The Association Between Ki-67 Proliferation Index and Disease Progression in Gastro Intestinal Stromal Tumors: A Retrospective Study

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Abstract:

Introduction: Approximately 40% to 50% of high risk Gastro intestinal stromal tumors progress even with complete surgical resection. Therefore, prognostication of patients is essential to determine the risk of recurrence and influence management decisions. Many risk stratification systems have developed. Tumor size, site and mitotic count are the major clinicopathologic risk factors together with tumor rupture. The nuclear proliferating protein ki-67 has demonstrated its prognostic significance in the outcome of various malignancies but in gastro intestinal stromal tumors, the relationship between its overexpression and the risk of disease recurrence remains poorly defined.

Aim of the work: To investigate whether Ki-67 labelling index can be considered an independent predictor for disease progression or not.

Patients and methods: This is a retrospective study that enrolled patients with localized stage of gastrointestinal stromal tumors treated by surgical excision and adjuvant Imatinib mesylate in the Governorate of Sohag between January 2012 and January 2022.

Results: A total of 74 Egyptian patients with localized gastrointestinal stromal tumors treated with excision and adjuvant Imatinib mesylate have been retrospectively analyzed. The median age was 53 year and the median follow up period was at 40 months. During follow up, 27% has developed progressions both local and distant and 12% has died. Among the studied risk factors, only the extra gastric location and Ki-67% labelling index >7% were associated with more disease recurrences in univariate analysis. A labelling index ≤7% was associated with better local and distant control in the studied subgroups but with no effect on overall survival.

Conclusion: Ki-67% labelling index >7% is an important prognostic indicator of high risk of disease progression after surgical excision of localized GIST and more larger studies are warranted.

Keywords: Ki-67 proliferation index, disease progression, GIST

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Introduction:

Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors that arise from the gastrointestinal tract, showing differentiation toward the interstitial cells of Cajal and accounting for <1% of all gastrointestinal neoplasms [1]. GISTs usually occur in older adults (median age 55–60 years) and rare in children in the second decade [2]. The incidence does not differ with sex [3]. Most of GISTs (80%-90%) develops as a result of activating mutations in two receptor protein tyrosine kinases: KIT and/or PDGFRA [4].

Other markers for GISTs include CD34 antigen (70%), smooth muscle actin (30%-40%), desmin (<5%), and S100 protein (approximately 5%) [5]. Despite all these diagnostic markers, difficulties could develop in some cases. DOG1 is used as a marker for differential diagnosis in many studies [6]. Sixty percent of GISTs arise in the stomach, 35% in the small intestine, and less than 5% in the rectum, esophagus, omentum, or mesentery, most GISTs in the latter 2 sites are metastatic [2]. Surgical resection achieving negative margins (R0 resection) is the standard curative

treatment for primary, localized GIST [7]. Despite radical resection with clear margins, 40% - 80% recur within the abdomen most commonly in the peritoneum and liver [8].

Therefore, prognostication of patients with GIST is essential to determine the risk of recurrence and influence management decisions concerning the use of adjuvant imatinib treatment and intensity of surveillance in order to delay or prevent recurrence [9]. Many risk stratification systems have developed in the last two decades. Tumor size, site and mitotic count are the three major clinicopathologic risk factors for assessment of the risk of recurrence after surgical resection of GIST [10]. The National Institute of Health (NIH) consensus classification system was the first widely accepted one and based only on tumor size and mitotic index (MI) [5]. The tumor site was subsequently incorporated in risk stratification system referred to as Miettinen-Lasota/Armed Forces Institute of Pathology (M-L/AFIP) classification system that can be considered the most widely used risk stratification system for GIST management [2, 11, 12].

Although this system has been criticized for non-incorporating tumor rupture as a risk factor for recurrence and for its dependence on relatively old microscopes in assessment of the MI, its prognostic value was reported to be superior than NIH classification system [7, 13]. In 2008 the NIH system was modified with incorporation of tumor location and tumor rupture as prognostic variables [14]. However, the newly modified NIH system was criticized for its dependence on expert opinion and for absence of statistical validation [9]. In 2009, the Memorial Sloan Kettering Cancer Center (MSKCC) has developed a prognostic nomogram to predict the risk for tumor recurrence after excision of localized primary GIST based on tumor size, location and MI [15 - 17].

According to this nomogram, the concordance probabilities between the predicted and the observed recurrence free survival (RFS) was better than that provided by the two NIH risk stratification systems (p=0·04) but similar to that of AFIP Miettinen system [15]. Both the MSKCC nomogram and AFIP criteria were considered by authors to have the best predictive accuracy for tumor recurrence compared to the NIH and Joensuu risk classification systems in Asian patients [16]. Until now, the optimal risk stratification system for surgically treated localized primary GIST remains controversial [9]. As some low risk (LR) GIST with small size and low MI may recur and spread after radical surgery, it is important to investigate other risk factors of disease progression that refine the current risk stratification standards and help determine the appropriateness of adjuvant treatment and the intensity of postoperative surveillance [18].The nuclear proliferating protein ki-67 is a non histone nuclear protein that is present in the cell nucleus throughout all phases of the cell cycle apart from G0 phase. This makes it a widely used biomarker of tumor proliferation and crucial factor in pathologic assessment [19]. Its over expression is observed in tumor cells, and it has been considered as a marker for cancer prognosis [20].

