Relation of PTEN and Ki67 expression with prognosis in gastrointestinal stromal tumors

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Abstract

Aim: The purpose of the present study of ours was to examine the relation of Ki67 and PTEN expression with the prognosis in the pathologies of patients who undergo gastrointestinal stromal tumor surgery in our clinic.
Gastrointestinal stromal tumors; In addition to being small incidental tumors; can be tumors with large size and malignant behavior. For this reason, the relation of a great number of macroscopic and microscopic parameters with prognosis was examined. The diameter, location and mitotic index of the tumor rank the first among these parameters; however, several pathological parameters like PTEN and Ki67 expression are also important.
Material and Methods: The present study was conducted between January 2005 and April 2015 with patients diagnosed with gastrointestinal stromal tumors. The demographic data (age and gender), tumor localizations, tumor sizes, mitotic activities, prognostic risk classification, follow-up times, PTEN expressions, Ki67 proliferation indices, relapse and metastasis status, and survival data of the patients were examined retrospectively.
Results: A total of 76 patients were included in the present study. A total of 33 (43.4%) of the participants were women, and the average age was 59.7±11.7 years. The relations of PTEN with risk scores (p=0.330), tumor diameter (p=0.360), location (p=0.169), and mitosis count (p=0.579) were not significant. The relation of PTEN staining rate with relapse (p=0.832), metastasis (p=0.626) and survival (p=0.069) was not found to be statistically significant. The relation of Ki67 expression with risk scoring (p=0.018) and mitosis count (p=0.003) was significant. Although the relation between Ki67 and relapse (p=0.019) and metastasis (p=0.003) was significant, the relation between survival (p=0.655) was not significant at a statistical level.
Conclusion: The Ki67 expression was a prognosis-indicating index for relapse and metastasis when the baseline value was taken as 5%; however, no relation was detected between PTEN expression and prognosis.

Keywords: Immunohistochemical; GIST; mesenchymal; tumor; cancer; pathology

INTRODUCTION

Cutaneous leishmaniasis (CL) is a disease caused by Leishmania-type parasites that progress with nodular ulcerative lesions on the skin and heals with atrophic scarring. (1-3) CL is generally divided into acute and chronic forms. Chronic form is divided into two as lupoid CL and recidivan CL. Acute CL lesions usually heal within 1-2 years, leaving a collapsed scar at the lesion site. Chronic CL occurs at a rate of 5-10% and lesions last for more than two years. Primary cutaneous lesions which persist for more than 2 years are called lupoid CL but lesions which reactivate after years at the edge of the healing primary lesion scar is called recidivan CL. (4,5) Although numerous studies have been conducted on the clinical features of patients with CL,(6-8) the number of studies examining the clinical features of patients with recidivan CL is scarce. (9)

In this study, clinical features of patients admitted to our clinic and diagnosed as recidivan CL were investigated.

MATERIAL and METHODS

The slide, paraffin block and report archives of the 76 cases that were diagnosed with GIST between January 2005 and April 2015 with morphological and Immuno-

Histochemical (IHC) findings were examined in the Pathology Unit of Kartal Dr. Lutfi Kirdar Training and Research Hospital. The demographic data (age and gender), size and localization of the tumors, mitotic activities, prognostic risk classifications, follow-up times, PTEN expressions, Ki67 proliferation indices, relapse and metastasis status, and survival data of the patients were also examined. The Ki67 indices of the paraffin blocks of the patients were re-evaluated, and the results were analyzed with light microscopy by employing PTEN in an IHC manner in the paraffin blocks. In the present study of ours, the risk classification of the tumors was made according to the risk classification given in the AFIP archives of Miettinen and Lasota. A total of 8 patients who had synchronous tumors were not included in the statistical analyses to evaluate relapse and metastases with PTEN and Ki67 expression; and therefore, the evaluations were carried out over 68 patients. The PTEN expression was evaluated in 4 modalities (-/+/++/+++) by multiplying the staining, density, and prevalence values. The Ki67 expression evaluations were made in two groups by taking <5% and >5% and 5% as the baseline values. In addition, the patients who had secondary primary cancers other than GIST were not included in the survival analyses and the recurrence-metastasis status evaluations.

