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Immunohistochemical Expression of Ki67 in Gastrointestinal Stromal Tumors

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ABSTRACT

Background: One of the most common types of gastrointestinal neoplasms is a GIST (gastrointestinal stromal tumourKi67 is a proliferation marker that can detect all but G0 proliferating cells.

Objective: To evaluate Ki67 expression in GIST and its correlation with the clinicopathologic parameters including survival using immunohistochemical method.

Patients and Methods: In this study, Ki67 immunohistochemistry was performed on sections cut from 56 formalin-fixed, paraffin-embedded GIST. Diagnosis of GIST was confirmed by positive expression of C-kit in all cases except 5 negative cases that were positive for PDGFRA. Clinical data included age, gender, tumour location (gastrointestinal and extra gastrointestinal), tumour size, lymph node status, presence of distant metastasis, and patient status. The assessed pathological parameters included mitotic count, cell type (spindle, mixed spindle and epithelioid, epithelioid) and necrosis. Cases are divided into very low, low, moderate and high-risk groups according to National institute of health and 2006 risk stratification.

Results: Ki67 expression ranged between 0.5-6.6 % with a median of 3 % and a mean±SD of 3.08±1.77 %. 60.7% had low Ki67 labelling index (LI) (below the median, ≤3%), 39.3% had high Ki67 LI (above the median ≥3%). Ki67 LI was significantly correlated to mitosis (P=0.010), 2006 risk stratification (P value=0.027) and with young patients (P=0.019). However, it has no correlation with the overall survival and rest of clinicopathological parameters.

Conclusion: Ki67 could be included in risk stratification of GIST since its expression is correlated with GIST risk stratification.

Keywords: Immunostaining, GIST, Ki67, Mitosis, Risk stratification.

INTRODUCTION

Mesenchymal tumors in the digestive tract, such as gastrointestinal stromal tumors (GIST), are the most common, accounting for 0.1–3% of all cases of gastrointestinal cancer. GIST are clinically relevant, despite their rarity, because at least 10–30% of them are malignant **(1).**

More than 60% of tumors are found in the stomach, which is followed by the duodenum (4-5%), colon (4%), oesophagus (1-2%), and rectal (1-2%) regions (1-2%) **(2).** Appendix and gallbladder GIST are extremely rare. Extra-visceral GIST, such as in the mesentery, pelvis, omentum, or retroperitoneum, is extremely rare. Extra gastrointestinal GIST is the name given to tumors found in the preceding locations **(3).**

An indicator of cell division and proliferation, the nuclear antigen Ki67 is closely linked to cell mitosis. Ki67 is only found in cells that are actively proliferating, and it is present throughout the cell cycle at stages G1, S, and G2 **(4).** Except for G0, the mitotic index only reflects the M stage of mitosis, which Ki67 can recognise. As a result, any malignant tumour, including GIST, can use Ki67 as an appropriate proliferation marker **(5).**

The aim of the present study was to evaluate Ki67 expression in GIST and its correlation with the clinicopathologic parameters including survival using immunohistochemical method.

PATIENTS AND METHODS

There were 56 patients with gastrointestinal stromal tumors included in the research (GIST). The National Cancer Institute, Cairo University's archive of formalin-fixed, paraffin-embedded tissue blocks was searched from 2006 to 2013. Data from the medical files of patients at Cairo University's National Cancer Institute were retrieved for this study. The patients' medical records and/or personal contact provided the source of the follow-up data.

Clinical data included age, gender, tumour location (gastrointestinal and extra gastrointestinal), tumour size, lymph node status, presence of distant metastasis, and patient status. Necrosis, mitotic count, cell type (epithelioid, epithelioid mixed), and cell type were all examined as pathological parameters. It was determined

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that the largest tumour size was taken into account. Fifty high power fields (HPFx) were used to count the mitoses (400x).

This study's GIST risk stratification models adhered to the NIH's consensus on GIST risk stratification[6], and the Armed Forces Institute of Pathology (AFIP) classification of 2006 risk stratification[2].