Although Numerous studies have demonstrated the prognostic significance of ki-67 labelling index (LI) in the outcome of various malignancies such as breast, prostate, cervix, stomach, esophagus [21], hepatocellular carcinoma, lymphoma and, lung cancers where higher levels of ki-67 LI was associated with shorter progression free survival (PFS) and overall survival (OS) [22], the relationship between over overexpression of ki-67 LI and the risk of GIST recurrence remains poorly defined [20]. To date, several studies have investigated the role of Ki-67 LI levels in the prediction of GIST prognostic risk, with higher values indicating an increased risk of tumor recurrence and the need of intensive management and observation [18, 20-23]. The present study aims to investigate the association between Ki-67 LI expression and disease progression after surgical resection and adjuvant Imatinib therapy in patients with localized GIST.

Patients and Methods:

In this retrospective study, we had screened out the medical records of 74 patients with primary gastrointestinal stromal tumors (GIST) diagnosed and/or treated in Sohag University Hospital and Health Insurance Hospital from the Governorate of Sohag, in the South of Egypt and nearby Governorates between January 2012 and January 2022.

The inclusion criteria in our study have included patients aged between 18 and 80 yr of either sex with localized tumors at risk of recurrence treated with surgery and adjuvant Imatinib. The technique of resection was at the discretion of the individual surgeon. Tumor diagnosis has been done by means of tru cut needle biopsy (TCNB) and final diagnosis have carried on in post operative tissue specimens. The patients should have complete clinical, radiological and, pathological data that confirm the diagnosis and stage of the disease. Presence of necrosis, hemorrhage, MI (defined as the number of mitoses / 50 microscopic high-power fields (hpf) and, tumor width have been revised in hematoxylin-eosin (HE) slides by the pathologist. Immunohistochemical reports confirming the diagnosis of GIST and the positivity of CD 117 (C-kit) and/or DOG-1 have been required for enrollment in the study. Paraffin blocks have been immunostained for cKit, DOG 1, SMA, CD 34, S 100 protein , CK and Ki-67 LI using Ventana Brand Benchmark Autostainer (Ultra or GX model) and Ventana ultraView or Optiview DAB detection Kit system. Ki-67 LI was defined as the percentage of Ki-67 antigen positive cells. Staging work up was made by chest and abdominopelvic CT

Eligible patients must have follow-up data recorded in their medical sheets. A minimum of 3 m follow up after surgery have been required. Exclusion criteria have included pediatric patients, patients with prior history of cancers, chemo or radiotherapy, end organ failure or patients with locally advanced, recurrent or metastatic tumors.

We have used the Modified National Institute of Health Risk Stratification Criteria for GIST proposed by

Joensuu as a risk stratification system [14]. DFS that was assessed as the primary end point of the study was calculated from the date of surgery to the date of developing new lesions or to the date of last follow up. OS was calculated from the date of diagnosis to the date of death or date of last follow up and studied as the second end point.

For the statistical analysis, IBM SPSS ver. 22 was used. A chi-square test or a Fisher exact test (as indicated) was used for categorical variables comparisons. Independent t - test and Mann Whitney tests (as indicated) were used for continuous variables analysis. For survival analysis, Kaplan-Meier and log-rank tests were used. The Cox regression hazard model was used for finding independent prognostic factors for PFS and OS. A two sided p value < 0.05 is considered significant.

This study was approved by the Medical Research Ethics Committee, faculty of Medicine, Sohag University under IRB Registration Number: Soh-Med-23-01-28.

Informed consent was exempted owing to the use of retrospective clinicopathologic data and all information was anonymous.

Results:

This study has enrolled a total of 74 patients with localized GISTs. Forty five patients (61%) were males while 29 (39%) were females. The median age on presentation was 53 yr (range from 19 to 80 yr). Abdominal pain was the main presenting symptoms (53%) followed by abdominal mass (8%) and bleeding per rectum (8% for each).

The primary tumor site was gastric in 40 (54%) and intestinal 30 patients (40%).

All cases have been treated by open surgical excision and adjuvant Imatinib. Two cases recorded tumor rupture during excision.

Spindle cell morphology has been reported in 90% of pathology reports. C-Kit +/- DOG 1 mutations have been reported in all pathology reports. CD 34 was reported +ve in 33 and –ve in 12 cases (44% and 16% respectively). Ki 67 LI was reported in 39 (53%) of cases, It has ranged from 1 % –50 % with a mean at 7%. Index at ≤ 7% and > 7% was found in 29 (39%) and 10 (13.5%) of cases respectively.

According to the modified NIH classification, there have been 29 cases (39%) in the high risk (HR) group, 11 (15%) in the intermediate risk (IR) group and, 15 cases (20%) in the low risk (LR) group. In the remaining cases, no complete data was found to determine the risk (being a retrospective study). No very low risk tumors were encountered.