Immunohistochemical examination
The 3-micron-thick sections of the paraffin blocks were taken into the slides that were coated with poly-l-lysine; and were deparaffinized at 60OC in the drying oven. The next stages were carried out with the Tissue Microarray (TMA) (Leica Bond Max, Germany) Technique. The slides were kept at 0.5% hydrogen peroxide for 15 minutes to block the endogenous peroxidase. The sections were incubated for 30 minutes with rabbit monoclonal anti-PTEN antibody (Cell signaling technology, code 138G6, 1/200 dilution, US) and liquid mouse monoclonal antibody with Ki67 antigen (Leica, Code NCL-L-Ki67-MM1, 1/100 dilution, UK). Then, post-primary antibody, polymer antibody and DAB mixtures (Leica; LOT 11776) were applied for 10 minutes, respectively. Contrast staining was carried out with Mayer Hematoxylin, and the slides were closed with the closing agent.

Evaluation of immunoreactivity
The proliferation index was computed by counting 1000 cells in the area where the highest nucleus was stained with this antigen and by giving the percentile rate of these stained ones for the Ki67 that was applied to the blocks where mitosis was observed at the highest intensity. The tonsil sections were employed as positive tissue control for Ki67. Endometrial sections were employed as positive tissue controls for PTEN, and cytoplasmic staining was detected in them. The PTEN expression scoring was carried out by considering the staining prevalence and intensity. The prevalence of staining was graded as follows; when 1% of the tumor cells were stained, 0; when 1-25% of the tumor cells were stained 1; when 26-50% of the tumor cells were stained, 2; when 51-75% of the tumor cells were stained 3; when >75% of the tumor cells were stained, 4. The staining density was classified as follows; negative staining 0; poor staining 1; moderate staining 2, strong staining 3. The Histological Score (H-score) was computed as follows: The staining prevalence score and staining intensity score were multiplied. In this context, the results between 0 and 12 were scored as; Score 0 (-/negative); score 1-4 (+/weak positive); score 5-8 (++/moderate positive); score 9-12 (+++/strong positive).

Statistical Analysis
The statistical evaluation of the data was carried out on the computer by employing SPSS 20.0 for Windows. The average of the quantitative data was shown as median (minimum-maximum) and mean - standard deviation. The qualitative data were expressed as numbers (n) and percentages (%). In the present study of ours, the quantitative data were compared with the Student's t-test and One Way Anova Test; and the qualitative data were compared with the Chi-Square Test. Survival analyzes were carried out with the Kaplan-Meier Method.

RESULTS

The study group consisted of 33 women (43.4%) and 43 (56.6%) men who were diagnosed with GIST between January 2005 and April 2015; and the average age was 59.7±11.7 years. The localization of most of the tumors was the stomach (56%); and it was determined that the non-GIS localization was retroperitoneal in one patient, the pelvis in one patient, and the abdomen in two patients. The tissue they stemmed from could not be determined clinically and radiologically.

It was determined that excisional biopsy was applied in 75 (98%) of the cases, an incisional biopsy was performed in one patient (1%); and in 20 (26%) of the patients, it was observed that the surgical limit was positive in the pathologic examination. Since incisional biopsy was applied in one of the tumors, it was determined that one of the tumors could not be evaluated in terms of growth pattern. A total of 62 (82%) of the remaining tumors were expansive, and 13 (17%) were in the infiltrative growth pattern.