Immunohistochemical staining: A standard microtome was used to cut four micron-thick sections, which were then mounted on positively charged slides. Xylene was used to deparaffinize and rehydrate paraffin-embedded tissue sections. Tris-EDTA high PH retrieval solution (Dako, Ref K8000, Glostrup, Denmark) was applied to the sections for 20 minutes. A peroxidase-blocking reagent was used to stop the endogenous peroxidase from working.

A dilution of 1:200 of the primary antibody for Ki67 (LAB VISION, clone SP6, Fremont, USA) was used. DAB was used as a substrate chromogen solution to detect a positive reaction. Counterstaining with Mayer's haematoxylin was then performed on the sections. A coverslip was used to protect each slide after it had been dehydrated using various grades of alcohol. Tonsils were used as positive controls. Each run of immunohistochemical staining included a negative control in which the primary antibody was replaced by PBS (phosphate buffer solution).

Using the Ki67 antigen, the proliferative index was calculated as the proportion of cells with a positive nuclear reaction out of 100 GIST cells.

Ethical consent:

An approval of the study was obtained from Menoufia University Academic and Ethical Committee.

Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The IBM SPSS software package version 20.0 was used to analyse the data that was fed into it. (IBM Corp., Armonk, New York) Quantitative data were presented in the form of percentages and numbers. The normality of the distribution was confirmed using the Kolmogorov-Smirnov test. The range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR) were used to describe quantitative data (IQR).

It was determined that the findings had a significance level of 5%. Chi-square test for comparing different categories of categorical variables. Fisher's Exact or Monte Carlo correction for more than 20% of the cells have expected counts less than 5; apply the chi-square correction. Student t-test it's possible to compare two groups of subjects using normally distributed quantitative data. Mann Whitney test for quantitative variables with an abnormal distribution, a comparison between two research groups is necessary. Kruskal Wallis test for the case of quantitative variables with an abnormally wide distribution, comparing the results of more than two groups is recommended. P value < 0.05 was considered significant.

RESULTS

The clinicopathological parameters of studied GIST cases are presented in **table 1**.

Ki67 expression in the studied cases ranged between 0.5-6.6 % with a median of 3 % and a mean±SD of 3.08±1.77 %. Thirty-four cases (60.7%) had low Ki67 proliferation index (below the median, ≤3%), and 22 cases (39.3%) had high Ki67 proliferation index (above the median ≥3%).

Table (1): Clinicopathological data of the studied GIST cases (n = 56)

		No.	%
Age (years)	≤54 years	23	41.1
	>54 years	33	58.9
	Min. – Max.	24.0 – 82.0	
	Mean ± SD.	54.13 ± 11.51	
	Median (interquartile range)	57.0 (46.0 – 61.0)	
Gender	Male	28	50.0
	Female	28	50.0
Site	Gastrointestinal	41	73.2
	Extra gastrointestinal	15	26.8
Size (cm)	≤14	33	58.9
	>14	23	41.1
	Mean ± SD.	14.46 ± 7.67	
	Median (IQR)	12.0 (9.0 – 19.0)	
Lymph node (L.N)	Not available	37	66.1
	Negative	17	30.4
	Positive	2	3.6
Primary tumor (pT)	T2	5	8.9
	T3	14	25.0
	T4	37	66.1
Status (n = 49)	Censored	36	73.5
	Alive	5	10.2
	Dead	8	16.3
Distant metastasis	Not available	4	7.1
	Negative	36	64.3
	Positive	16	28.6
Progression	Not available	4	7.1
	No	33	58.9
	Yes	19	33.9
Morphology	Epitheloid	8	14.3
	Spindle	39	69.6
	Mixed	9	16.1
Mitosis	≤5	31	55.4
	>5	25	44.6
Necrosis	Negative	21	37.5
	Positive	35	62.5
National institute of health risk category	Low risk	3	5.4
	Intermediate risk	9	16.1
	High risk	44	78.6
2006 risk category	Very low	2	3.6
	Low	4	7.1
	Moderate	18	32.1
	High	32	57.1

Higher mean and median percentage of Ki67 expression were significantly associated with young patients (<54 years) (P=0.019), cases with high mitotic figures (>5/HPF) (P=0.010), and risk category (according to 2006-armed forces institute risk stratification) (P value=0.027) **(figures 1&2) (table 2).**

On the other hand, there was no statistically significant relationship between Ki67 proliferation index and the rest of different clinicopathological parameters, patient status, progression or distant metastasis **(Table 2).** Also, there was no significant statistical association between Ki67 proliferation index and overall survival (OS) (data not shown).