Maximum tumor dimension ranged from 3 to 25 cm (median 8 cm). Maximum dimension < 5 cm, between 5 and 10 cm and > 10 cm have been reported in 9 (12%), 10 (13%) and 12(16%) specimens respectively. Concerning MI, it has ranged from 3 -- 25 /50 hpf with a median at 6/50 hpf. An index ≤ 5, >5 - 10 and > 10/ 50 hpf have been reported in 21 (28%), 9 (12%) and 15 (20%) cases respectively.

The details of clinicopathologic characteristics of the whole cohort were shown in table 1.

The distribution of these characteristics in both sexes showed no significant differences apart from the significant association between male gender and intestinal location of the tumor (p=0.016).

Survival analysis

The follow up period ranged from 3 to140 m with a median at 40 m. During the follow up, 9 (12%) died due to the disease with a median time at 40 m, and 20 (27%) patients had developed disease recurrence with a median time at 34 m. Distant progression (hepatic and extrahepatic) had developed in 14 (19%) patients while local progression developed in 6 (8%) patients.

The curves of OS and, local progression free survival (LPFS) rates are shown in figure 1A and 1B respectively while distant progression free survival (DPFS) rate in figure 2A. These figures show that the 5y OS, LPFS and, DPFS for all patients were at 82%, 90% and 68% respectively.

Table 2 shows the univariate analysis of the studied potential prognostic factors that might affect tumor recurrence. On the level of LPFS and DPFS, as seen in table 2, among the studied potential prognostic factors, only the level of Ki-67 LI and tumor site that obviously impacted these rates. Ki-67 LI > 7% was significantly associated with more probability of local and distant failures compared with tumors with LI ≤ 7% as predicted from the lower 5 y LPFS rate (60% vs 90% , p = 0.05) and lower 5 y DPFS rate (50% vs 90% , p = 0.05) shown in figure 1C and 2B respectively.

Intestinal location of the tumor was also significantly associated with higher probability of distant progression with significantly lower 5 y DPFS rate compared with gastric location (54% vs 81%, p=0.05) as seen in figure 2C.

On multivariate analysis using Cox proportional hazard model, Ki-67 LI ≤ 7% was associated with marked reduction in the hazard of distant progression (HR: 0.19, CI: 0.031 – 1.017, p: 0.074) while gastric location of the tumor failed to show such reduction in the hazard of distant progression compared with intestinal location (HR: 0.28, CI: 0.047 – 1.72, p = 0.17).

Concerning the OS, as seen in table 2, the 5 y OS rate was not significantly associated either with the patients characteristics (age, sex and, comorbidities) nor with the disease characteristics (site, size, MI, risk group and, Ki-67 LI).

On categorization of the cases into subgroups, as seen in table 3, it appears that male patients with Ki-67 LI ≤ 7% showed significantly higher 5 y LPFS rate compared with males with LI > 7% (100% vs 75%, p = 0.03) as seen in figure 1D and female patients with Ki-67 LI ≤ 7% significantly scored higher 5 y DPFS rate compared with females with LI > 7% (100% vs not reached, p=0.02) as seen in figure 2D.