When the report archive was examined, it was seen that there were synchronous tumors that accompanied GIST in 8 (10%) of the patients. It was determined that three of these synchronous tumors were gastric signet ring cell-adenocarcinoma, two were gastric adenocarcinoma, one was adenocarcinoma in the colon, one was a pancreatic neuroendocrine tumor, and one was a gastric neuroendocrine tumor. While there was only one GIST focus in 70 (92%) of the cases, there were more than one focus in 6 (8%) patients. The number of foci, which was 2 in 4 patients, was 5 in 1 patient, and 10 in 1 patient.

Table 1. Clinical and pathologic parameters		Stomach (%)	Small Intestine (%)	Colon (%)	Non-GIS (%)
N (%)		43 (56.5%)	27 (35.5%)	2 (2.6%)	4 (5.2%)
Gender	Male	20 (%46.5)	11 (%40.8)	- (%0)	2 (%50)
	Female	23 (%53.5)	16 (%59.2)	2 (%100)	2 (%50)
Age	<40	- (%0)	3 (%11)	- (%0)	- (%0)
	≥40	43 (%100)	24 (%89)	2 (%100)	4 (%100)
Tumor Size	≤2cm	9 (%21)	2 (%7.5)	- (%0)	- (%0)
	>2cm ≤5cm	14 (%32.5)	7 (%25.9)	- (%0)	- (%0)
	>5cm ≤10cm	17 (%39.5)	8 (%29.6)	- (%0)	1 (%25)
	>10cm	3 (%7)	10 (%37)	2 (%100)	3 (%75)
Mitotic Index	≤5/50BBA	28 (%65.1)	17 (%63)	- (%0)	4 (%100)
	>5/50BBA	15 (%34.9)	10 (%37)	2 (%100)	- (%0)
Risk Score	No Risk	9 (%21)	2 (%7.4)	- (%0)	- (%0)
	Very Low	10 (%23.3)	- (%0)	- (%0)	- (%0)
	Low	9 (%21)	5 (%18.5)	- (%0)	1 (%25)
	İntermediate	4 (%9.2)	4 (%14.8)	- (%0)	- (%0)
	High	11 (%25.5)	16 (%59.3)	2 (%100)	3 (%75)
Recurrence		3 (%7)	4 (%14.9)	1 (%50)	- (%0)
Metastasis		4 (%9.3)	8 (%29.7)	1 (%50)	- (%0)

Table 2. IHC Results		N	%
CD117	Positive	76	100
	Negative	-	-
CD34	Positive	66	86.9
	Negative	10	13.1
SMA	Positive	36	47.4
	Negative	40	52.6
Desmin	Positive	2	2.6
	Negative	74	97.4
S100	Positive	9	11.8
	Negative	67	88.2
Ki67	<%5	40	52.6
	≥%5	36	47.4
PTEN	-	2	2.6
	+	17	22.4
	++	29	38.2
	+++	28	36.8

When the cell types in the tumors were evaluated, it was determined that 34 (44%) were spindle, 35 (46%) were mixed, and 7 (9%) were epithelioid cells. While cellularity was apparent in 60 (79%) of the tumors, and it was lower in 16 (21%). While Cytological Atypia was mild in 58 (76%) of the tumors, it was at a significant level in 18 (23%) tumors. Although necrosis was detected in 28 (36%) of the tumors, no necrosis was observed in 48 (63%).

The demographic data, pathology results, risk classifications, distribution of metastasis and recurrence results by location of the patients are given in Table 1. CD117, CD34, SMA, Desmin, S100, PTEN staining rates of the tumors and their Ki67 proliferation rates are given in Table 2. The distribution of the PTEN expression and Ki67 proliferation indices according to the groups are given in Tables 3 and 4. The results showing the relation of PTEN and Ki67 expression with relapse, metastasis, and survival are given in Table 5-6 and Figure 1-2.

