7]. However, the mechanisms that support the relationship between survival and necrosis are unclear.

The main propose of this research was to investigate the potential value of these two markers on recurrence and survival in stage III RC patients.

Material and Methods

Patients selection

The study was approved by Kırıkkale University Health Research Ethics Committee (2019.05.12). In this study, all procedures were consistent with the 1964 Helsinki declaration and the ethical standard of the national/institutional research committee. Informed consent was obtained from patients individually. The author do not have any financial participation and there is no conflict of interest.

In this retrospective research, all patients who surgically resected for stage III RC in Kırıkkale University between 1999-2012 (n = 145) were identified. In this database; age, pT, size, number of lymph node metastasis, neural/vascular invasion, grade, and stage were collected retrospectively. RCs were categorized according to the following criteria: Age (mean age was 75; <75 and ≥75), pT (pT1/pT2 and pT3/pT4), size (mean size was 5,5 cm; ≥ 5.5 cm and < 5.5 cm), perineural invasion (Yes and No), angiolymphatic invasion (Yes and No), Number of Lymph Node Metastasis (<7 and ≥7), stage (stage IIIA/IIIB and stage IIIC) and grade (Low/Moderate grade and High grade). All cases were re-evaluated according to American joint committee on cancer classification, 7th [8].

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Tissue Processing

Fixed in formalin, embedded in paraffin, archival tumor specimens from a hundred forty-five patients who operated for CRC during 1999-2012 at the Pathology department were retrieved. From each patient, one tumor block demonstrated the deepest invasively area was chosen. Cases were only admitted finally when sufficient tissue remained in the paraffin block for future review. A 4 µm thickness section (n=145) was taken from each block, and hematoxylin & eosin (H&E) was stained.

Estimates of IGP and TN

IGP was evaluated by categorizing the tumors at the invasive front for a pushing or an infiltrating tumor border. The recognition of these categories was made at x 10 objective (4.9 mm^2). The well circumscribed invasive edge and the absence of tissue dissection were defined as the pushing tumor border, whereas the widespread dissection of the tissue with loss of a clear boundary and mesenteric adipose tissue dissection by tumour glands or cells were defined as the infiltrative tumor border.

TN was visually noted for all tumour sections. Our method was the same with two previous studies [6, 7]. In H&E stained sections, a tumor area that shows nuclear shrinkage, increased eosinophilia, disappearance, fragmentation, and shadows of tumor cells was recognized as TN. Although not required for definition, the neutrophilic inflammatory infiltrates at tumor boundaries was identified to support classification as necrosis. Intraluminal necrosis was included in the evaluation of TN and fulfilled the criteria. All the histological evaluations were made blinded to the clinical information. Representative examples of IGP and TN are shown in Figure 1a-1b.

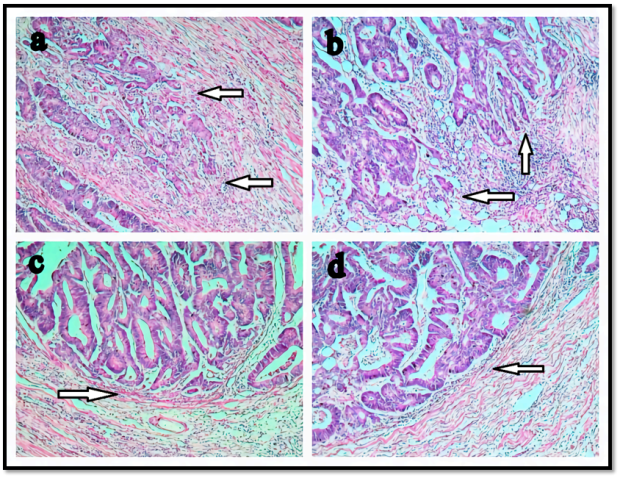

Figure 1a: The recognition of Invasive growth pattern (IGP) was made at a x 10 objective (4.9 mm^2). IGP was recognized when there was a widespread dissection of the tissue at the tumor margins with loss of a clear boundary between tumor and host. Then, one area where the IGP was the most extensive was selected and, the clusters were classified as invasive (a-b) and expansive (c-d) at×20 objective.