Table (2): Association between Ki67 percentage of expression and different clinicopathological parameters of studied GIST cases (n=56)

	N	Ki67 % Mean ± Standard deviation	Test of Sig.	P value
Age (years)				
≤54 years	**23**	3.72 ± 1.70	Mann Whitney test = 239.5*	0.019*
>54 years	**33**	2.63 ± 1.70		
Gender				
Male	**28**	3.03 ± 1.72	Mann Whitney test =383.50	0.889
Female	**28**	3.13 ± 1.85		
Site				
Gastrointestinal	**41**	2.85 ± 1.75	Mann Whitney test = 219.5	0.102
Extra gastrointestinal	**15**	3.70 ± 1.72		
Size				
≤14 (cm)	**33**	2.84 ± 1.79	Mann Whitney test = 305.50	0.216
>14 (cm)	**23**	3.42 ± 1.72		
Lymph node (L.N)				
Negative	**17**	2.49 ± 1.21	Mann Whitney test = 6.50	0.187
Positive	**2**	3.75 ± 1.06		
Primary tumor (pT)				
T2	**5**	3.30 ± 2.11	Kruskal Wallis test = 0.142	0.932
T3	**14**	2.97 ± 1.90		
T4	**37**	3.09 ± 1.73		
Status				
Alive + Censored	**41**	3.23 ± 1.83	Mann Whitney test = 136.50	0.464
Dead	**8**	2.66 ± 1.38		
Distant metastasis				
Negative	**36**	3.04 ± 1.89	Mann Whitney test = 265.0	0.647
Positive	**16**	3.04 ± 1.20		
Progression				
No	**33**	2.88 ± 1.85	Mann Whitney test = 248.0	0.211
Yes	**19**	3.32 ± 1.40		
Morphology				
Epitheloid	**8**	3.31 ± 2.22	Kruskal Wallis test = 0.898	0.638
Spindle	**39**	2.94 ± 1.76		
Mixed	**9**	3.48 ± 1.50		
Mitosis				
≤5	**31**	2.58 ± 1.69	Mann Whitney test = 232.0*	0.010*
>5	**25**	3.70 ± 1.69		
Necrosis				
Negative	**21**	2.96 ± 1.79	Mann Whitney test = 344.0	0.689
Positive	**35**	3.15 ± 1.78		
National institute of health risk category				
Low risk	**3**	3.83 ± 2.75	Kruskal Wallis test = 1.839	0.399
Intermediate risk	**9**	2.46 ± 1.77		
High risk	**44**	3.16 ± 1.72		
2006 risk category				
Very low	**2**	0.75 ± 0.35	Kruskal Wallis test = 9.182*	0.027*
Low	**4**	2.25 ± 1.26		
Moderate	**18**	2.66 ± 1.64		
High	**32**	3.57 ± 1.77		