Table 3. Risk scoring with PTEN; the relation between tumor diameter, localization and mitosis count

		PTEN (%)				Total (%)	P
		-	+	++	+++		
Risk Score	No Risk	0 (0)	4 (5)	5 (6)	2 (2)	11(14)	
	Very Low	1 (1)	0 (0)	2 (2)	7 (9)	10 (13)	
	Low	0 (0)	2 (2)	6 (8)	7 (9)	15 (19)	0.33
	Moderate	0 (0)	2 (2)	4 (5)	2 (2)	8 (10)	
	High	1 (1)	9 (12)	12 (16)	10 (13)	32 (42)	
Tumor Diameter	Range (cm)	4-10	0,4-35	0,1-19,5	0,8-15		0.36
	Median (cm)	7	9	6	5.7		
Localization	Stomach	2 (2)	8(10)	14 (18)	19 (25)	43 (56)	
	Small Intestine	0 (0)	7 (9)	15 (19)	5 (6)	27 (35)	0.17
	Colon	0 (0)	1 (1)	0 (0)	1 (1)	2 (2)	
	Non-GIS	0 (0)	1 (1)	0 (0)	3 (4)	4 (5)	
Mitosis	≤5	1 (1)	9 (12)	21 (27)	18 (23)	49 (64)	0.58
	>5	1 (1)	8 (10)	8 (10)	10 (13)	27 (35)	

Table 4. Risk scoring with Ki67, relation between tumor diameter, localization and mitosis count

		Ki67 Group (%)		Total (%)	P
		<%5	≥%5		
Risk Score	No Risk	10 (13)	1 (1)	11 (14)	
	Very Low	5 (6)	5 (6)	10 (19)	
	Low	10 (13)	5 (6)	15 (19)	**0.01**
	Moderate	4 (5)	4 (5)	8 (10)	
	High	11 (14)	21 (27)	32 (42)	
Tumor Diameter	Range (cm)	0,1-35	2-23		0.10
	Median (cm)	5.3	7		
Localization	Stomach	25 (33)	18 (23)	43 (56)	
	Small Intestine	12 (16)	15 (19)	27 (35)	0.60
	Colon	1 (1)	1 (1)	2 (2)	
	Non-GIS	2 (2)	2 (2)	4 (5)	
Mitosis	≤5	32 (42)	17 (22)	49 (64)	**0.00**
	>5	8 (10)	19 (25)	27 (35)	

Table 5. Effects of PTEN on relapse, metastasis and survival

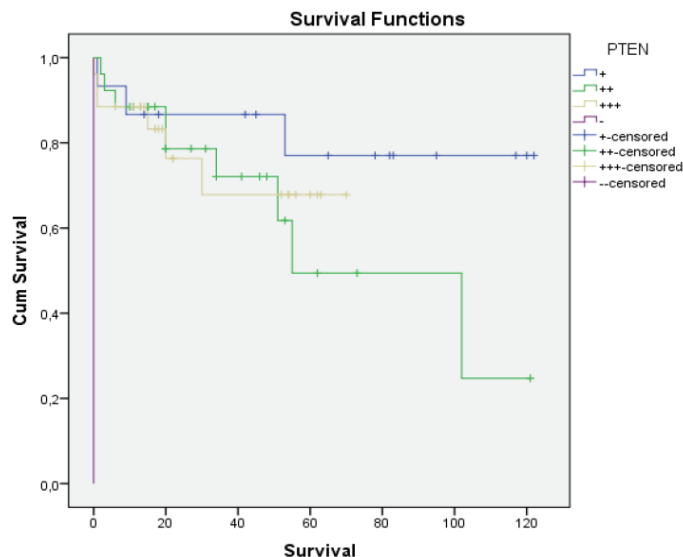|	PTEN (%)				P
	-	+	++	+++	
Relapse	0 (0%)	2 (13%)	4 (15%)	2 (7%)	0.83
Metastasis	0 (0%)	2 (13%)	7 (27%)	4 (15%)	0.62
Median survival (months)	27	52	21	19	0.07