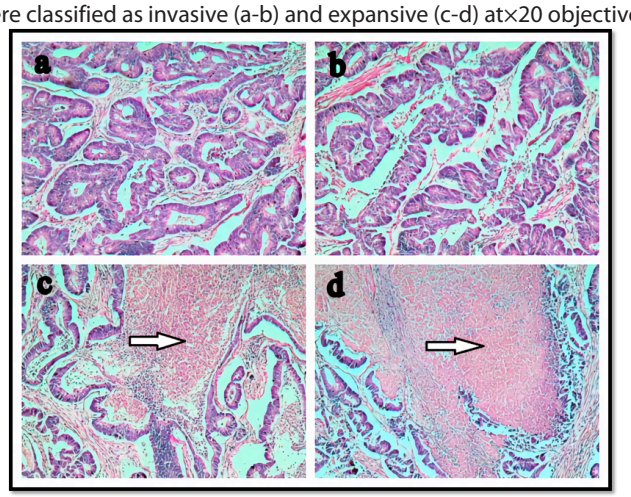

Figure 1b: Tumor necrosis (TN) was specified as an area with increased eosinophilia and nuclear shrinkage, fragmentation and disappearance, with shadows of tumor cells visible to a variable extent. Then, one area where the TN was the most extensive was selected and, the clusters were classified as negative (a-b) and positive (c-d) at a ×20 objective.

Patients follow-up

Survival information was obtained from the archives of Kırıkkale University. In this study, survival rates were evaluated for outcome measures. The event end-point time was calculated from the primary surgery day. The follow-up period was selected as a wide range of thirteen years in order to make a more reliable decision about the relapse of the cases. The time from surgery to death or distant and local-regional recurrence time was called recurrence-free survival (RFS). The time from primary surgery to the last follow-up day or the death day was defined overall survival (OS). After sixty months, the events were censored as sixty months.

Statistical Analysis

Descriptively data were listed using ranges, means and standard deviation for continuous data and percentages and frequencies for categorical data. Analyses of clinicopathological variables of IGP and TN were carried by using the test of the Chi-Square, and the test of Fisher's Exact was applied when the Chi-Square test was not available. The log-rank test was used for significant differences between univariable survival groups and the Kaplan-Meier method was used in survival curves. The Cox regression model with a 95% confidence interval (CI) and a hazard ratio (HR) of 1.0 as a reference was used for the significant difference between multivariable survival groups. All tests were bilateral and P values less than 0.05 were noted as significant. SPSS 21.0 (IBM Institute, North Castle, USA) was used in the analysis.

Results

Patients characteristics

A hundred forty-five cases that surgically resected for RC were included in the study. 92 (63.4%) of the cases were male and 53 (36.6%) were female. Average of age and size were 75,08 ± 7.45 (range:39-86) and 5.50 ± 1.85 (range: 2-10), respectively. 24 (16.6%) of the cases were detected as PT1/PT2, 121 (83.4%) as PT3/PT4 and 98 (67.6%) of the tumor was low/moderately differentiated and 47 (32.4%) as poorly differentiated.

Assessment of IGP and TN

IGP and TN were scored on H&E stained sections mentioned above. At low-power magnification, the distribution of these parameters was not relatively homogeneous within slides. One suitable block was selected from each tumor that had a good level of homogeneity of invasive edge and necrosis.

For IGP, 49 (33.8%) of patients were considered as positive whereas 96 (66.2%) of patients were considered as negative. Significantly association existed between the IGP and large size (p<0.001), high pT (p=0.016), perineural invasion (p=0.025), high number of metastatic lymph nodes (p<0.001), advanced grade (p<0.001) and advanced stage (p<0.001). For TN, 35 (24.1%) of patients were considered as positive whereas 110 (75.9%) of patients were considered as negative. Significantly association existed between the TN and large size (p=0.033), high pT (p=0.047), high number of metastatic lymph nodes (p=0.001), and advanced stage (p=0.018). The relationship between IGP/TN and clinicopathological characteristics are shown in Table 1.

Table 1 The relationship between IGP/TN and clinicopathological characteristics.

		Invasive Growth Pattern (n=145) (%)			Tumor Necrosis (n=145) (%)		
		Positive	**Negative**	**P value**	**Positive**	**Negative**	**P value**
PT-stage	pT1/pT2	21	3		15	9	
		87.5%	12.5%	0.016*	62.5%	37.5%	0.047*
	pT3/pT4	75	46		49	72	
		62.0%	38.0%		40.5%	59.5%	
Age	<75	36	19		25	30	
		65.5%	34.5%	0.881	45.5%	54.5%	0.803
	≥75	60	30		39	51	
		66.7%	33.3%		43.3%	56.7%	
PN invasion	Negative	82	37		54	66	
		68.9%	31.1%	0.141	45.0%	55.0%	0.647
	Positive	14	12		10	15	
		53.8%	46.2%		40.0%	60.0%	
Size	<5.5 cm	70	21		34	57	
		76.9%	23.1%	<0.001*	37.4%	62.6%	0.033*
	≥5.5cm	26	28		30	24	
		48.1%	21.9%		55.6%	44.4%	
AL invasion	Negative	86	37		54	69	
		69.9%	30.1%	0.025*	43.9%	56.1%	0.893
	Positive	10	12		10	12	
		45.5%	54.5%		45.5%	54.5%	
LN Status	<7	61	16		44	33	
		79.2%	20.8%	<0.001*	57.1%	42.9%	0.001*
	≥7	35	33		20	48	
		51.5%	48.5%		29.4%	70.6%	
Grade	Low/Moderate grade	79	19		40	58	
		80.6%	19.4%	<0.001*	40.8%	59.2%	0.245
	High grade	17	30		24	23	
		36.2%	63.8%		51.1%	48.9%	
Stage	Stage IIIA/IIIB	70	21		47	44	
		76.9%	23.1%	<0.001*	51.6%	48.4%	<0.018*
	Stage IIIC	26	28		17	37	
			51.9%		31.5%	68.5%	