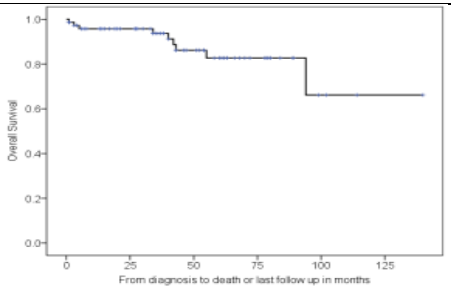

Fig 1A

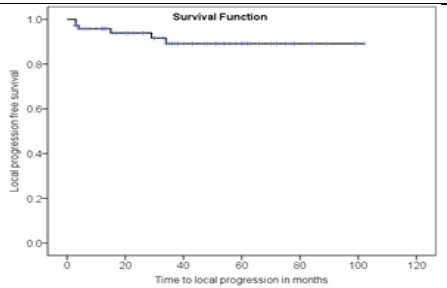

Fig 1B

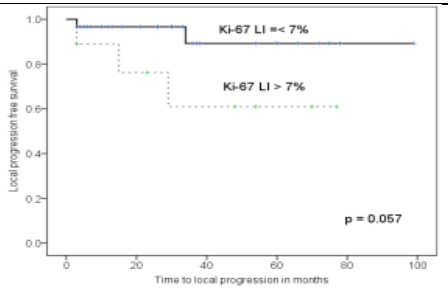

Fig 1C

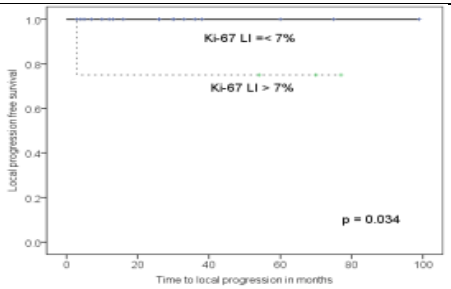

Fig 1D

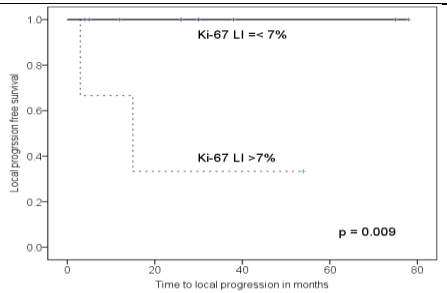

Fig 1E

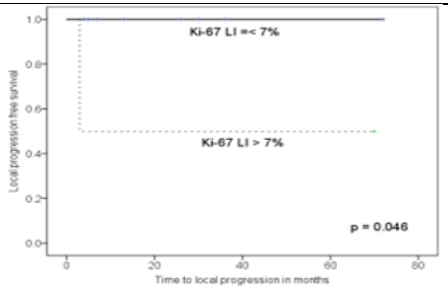

Fig 1F

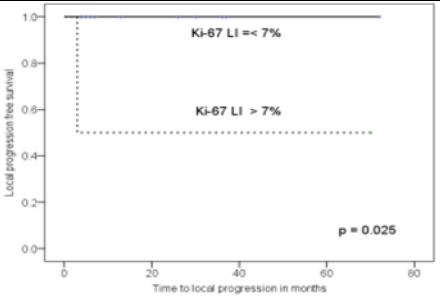

Fig 1G

Figure 1: Overall survival of the whole cohort (1A). Local progression free survival rate of the whole cohort (1B). The 5 Y LPFS is obviously higher with Ki-67 LI < 7% vs ≥ 7% in the whole cohort (1C) and in the sugroups studied: male patients (1D), intestinal GIST subgroup (1E), low risk GIST (1F), mitotic index ≤ 5/ 50 hpf (1gG).

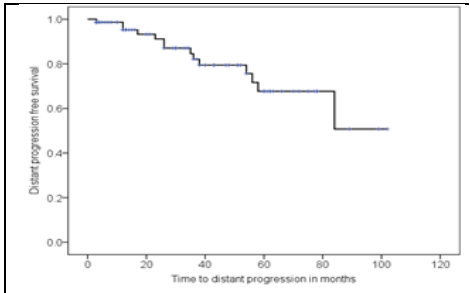

Fig 2A

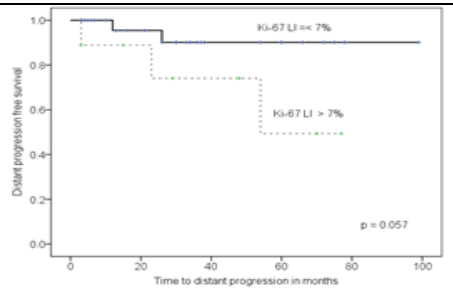

Fig 2B

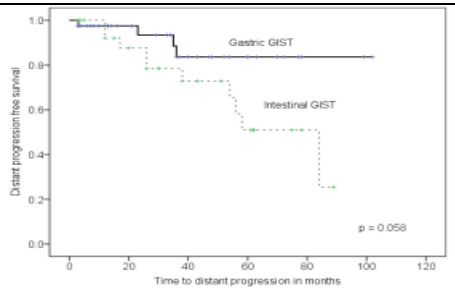

Fig 2C

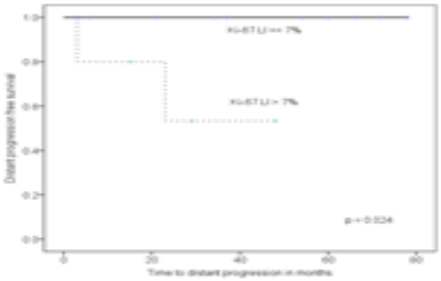

Fig 2D

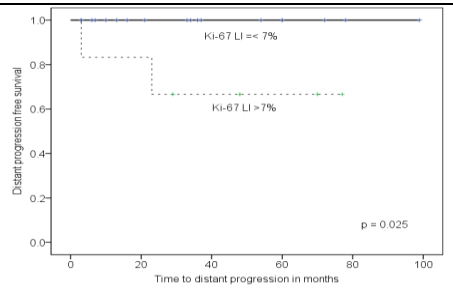

Fig 2E

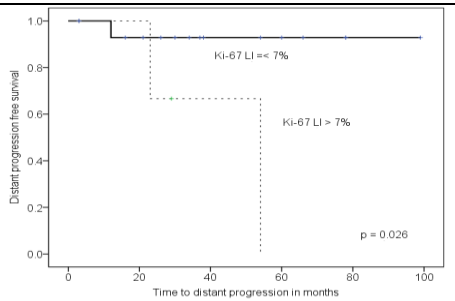

Fig 2F

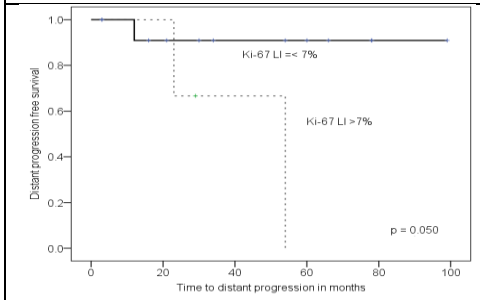

Fig 2G

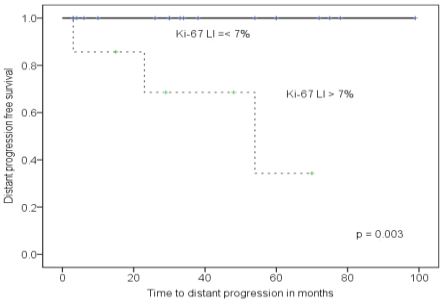

Fig 2H

Figure 2: DPFS in the whole cohort (2A) and its significant association with Ki-67 LI level (2B) and tumor location (2C) in the whole cohort. DPFS with Ki-67 LI ≤ 7% is obviously higher than > 7% in subgroups of females (2D), gastric GIST (2E), High risk GIST(2F), MI > 10 / 50 hpf (2G) and R0 resection margins (2H).