Figure 1. Survival graph of PTEN
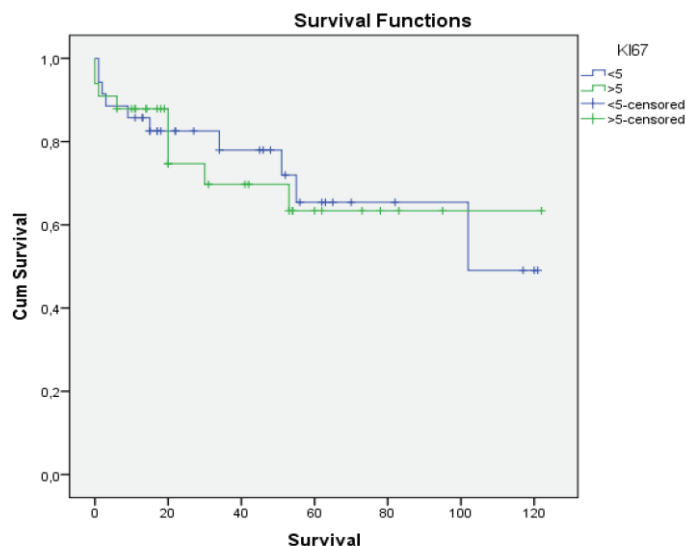
Figure 2. Survival graph of KI67

Table 6. Effects of Ki 67 on relapse, metastasis and survival			
	Ki 67		**P**
	<%5	**≥%5**	
Relapse	1 (3%)	7 (21%)	**0.02**
Metastasis	2 (5%)	11 (33%)	**0.00**
Median survival (min-max)	31 months (1-121)	20 months (0-122)	0.65

DISCUSSION

Typically, GISTs are mesenchymal tumors that do not resemble leiomyomas and schwannomas; and their clinical, histological and IHC characteristics stemming from ICC cells differ. They are frequently seen in the 50-60 age group, which is also the case in our study, without gender dominance (6). Although they are frequently detected in GIS, they might rarely be detected outside the GIS, as it is the case in our study. Although their prognosis is better when compared to that of the epithelioid tumors, they may yield bad prognostic results in some cases. A great number of IHC markers were evaluated together with clinicopathological findings, which might predict the progression of these tumors that might occur due to many molecular factors and that cannot be differentiated as benign or malignant. The PTEN, which is the phosphatidylinositol-3 kinase (PI3K)/Akt inhibitor, which was determined to have major effects on the signal transduction pathways in GISTs, was evaluated for this purpose.

In the literature; it was found that weak/no PTEN expression, gastrointestinal bleeding (in the form of hematemesis, melena or fecal occult bleeding), non-gastric tumor localization, large tumor diameter, high mitotic index, increased cellularity, tumor rupture, and Ki67 proliferation index being >1% were bad prognostic factors in terms of survival without relapse. PTEN is expressed in both nuclear and cytoplasmic ways in most of the tumors; however, it is expressed in a cytoplasmic way in some tumors; and for this reason, only nuclear staining was not adequate. PTEN is known to show both nuclear and cytoplasmic staining in most tumors, but not only nuclear staining (7,8). In our study, there was both cytoplasmic and nuclear-cytoplasmic staining only in one patient; and there was only cytoplasmic staining in the other patients. Unlike the literature, the disease-specific survival was determined in the PTEN + group (52 months) with the highest percentage, and it was observed that as the expression increased, survival decreased; however, it was determined that this relation was not significant in our study. No significant relation was detected between relapse and metastasis and the expression of PTEN. There was no apparent relation between the risk scoring, tumor size, and mitosis count and PTEN expression. However, it was considered that in the gastric tumors which have less relapse and metastasis rates compared to the small intestine, the PTEN being largely +++ supports good prognosis; however, no similar differences were detected in other localizations.