*: Significant

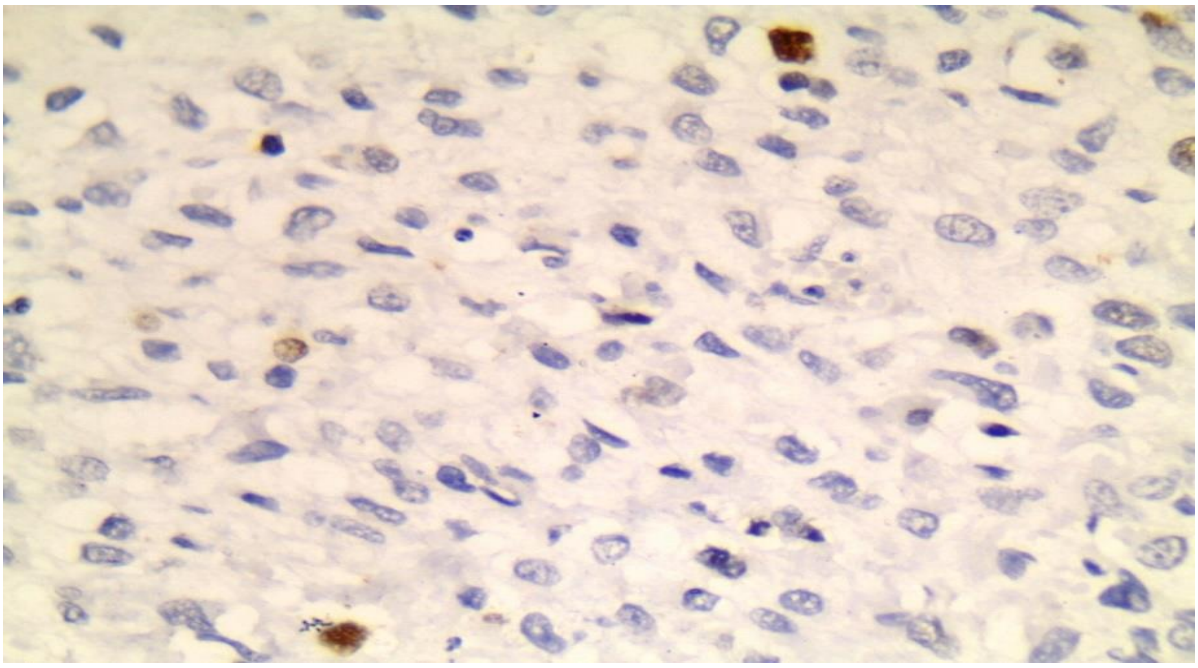

Figure (1): Low percentage of Ki 67 expression (1%) in a case of GIST with very low risk (KI67 IHC x400)