Table 1: Clinicopathologic characteristics of the whole cohort.

Variable	Males (45 patients)	Females (29 patients)	p
Mean age	54.7 y	52.7 y	0.56
Mean follow up period in month	32 month	41 month	0.154
Site Stomach Small intestine	 19/40 (47.5%) 23/30 (76.7%)	 21/40 (52.5% 7/30 (23.3%)	 **0.016**
Maximum dimension (Mean)	8.11 cm	10.92 cm	0.138
Ki-67 LI (mean)	6.68 %	7.59 %	0.784
Mean Mitotic index (IM)	9/50 hpf	10/50 hpf	0.385
Risk Low Intermediate High	 12 (80 %) 6 (54.5%) 17 (58.6%)	 3 (20%) 5 (45.5%) 12 (41.4%)	 0.295
Comorbidities (DM & hypertension) Yes No	 11 (69%) 20 (57%)	 5 (31%) 15 (43%)	 0.543

Table2. The association between clinicopathologic factors and local progression, distant progression free survival and overall survival.

Variable	5 Y LPFS		5 Y DPFS		5 Y OS	
	***Num* & rate**	**p-value**	***Num* & rate**	**p-value**	***Num* & rate**	**p-value**
Age						
≤ 54 yr	3/39 & 90%	0.77	7/39 & 60%	0.95	4/39 & 85%	0.32
> 54 yr	3/32 & 88%		6/32 & 75%		5/32 & 80%	
Sex						
Male	2/45 & 90%	0.23	9/45 & 63%	0.42	4/45 & 85%	0.69
Female	4/29 & 82%		5/29 & 72%		5/29 & 80%	
Comorbidities						
Yes	1/16 & 89%	0.37	2/16 & 76%	0.31	1/16 & 89%	0.18
No	5/35 & 82%		7/35 & 76%		7/35 & 75%	
Ki-67 LI						
≤ 7%	2/30 & 90%	0.05	2/30 & 90%	0.05	3/30 & 83%	0.47
> 7 %	3/9 & 60%		3/9 & 50%		3/9 & 76%	
Site						
Stomach	4/40 & 87%	0.63	4/40 & 84%	0.05	5/40 & 86%	0.98
Small intestine	2/30 & 91%		10/30 & 51%		4/30 & 79%	
Mitotic index MI						
≤ 5/50 hpf	2/18 & 89%	0.48	2/18 & 80%	0.97	4/18 & 63%	0.17
> 5 – 10/50 hpf	0/11 & 100%		2/11 & 57%		1/11 & 83%	
>10/50 hpf	3/26 & 85%		5/26 & 78%		2/26 & 94%	
Maximum tumor dimension						
< 5 cm	0/5 & 100%	0.56	0/5 & 100%	0.36	0/5 & 100%	0.60
5 -- 10 cm	1/14 & 85%		0/14 & 100%		1/14 & 88%	
> 10 cm	0/12 & 100%		2/12 & 88%		0/12 & 100%	
Modified NIH risk category : High	3/29 & 85%	0.56	5/29 & 80%	0.42	2/29 & 95%	0.15
Intermediate	0/11 & 90%		2/11 & 58%		1/11 & 86%	
Low	2/15 & 86%		2/15 & 75%		4/15 & 58%	

Table 3. The association between Ki-67 index and local, distant progression free survival and overall survival in study subgroups.