Ki67 is one of the most important IHC indicators that are employed to determine tumor proliferation. It is also the most commonly used indicator in the literature as a prognostic marker in GISTs; however, an absolute Ki67 threshold value that might make the clinicians to consider malignancy is still controversial. Different threshold values were proposed in different studies, and the most frequently used one among them was 5% (9-11). In our study, the threshold value was taken as 5%; there was a significant relation was detected with relapse (p=0.019) and metastasis (p=0.003). In addition to these, it was determined that the Ki67 values showed a correlation with the risk group classification (p=0.018) and mitosis count (p=0.003). In the study that was conducted by Carillo et al., it was shown that the Ki67 was an independent prognostic factor; however, it was also shown that it was not as significant as tumor size and mitosis count in determining the prognosis (9). Similarly, in the study that was conducted by Wong et al., although it was stated that the Ki67 was a useful marker in evaluating the proliferation of GISTs, it was also emphasized that it was less reliable than the mitotic index (12).

GISTs show different prognostic behavior depending on the location. In our study, it was determined that small intestine GISTs had a higher relapse and metastasis rates, higher tumor diameters compared to those localized in the stomach; and progressed more aggressively. Liang et al. conducted a study and evaluated the prognostic significance of the PTEN and Ki67 in GISTs. The Ki67 proliferation index being <5% was related with the prolonged disease-specific survival in both gastric and small intestinal GISTs, the expression of PTEN in ≥50% of the tumor cells was related with the prolonged disease-specific survival in only gastric GISTs. It was reported that small intestine GISTs progressed more aggressively than the GISTs in the stomach. The Ki67 was high at a significant level in the tumors of the patients who died due to the GISTs localized in the stomach and small intestine at a significant level when compared to the tumors of the living patients, and the PTEN was lower. The survival was determined to be significantly longer in the gastric and small intestine tumors that had PTEN ≥50%, Ki67 proliferation index <5%, and CD44 positive, when compared to the other immunophenotypes (10). In the present study of ours, where the Ki67 was evaluated in two different groups as <5% and ≥5% as in many other studies in the literature, and no differences were detected in terms of survival. In the study that was conducted by Nilsson et al., it was reported that tumor diameter and Ki67 proliferative index were effective factors in survival; and it was emphasized that the GISTs that were bigger than 6 cm -regardless of the proliferative index-, and the ones that had Ki67 proliferative index bigger than 5% were potentially malignant -regardless of tumor size (11). In the study that was conducted by Zhao et al., two different Ki67 threshold values were evaluated as >5% and >8%. It was determined that the ≤5%, 6-8%, >8% values of the Ki67 were independent prognostic factors in survival without

relapse; and Ki67 being >8% had a negative effect on the adjuvant imatinib treatment that was used in patients with high risks. It was also determined that these patients had a bad prognosis in spite of the drug treatment (13). In the study conducted by Oliveira et al., the relation of endoglin (CD105), CD31 and vascular endothelial growth factor (VEGF), which are angiogenic markers, and Ki67 with prognosis was evaluated. In the tumors, the CD105 and CD31 were expressed above the cut-off values in tumors that had poor prognosis (>1.2% and> 2.5%, respectively); and VEGF was not expressed or was expressed poorly in the group that had a good prognosis. With these findings, it was emphasized that the Ki67 expression being ≥5% was strongly correlated with bad prognosis, and was independent from the tumor localization, which is different from the mitotic index (14). In a study that was conducted with 111 GIST, the relation of CD133 and Ki67 expression with tumor prognosis was evaluated, and it was determined that the 1, 3 and 5-year survival rates of CD133 (+) tumors were lower than in CD133 (-) tumors; and similarly, the 1, 3 and 5-year survival rates of Ki67 (+) tumors were lower than the survival rates of the Ki67 (-) tumors (15). With these results, our study has shown that the Ki67 expression is correlated with poor prognostic criteria like the relapse and metastasis, in line with the literature data, and the 5% limit might be a threshold value for separating GISTs into prognostic groups.

CONCLUSION

While Ki67 expression is an index showing prognosis in terms of relapse and metastasis when the basis is taken as 5%, no significant results were detected to predict the GIST prognosis by PTEN expression. Competing interests: The authors declare that they have no competing interest.

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