The significant limit for Chi-square test is 0.05. Results are italic when the results are significant. Abbreviations: IGP: Invasive growth pattern, TN: Tumor necrosis, PT: Pathologic tumour stage, PN: Perineural, LN: Lymph Node, AL: Angiolymphatic.

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Follow-up

For IGP, in the follow-up period of thirteen years, eighty- five patients died (58.6%; n=65 in positive, and n=20 in negative), and ninety-six patients had relapsed (66.2%; n=72 in positive, n=24 in negative). The 5-year RFS and OS ratios were 50.4% and 55.2% in positive cases, versus 83.5% and 86.3% in negative patients, respectively. For TN, eighty- five patients died (58.6%; n=61 in positive, and n=24 in negative), and ninety-six patients had relapsed (66.2%; n=68 in positive, n=28 in negative). The 5-year RFS and OS ratios were 53.2% and 58.0% in positive cases, versus 80.7% and 83.5% in negative patients, respectively (Table 2).

Table 2.

		Univariate survival analysis (n=145) (%)				Multivariate survival analysis (n=145) (%)			
		OS		RFS		RFS		OS	
		5-year (%)	P value	5-year (%)	P value	HR (95% CI)	P value	HR (95% CI)	P value
PT-stage			0.555		0.378		NC		NC
	pT1/pT2	71		70		-		-	
	pT3/pT4	70		63		-		-	
Age			0.718		0.785		NC		NC
	<75 cm	66		63		-		-	
	≥75 cm	75		70		-		-	
PN invasion			0.466		0.553		NC		NC
	No	75		68		-		-	
	Yes	66		65		-		-	
Size			0.357		0.263		NC		NC
	<5.5 cm	78		73		-		-	
	≥5.5cm	63		60		-		-	
AL invasion			0.243		0.145		NC		NC
	No	81		75		-		-	
	Yes	60		58		-		-	
LN Status			0.033*		0.025*		0.334		0.052
	<7	84		79		1		1	
	≥7	57		54		2.58 (0.73-6.52)		1.58 (0.97-2.15)	
Grade			0.043*		0.009*		0.199		0.033*
	Low/Moderat-egrade	83		80		1		1	
	High grade	58		53		3.47 (0.58-5.47)		1.47 (1.13-3.35)	
Stage			0.007*		0.003*		0.036*		0.025*
	Stage IIIA/IIIB	85		81		1		1	
	Stage IIIC	56		52		1.54 (1.10-2.72)		1.64 (1.08-2.47)	
IGP			0.003*		0.001*		0.008*		0.005*
	Positive	55		50		1		1	
	Negative	86		83		1.55 (1.11-3.18)		1.58 (1.05-2.66)	
TN			0.017*		0.005*		0.024*		0.013*
	Positive	83		80		1		1	
	Negative	58		53		1.38 (1.09-2.28)		1.44 (1.07-2.34)	

The significant limit for Chi-square test is 0.05. Results are italic when the results are significant. Abbreviations: IGP: Invasive growth pattern, PT: Pathologic tumour stage, PN: Perineural, LN: Lymph Node, AL: Angiolymphatic, NC: Not calculable, CI: Confidence interval, HR: Hazard ratio, OS: Overall survival, RFS: Relapse-free survival

Survival analyses

In univariate analysis, for IGP, significant differences between survival groups were observed for RFS (p=0.001) and OS (p=0.003). For TN, in univariate analysis, significant differences between survival groups were observed for RFS (p=0.005) and OS (p=0.017). Lymph nodes status, grade and stage were significantly related to an adverse outcome for RFS and OS (Table 2, Figure 2-3).