Variable	Ki-67 LI ≤ 7%			Ki-67 LI > 7%		
	5 y LPFS *N*um & rate ; p	**5 y DPFS *N*um & rate ; p**	**5 y OS *N*um & rate ; p**	**5 y LPFS *N*um & rate ; p**	**5 y DPFS *N*um & rate ; p**	**5 y OS *N*um & rate ; p**
Sex : Males	0/18 & 100%; 0.03	2/18 & 83%; 0.91	1/18 & 80% ; 0.47	1/4 & 75% ; 0.03	1/4 & 67 %; 0.91	1/4 & 75% ; 0.47
Females	2/12 & 80% ; 0.18	0/12 & 100%; 0.02	2/12 & 81% ; 0.90	2/5 & NR; 0.18	2/5 & NR; 0.02	2/5 & 75%; 0.90
Age : =< 54 y	1/15 & 92% ; 0.22	1/15 & 90% ; 0.21	1/15 & 100% ; 0.75	2/6 & 40% ; 0.22	2/6 & 40% ; 0.21	2/6 & 82% ; 0.75
> 54 y	1/14 & 86% ; 0.16	1/14 & 90% ; 0.12	2/14 & 81% ; 0.51	1/3 & 67% ; 0.16	1/3/& 67% ; 0.12	1/3 & 65% ; 0.51
Co morbid : Yes	1/8 & 77% ; 0.61	0/8 & 100% ; 0.08	1/8 & 77% ; 0.61	0/1 & NR ; 0.61	1/1 & NR ; 0.08	0/1 & 100% ; 0.61
No	1/15 & 92% ; 0.08	2/15 & 81% ; 0.48	2/15 & 78% ; 0.93	3/8 & 55% ; 0.08	2/8 & 70% ; 0.48	3/8 & 73% ; 0.93
Site : Stomach	2/19 & 85% ; 0.76	0/19 & 100% ; 0.02	2/19 & 87% ; 0.90	1/6 & 75%; 0.076	2/6 & 68% ; 0.02	1/6 & 100% ; 0.90
Intestine	0/10 & 100%; 0.00	2/10 & 75% ; 0.62	1/10 & 75% ; 0.24	2/3 & NR ; 0.00	1/3 & NR ; 0.62	2/3 & 35% ; 0.24
Risk: High	2/16 & 84% ; 0.33	1/16 & 92% ; 0.02	1/6 & 90% ; 0.65	1/3 & NR ; 0.33	2/3 & NR ; 0.02	1/3 & 100% ; 0.65
Intermediate	0/4 & Not applic	0/4 & Not applic	0/4 & Not applic	0/2 & Not applic	0/2 & Not applic	0/2 & Not applic
Low	0/8 & 100% ; 0.04	1/8 & 75% ; 0.61	1/8 & 50% ; 0.45	1/2 & 50% ; 0.04	0/2 & 100% ; 0.61	1/2 & 50% ; 0.45
MI: =< 5 / 50 hpf	0/10 & 100%; 0.02	1/10 & 80% ; 0.65	1/10 & 50% ; 0.40	1/2 & 50% ; 0.02	0/2 & 100% ; 0.65	1/2 & 50% ; 0.40
>5 - 10 / 50 hpf	0/5 & Not applic	0/5 & Not applic	0/5 & Not applic	0/2 &Not applic	0/5 & Not applic	0/5 & Not applic
> 10 / 50 hpf	2/13 & 80% ; 0.45	1/13& 92% ; 0.05	1/13 & 80% ; 0.70	1/3 & NR ; 0.45	2/3 & NR ; 0.05	1/3 & 100% ; 0.70
Max dim: < 5 cm	0/2 & Not applic	0/2 & Not applic	0/2 & Not applic	0/2 & Not applic	0/2 & Not applic	0/2 & Not applic
5 – 10 cm	1/9 & 67% ; 0.41	0/9 & Not applic	1/9 & 75% ; 0.48	0/2 & 100%; 0.41	0/2 & Not applic	0/2 & 100%; 0.48
> 10 cm	0/6 & Not applic	0/6 & Not applic	0/6 & Not applic	0/6 & Not applic	0/6 & Not applic	0/6 & Not applic
R0	2/14 & 81% ; 0.38	0/14 & 100% ; 0.00	1/14 & 90% ; 0.52	2/7 & 62% ; 0.38	3/7 & 33% ; 0.00	2/7 & 83% ; 0.52

Abbreviations : Not applic : not applicable, NR: not reached.

Table 4. Retrospective studies relevant to our study.

	Study details							
Author [Ref]	**Patients num**	**Mean patient age**	**Mean / Median follow up**	**Ki 67 cutoff**	**Correlation between Ki 67& PFS**	**Correlation between Ki67 &OS**	**Other factors and recurrence**	**Other factors and OS**
Hao Wang [24]	175	61.5 y	40 m	> 1%	Exist	NR	No / low exp of PTEN, Rup, GIT bl, MI > 5/50 hpf, S >10 cm, EGL, hyper cell, HR	NR
Canrong L [25]	111	57 y	22 m	>5%	Exist	Exist	CD133, S: > 5cm, EGL,MI > 5 /hpf, inv depth, Inc R, rup, No AT	CD 133
Huali Li [22]	151	61 y	NR	> 5 %	Exist	NR	NR	NR
Liwen Hong [26]	62	53 y	66 m	>5%	Exist	Exist	S > 5 cm, HR, MI > 10/50 hpf, presence of symptoms	S > 5 cm, HR MI > 10/50 hpf
Robert B [27]	52	61.5 y	50 m	> 5 %	Exist	NR	EGL, MI > 10/50 hpf,	NR
Elif Y [28]	105	64 y	NR	> 5%	Exist	Absent	Nec, S ≥ 8cm, MI ≥5/50 hpf, bl., ulc	MI ≥5/50 hpf, ulc, IR/HR, met
Hui Qu [29]	82	59 y	43m	> 5%	NR	Exist	NR	No AT,S > 10 cm, MI > 5/50 hpf, rup
Borislav B [30]	100	60.5 y	60 m	6%	Exist	NR	SMA exp	NR
Merih T [31]	65	62 y	88 m	≥6%	NR	Exist	NR	S > 10 cm, nec, EGL, MI > 5/50 hpf, mets, CD 34 –ve, HR
J-p Wang [18]	204	59 y	29 m	≥ 6%	Exist	Absent	EGL,S >10cm, MI >5/50 hpf , hg, nec, inv.	NR
Tao Chen [32]	183	54 y	57 m	6%	Exist	NR	S >5 cm, MI >5/50 hpf, NGL, rup	NR