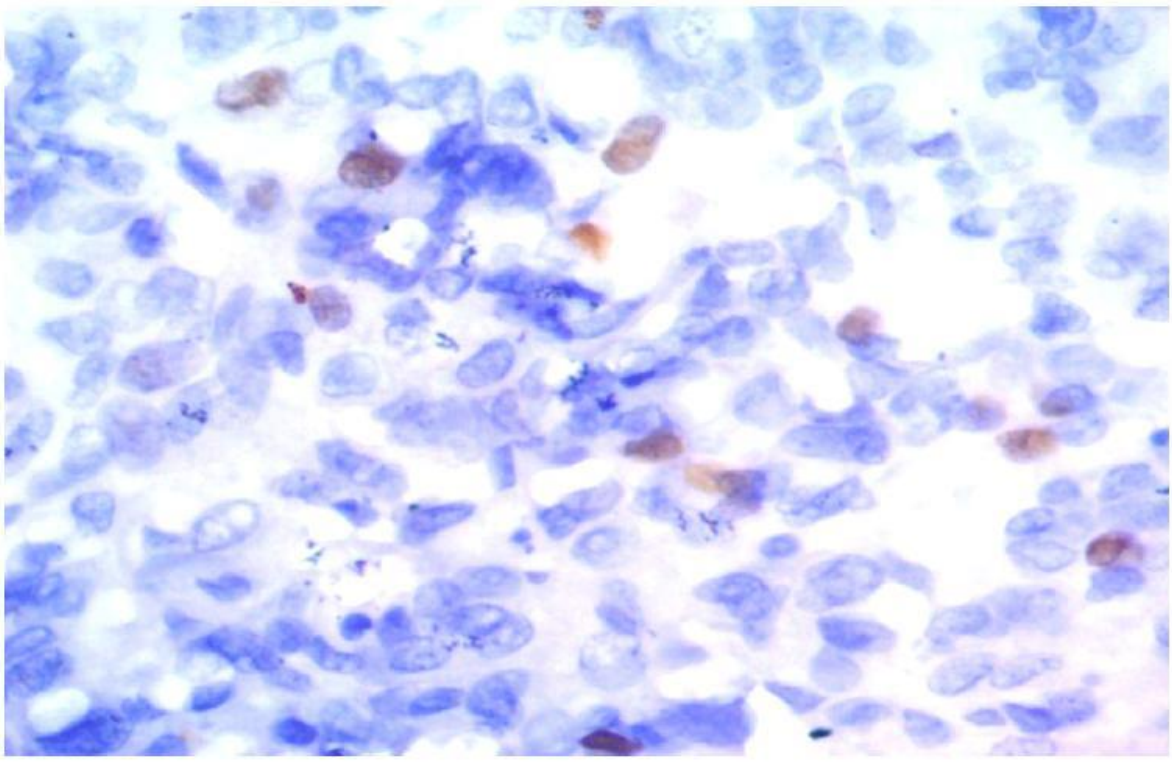

Figure (2): High percentage of Ki 67 expression (6%) in a case of GIST with high risk (KI67 IHC x400).

DISCUSSION

GISTs are well-known for their wide range of biological behaviours, and it is difficult to predict their malignancy potential [(8)]. Many aspects of GIST's biology have been studied extensively over the past decade [(9)].

The cut-off point of Ki67 (LAB VISION, clone SP6) in the present study was 3% which represents the median. Thirty-four cases (60.7%) had low Ki67 proliferation index, and 22 cases (39.3%) had high Ki67 proliferation index.

Cerski *et al.* [(10)] had reported that Ki67 cut-off point for proliferative index was determined as 3% in GIST. Fifty-seven cases presented Ki67 ≤ 3%, and 28 (33%) presented Ki67 > 3%. They used MIB-1 clone Dako, 1:50. These results are very close to our results.

Belev *et al.* [(11)] found that mean value in localized disease is 2.5 (which is close to the mean in present study), but they found the mean in recurrent and metastatic cases 10% with a cut-off point of 6%, they used (MIB-5 clone from DAKO, 1:25). Most of cases in present study (58.9%) had no metastasis or recurrence,

this could explain why our numerical values are close to their numerical values in localized disease but not in metastatic and recurrent one.

According to various studies, the expression of Ki67 varies greatly from one to the next due to a variety of authors' cutoffs and varying assessment methodologies [9-12].

The present results demonstrated a significant correlation between Ki67 proliferative index and GIST risk stratification category (2006 risk category) (P = 0.027).This finding was in agreement with **Carrillo *et al.*** [13], who stated that Ki67 is one of the most accurate predictors of clinical behaviour in GIST**. Wang *et al.*** [14] also found statistically significant differences in the Ki67 labelling index and the following factors: tumour size, mitotic index, tumour site which are components of the 2006 risk stratification. This conclusion is consistent with our findings.

Furthermore, **Zhou *et al.*** [9], supported the present study results because they found that compared to the low NIH group, intermediate and high NIH groups had more GIST patients with Ki67 overexpression, indicating that Ki67 overexpression could be a useful indicator of the likelihood that a GIST will progress to malignancy in the future.

Furthermore, in the present study there was statistically significant relationship between Ki67 proliferative index and mitosis. This finding was also in agreement with **Carrillo *et al.*** [13]**, Wang *et al.*** [14] **and Neto *et al.*** [15] who found that high MIB-1 index statistically correlated with high mitotic rate in GIST which supported our results**.**

In the present study, we found no significant relationship between Ki67 and overall survival, this finding was different from **Belev *et al.*** [11] who found that Ki67 was close to statistically affect overall survival in GIST patients. **Neto *et al.*** [15], also demonstrated the impact of Ki67 expression score on survival in patients with gastrointestinal stromal tumors (GIST). **Pyo *et al.*** [16] reported that a high Ki67 LI (labelling index) was significantly correlated with worse disease-free survival (DFS) and overall survival (OS) in GIST**.** This difference of results of the present study from previous ones can be explained by presence of factors affecting Ki67 LI such as differences in immunohistochemical methods, evaluation methods and observers.

CONCLUSION

Ki67 could be included in risk stratification of GIST since its expression is correlated with GIST risk stratification.

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