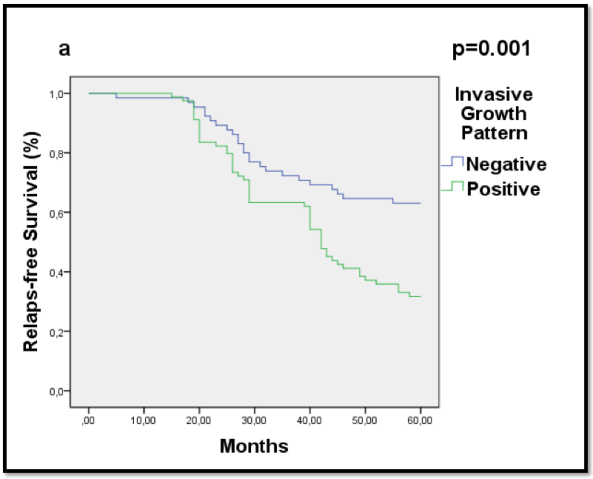

Figure 2a: Relapse-free-survival curves of Invasive growth pattern

Kaplan-Meier survival curves were used for Relapse-free survival. P value is significant at the 0,05 level.

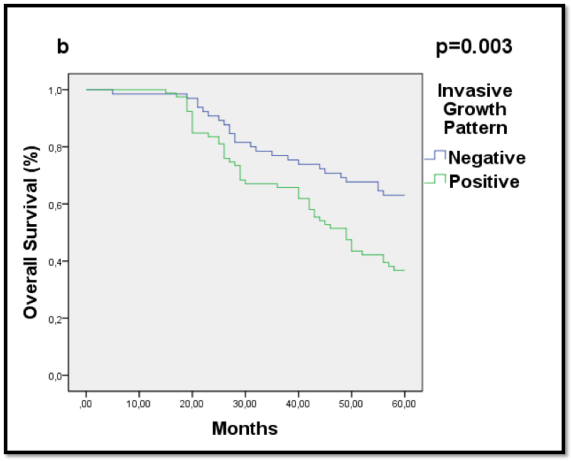

Figure 2b: Overall-survival curves of Invasive growth pattern

Kaplan-Meier survival curves were used for Overall survival. P value is significant at the 0,05 level.

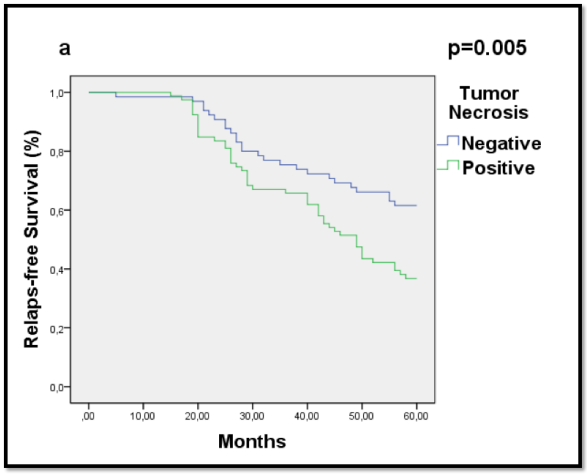

Figure 3a: Relapse-free-survival curves of Tumor necrosis

Kaplan-Meier survival curves were used for Relapse-free survival. P value is significant at the 0,05 level.

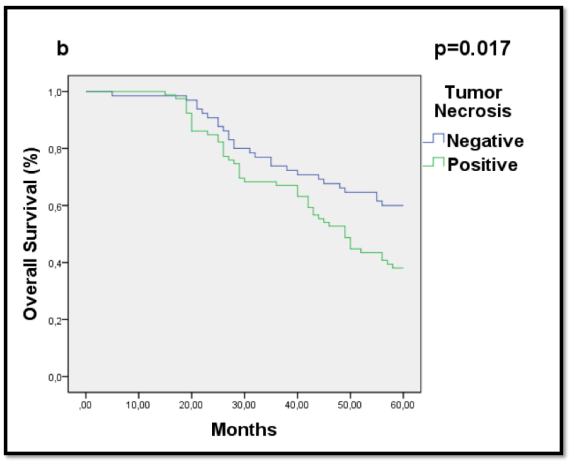

Figure 3b: Overall-survival curves of Tumor necrosis

Kaplan-Meier survival curves were used for Overall survival. P value is significant at the 0,05 level.

In multivariate analysis, IGP was an independent worse prognostic parameter for RFS (HR=1.58 [1.05-2.66], p=0.005) and OS (HR=1.55 [1.11-3.18], p=0.008). In addition, TN was also an independent worse prognostic parameter for RFS (HR=1.44 [1.07-2.34], p=0.013) and OS (HR=1.38 [1.09-2.28], p=0.024). PT-stage, lymph nodes status and stage were significantly related to an adverse outcome for RFS and OS (Table 2).