Author [Ref]	**Study details**							
	Patients num	**Mean age of atients**	**Mean / Median follow up**	**Ki 67 cutoff**	**Correlation between Ki 67& PFS**	**Correlation between Ki67 &OS**	**Other factors and recurrence**	**Other factors and OS**
Xuechao L [33]	1022	58 y	24 m	> 6%	NR	Exist	NR	S > 5 cm ,MI ≥ 5/50 hpf
Wen-Yi Zhao [34]	418	59 y	42 m	> 8%	Exist	NR	S > 10 cm and MI > 10/50 hpf	NR
Shintaro S [35]	92	66 y	NR	≥8%	Exist	NR	HR , MI >5/20 hpf, EGL	NR
Mario Z [36]	54	63 y	3.9 yr	>9%	NR	Exist	NR	NR
Vij M [37]	121	50 y	26.5 m	> 10%	Exist	NR	MI: >5/5 mm2, S > 10 cm, hyper cell, N pleo, Epi S, nec., presence of skeinoid fibers, S-100 : –ve, EGL, mets.	NR
Wisit K [38]	46	64 y	33 m	> 10%	Exist	NR	Male gender, age ≥ 60 y., RM: R1, S >10 cm, MI > 5/50 hpf, HR.	NR
Sevinç Ş [39]	100	58 y	45 m	> 10%	NR	Exist	NR	MI,ulc,hg,Dog1
Lingquan W [40]	1015	59 y	22 m	> 10%	Exist in Int/HR	Exist in Int/HR	EGL, S >10 cm, MI >10/50 hpf, type of surgery, irreg TKI, mixed histopathology	irreg TKI, EGL, ,S >10cm, MI >10/50 hpf, type of surgery.

Abbreviations: AT: adjuvant treatment, Bl: bleeding, EGL: extra gastric location, Epi S: epithelial subtype, GIT: gastro intestinal, Hg: hemorrhage, hyper cell: hyper cellularity, HR: high risk, Inc R : incomplete resection, Irreg: irregular, Inv : invasion, IR : intermediate risk, LR : low risk, Met : metastasis, MI: mitotic index, No AT: no adjuvant treatment, N Pleo: nuclear pleomorphism, Nec: tumor necrosis, NR : not reported, Over exp : over expression , RM : resection margin, Rup: rupture, S: tumor size, SmA: smooth muscle antigen, Ulc: ulceration, VEGF: vascular endothelial growth factor.

Concerning the tumor site subgroups, gastric GIST with Ki-67 LI ≤ 7% have demonstrated significantly higher 5y DPFS than those with LI > 7% (100% vs 68%, p = 0.02) as seen in figure 2E. In intestinal subgroup, Ki-67 LI ≤ 7% was associated with significantly higher 5 y LPFS rate in contrast to patients with LI > 7% (100% vs not reached, p=0.00) as seen in figure 1E.

As regards the risk groups, patients in the HR group with Ki-67 LI ≤ 7% showed significantly higher 5 y DPFS than those with LI > 7% (92% vs not reached, p = 0.02) as seen in table 3 and figure 2F. On the other hand, patients in the LR subgroup with Ki-67 LI ≤ 7% demonstrated a significantly higher 5 Y LPFS vs those with LI > 7% (100% vs 50%, p = 0.04) as seen in table 3 and figure 1F.

In the subgroup with MI ≤5 / 50 hpf , a significantly superior 5 Y LPFS rate was noticed with Ki-67 LI ≤ 7% vs LI > 7% (100% vs 50% , p = 0.02) as seen in table 3 and figure 1G. On the other hand, in GIST with MI > 10 / 50 hpf, the 5 Y DPFS was significantly higher with Ki-67 index ≤ 7% vs > 7% (92% vs not reached, p = 0.05) as seen in table 3 and figure 2G. In the subgroup of patients with R0 resection margins, a significantly superior 5 Y DPFS rate was noticed with Ki-67 LI ≤ 7% vs with an index > 7% (100% vs 33% , p = 0.00) as seen in table 3 and figure 2H.

Discussion:

We have searched the PubMed database for relevant articles using the terms ‘GIST’ and ‘Ki67’ In the last 10 years between 2013 and 2023. At the time of writing this article, a total of 159 study were identified whose titles and abstracts were reviewed. These have included 98 retrospective studies(61.6%), 36 case reports (22.6%), 6 animal study (3.7%), 4 meta analysis (2.5%), 3 preclinical (1.9%), 3 genetic (1.8%), 2 review (1.3%) studies while case control, case series, observational and descriptive studies accounted for 4 studies(2.4%),1 for each and 3 were not gist (1.85). Chinese, Japanese and Turkish studies comprised the majority of them with 60, 24 and 14 studies for each respectively.

On the other hand, we have also searched the PubMed database for Egyptian studies on that point and have found 28 articles but none of them has addressed our point. We have focused on retrospective studies and looked for those that have reported on an association between Ki-67 LI cut off value and PFS and/or OS. Nineteen studies have been found and their main results have been summarized in table 4.

As seen in table 4, various cut off points for Ki-67 LI that significantly associated with disease progression have been reported.

In a Chinese study, Hao Wang and colleagues have reported that ki -67 LI > 1% was significantly associated with disease recurrence [24]. Another cut off level at > 5% was reported to be associated with disease outcome in 6 studies as seen in table 4. Chinese investigators have found that a level > 5% was associated with disease progression and OS [25,26] , or with disease progression alone as reported by Huali Li et al[22] whose results were in agreement with other groups from Germany [27] and Turkey [28]. A significant association with OS alone was also reported by Hui Qu and colleagues in their study on Chinese patients [29].

Five studies as seen in table 4 have found that a cut off level of Ki-67 LI at 6% was significantly associated with treatment outcome. One study from Croatia [30], one from Turkey [31] and 3 from China [18, 32,33]. In these studies a significant association between a cut off LI at 6% and above was noticed with disease progression alone [18, 30,32] or with OS [31,33].

A different cut off level at ≥ 8% was found significantly associated with disease progression in 2 studies, a Chinese [34] and a Japanese one [35].

Another cut off level at ≥ 10% was reported to be significantly associated with GIST recurrence in 4 studies conducted on Indian [37], Japanese [38], Turkish [39]and Chinese cohorts [40]. In these studies, that level was associated with disease recurrence [37, 39, 40] and with OS [39, 40].

In our study that was conducted on Egyptian patients a level of LI at ≤ 7% was significantly associated with better treatment outcome.

On the level of survival rates, although the 5 y OS rate was higher in GIST with Ki-67 LI ≤ 7% vs > 7%, the difference was no significant (83% vs 76% , p = 0.47) in contrast to the 5 y LPFS and DPFS rates where tumors with Ki-67 LI ≤ 7% showed significantly higher survival rates compared with tumors with LI > 7% (90 % vs 60% and 90% vs 50% respectively) as seen in table 2, figure 1C and 2B respectively.

In comparison with other studies, we found that our results using this cut off level of LI at 7% lies in the range of results reported by other investigators using close cut off levels of Ki-67 LI. A 5y OS rate with LI > 5% was reported at 50% [25], 75% [26] and at 92% with a cut off level > 10% [38]. Our result at 76% for tumors with LI > 7% is going with these results. On the level of RFS, we have analyzed both local and distant failures separately unlike other studies that have analyzed all failures together. In spite of this difference in study design, our study has demonstrated close results. The 5 y LPFS and DPFS reported here at 60% and 50% are lying close to studies that have reported 5 y RFS at 72% [26] and 77% [27] using a cut off level at > 5% and others that have reported rate at 48.8% with a LI > 6% [35] and 84.6% with LI > 10% [38].

The value of Ki-67 LI as a significant prognostic factor at that cut-off level (≤7% vs >7%) has been noticed in the subgroups analyzed. The 5y LPFS was obviously higher with a LI ≤ 7% vs > 7%, in males (fig 1D), intestinal location of the tumor (fig 1E), LR GIST (fig 1F) and with MI ≤ 5/50 hpf (fig 1G). Also the 5 y DPFS was obviously higher with LI ≤ 7% vs > 7%, in female patients (fig 2D), gastric location (fig 2E), high risk tumors (fig 2F), with MI > 10 /50 hpf (fig 2G) and with R0 surgical resection margin (fig 2H).

Among the well known risk factors associated with GIST recurrence (size, MI, site and, rupture) our study has not found any significant association between these factors and the outcome except for the tumor site. As

seen in table 2, gastric location was significantly associated with higher 5y DFFS than the intestinal one (84% vs 51% respectively, p = 0.05). This finding is consistent with the literature and studies mentioned in table 4 [18, 24, 25, 27, 32, 35, 37, 40].

As regards the MI, it is not disputable that both the MI and Ki-67 LI are indicators reflecting the cell proliferation status but whether the Ki-67 LI can substitute the MI or not is disputable [18]. Some scholars believe that Ki-67 LI being expressed in all phases of cell cycle apart from G0 can represent cell proliferation more comprehensively than the MI that is only measured in M phase [18,34]. The results of our study showed that ki-67 LI was superior to MI in predicting tumor recurrence than the MI as seen in table 2.

Conclusion:

Prognostic factors for gastro intestinal stromal tumors recurrence after surgical excision are under investigations. The role of Ki-67 LI as an important complementary factor is growing in the literature but the optimal cut off level is controversial owing to the retrospective nature of the studies addressing this issue and the difference in nationality of studied populations. Although many studies have recommended cut off levels at 5%, 6%, 8% and 10% as optimal cut off levels, We recognize that our study has limitations because of its retrospective design with its known recall bias, small number of patients, relatively short follow up period and, lack of accessibility of the known panel of all biomarkers other than Ki-67 and DOG-1 , we recommend that a level at 7% is a valuable cut off level that should be taken into consideration in the risk stratification criteria along with the other well established risk factors and patients with GIST and Ki-67 LI > 7% should receive more intensive follow up.

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Authors′ contributions

First Author: Study design, writing and revision of the study.

Second author: data collection.

Third author: writing, revision, tables and figures editing.

Fourth author: Study design, writing and revision.

Fifth author: Study design and data collection

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