Discussion

The potential value of IGP and TN in stage III RC patients was examined in this retrospective research. Our findings suggest that IGP and TN acts a significant role in the metastatic process of RC. If this evidence is confirmed within a more advanced clinic study, these parameters can be preferred as a predictive biomarker in RC.

In the World, one of the very commonly encountered malignant cancers is RC. This highly malignant tumor is in the third row for men and in the second row for the woman [1, 2]. For risk stratification of cancer, the TNM classification is the main principle. Whereas there are several cases with the same TNM stage of diseases that shows a different clinical outcome and thus this order is not impeccable [2]. For example, it is well known that a few portions of advanced (especially stage III) tumors never recurrence which may a little of patients alone benefited from adjuvant therapy, while in additionally 20-25% of early stage (stage I/II) tumor indicate adverse clinical course [2]. For this reason, additional risk criteria including recent markers are researched by broad consensus. The benefit of IGP and TN in survival has been demonstrated by some recent researches, also distinct from the TNM system [3-7]. So, these parameters are a possible strongly survival marker.

Histomorphological different tumor growth patterns in RC were first reported by Jass in 1987 [3]. In several well-designed retrospective cohorts in the literature, IGP was defined as a negative prognostic factor in advanced stage RCs. These were found that IGP is an independent prognostic parameter for cancer-related death and recurrence (9-15). There are also studies investigating the growth pattern of early stage RCs in the literature. These studies report that IGP provides additional prognostic information in these patients. For example, Cianchi et al. reported a significant relationship between IGP and prognosis in early stage RC patients ($p < 0.01$) [16]. These findings were also confirmed in a large cohort by Ueno et al. ($P = 0.015$) [14]. In our study, we found that IGP is an independent prognostic factor for poor RFS and OS in stage III RC patients by supporting the above-mentioned studies.

In addition, another example of the prognostic value of IGP in RC is that it correlates with vascular invasion, which is common in malignant tumors. For example, Ueno et al. described a strong relationship of infiltrative growth pattern with the presence of angioinvasion in RC [14]. This finding is consistent with the correlation between IGP and increased vascular invasion, as defined by Zlobec et al. [15]. In this research, we also found a significant association between IGP and worse prognostic factors including angiolymphatic invasion.

TN is a common feature of solid tumors, thought to reflect intratumoral hypoxic environment due to rapid increase of tumor cell numbers, such as lung [17], urothelial carcinoma [18], breast [19], renal malignancies [20, 21], GIST [22] and ewing sarcoma [23]. However, there is little information about RCs and is limited to several studies [6, 7, 24]. Our observations indicate that extensive TN is a good predictor for cancer-specific survival and disease progression in RC.

TN shows a paradoxical prognostic relationship in which increased tumor cell death indicates a more aggressive cancer. This relationship can be explained by the fact that normal blood flow in the tissue does not suffice for rapidly growing tumor cells and therefore a hypoxic microenvironment is formed [17]. However, it is still unclear whether inadequate tumor vascularization and inadequate tumor oxygenation are the only factors that cause TN. A statistically significant relationship between the high TN rate and the high proliferation rate supports this hypothesis in tumors with low vascularity, e.g. papillary renal cell carcinoma [20, 21].

In this study, there are a few restrictions. The inherent of retrospective analyses are firstly and foremost limitation. In our study, cases were treated with previous methods before 2012, which produce a distinction between the treated researches of RC today. We investigated IGP and TN using one block and section, well known that this symbolizes only a little portion of all tumor. This sampling bias was impossible to overcome because of the retrospective researches, as the examination cases had already been sampled and used for diagnostic intention. Nevertheless, this study has been a large study of these parameters among the studies in our country so far.

Conclusion

In our research, the presence of IGP and TN is related with adverse prognostic factors in stage III RC. Therefore, these parameters can be a good survival marker in surgically operated RC cases. Adding these useful markers in the risk-status should benefit a good stratification for treatment of RC. In addition, IGP was found to be more useful than TN.

Abbreviations

RC: rectal cancer, AJCC: American Joint Cancer Committee, IGP: Invasive growth pattern, TN: Tumor necrosis, HPF: High power field, H&E: Hematoxylin and eosin, SD: Standard deviation, HR: Hazard ratio, OS: Overall survival, RFS: Relapse-free survival

Declarations

Acknowledgements

We thanks the Kırıkkale University, Department of Pathology members and attendance in this research.

Declaration of conflict of interest

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

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