



Prognostic utility of Ki-67 in gastric carcinoma



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Abstract

Introduction: Gastric carcinoma represents the third leading cause of cancer related mortality worldwide. Most of cases are diagnosed at the time of tumor metastasis. There is a discrepancy in the results regarding the role of Ki-67 proliferation index as a prognostic factor in gastric carcinoma.

Objectives: To assess the prognostic value of Ki-67 proliferation index and correlate Ki-67 expression with the overall survival (OS) in patients with gastric carcinoma.

Patients and Methods: This retrospective study included fifty cases collected randomly from archival paraffin blocks of gastric carcinoma. Cases were collected during the period from 2013 to 2018. Sections were prepared and the slides were immunohistochemically stained with Ki-67 antibody.

Results: This study showed a statistically significant correlation between higher percentage of Ki-67 expression and cases with tumors located in the fundus and body of the stomach and with distant metastasis. Also a statistically significant correlation between higher mean of Ki-67 positive cell % and cases of adenocarcinoma with pT1 tumors and with loco-regional recurrence ($P < 0.001$, $P = 0.02$). A higher percentage of Ki-67 expression was found in cases of adenocarcinoma grade III and cases with positive perineural invasion compared to other cases, yet the correlation was statistically insignificant. According to survival analysis, OS rate was 34.0% and the median OS time was 14.6 months. There was no statistically significant association between Ki-67 proliferation index and disease free survival (DFS) and OS.

Conclusion: Ki-67 proliferation index can be used as a predictor for distant metastasis and loco-regional recurrence of gastric cancer.

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Introduction

Gastric carcinoma is considered as one of the most common aggressive malignancies and represents the third leading cause of cancer related mortality worldwide (1). In Egypt, gastric cancer is the tenth most common cancer in both sexes. It represents 2.9% of the total primary malignant tumors (2).

Most of gastric carcinoma patients are diagnosed at advanced stages when the tumor has metastasized and the surgical intervention is unsuitable. The 5-years survival rate is less than 20% and the median overall survival (OS) time is less than 12 months (3,4).

Ki-67 antigen is expressed in close relation to cell cycle. It appears in the nuclei of cells in the active phases of the cellular cycle G1, S, G2 and M but not in the resting cells (G0 phase), so the immune-expression of Ki-67 has been considered a useful tool to determine the potential of tumor proliferation (5).

Ki-67 is known as a marker of cellular proliferation, and it is used to predict the prognosis of cancer and evaluate the response

Key point

- Ki-67 is one of the most widely used proliferation index markers and has a validated prognostic value in many cancers including breast cancer
- There is controversy regarding prognostic value of Ki-67 in gastric cancer
- The present work revealed a higher percentage of Ki-67 expression in cases of adenocarcinoma grade III and in cases with positive perineural invasion, however there was no statistically significant correlation with OS and DFS

to treatment. Estimation of Ki-67 proliferation index before starting chemotherapy is also a strong predictor of effectiveness of the therapy (6).

Many studies have reported the clinical and prognostic value of Ki-67 in several types of malignancies, such as breast cancer and lung cancer (7,8).

In gastric carcinoma, despite several investigations, the prognostic value of Ki-67 proliferation index remains inconclusive. A large number of studies have reported that the high level of Ki-67 is correlated with poor

prognosis (9,10). Although other studies reported that Ki-67 expression is irrelevant to the prognosis of gastric carcinoma (11,12).

Objectives

The aim of the present study is to assess the prognostic value of Ki-67 proliferation index and correlate Ki-67 expression results with OS in patients with gastric carcinoma.

Patients and Methods

Study design

This retrospective study was carried out on 50 cases of gastric adenocarcinoma obtained through collection of archival paraffin blocks of gastric carcinoma from the pathology lab of Ain Shams University Hospital during the period from 2013 to 2018. Thirty-one cases were gastrectomy specimens and 19 were gastroscopic biopsies. All H&E slides were examined and histopathological features were re-evaluated as follows:

- Gastric carcinomas were classified according to Lauren classification (intestinal and diffuse types) and WHO classification (2010) into adenocarcinoma, mucinous adenocarcinoma, and signet ring adenocarcinoma (13).
- Histological grading of gastric adenocarcinomas was divided into well, moderately and poorly differentiated.
- The depth of tumor invasion, lymph node metastasis, distant metastasis and staging of tumor were assessed depending on TNM classification (2017) (American Joint Committee on Cancer 7th staging system).
- The lymphovascular invasion, perineural invasion, and involvement of surgical margin by the tumor were assessed.

Sections of 4 μ m thick were cut from paraffin-blocks which contained formalin fixed tumor tissue. During the staining procedure the slides were treated with the fully automated Benchmark Staining System (Ventana Medical Systems) using the primary antibody (rabbit monoclonal anti Ki-67 human clone 30–09 Ventana Medical System).

According to the recommendations of the International Ki-67 in Breast Cancer Working Group; positive Ki-67 staining was defined as only positive nuclear staining, regardless of the staining intensity, and the average score is recorded by the assessment of complete sections including hot spots (if present). In full sections, at least three high-power fields ($\times 40$ objective) should be selected (14).

As recorded by Ko et al (15) specimens with no stained tumor cells or stained tumor cells $< 5\%$ were defined as negative for Ki-67 immunostaining.

The nuclear expression of Ki-67 was counted within 100 tumor cells in a hot spot area (the area of the highest density of Ki-67-positive nuclei) under a light microscope, and graded as gastric carcinomas with low Ki-67 score ($\leq 20\%$) and gastric carcinomas with high Ki-67 score ($> 20\%$) as done by Ko et al (15), El-Gendi et al (16) and

Armani et al (17).

Statistical analysis

Statistical analysis for the results of Ki-67 immunostaining in gastric carcinoma was correlated with clinicopathological data including age and sex; histopathological type and grade of the tumor, depth of invasion, lymph node metastasis, distant metastasis, TNM staging, lymphovascular and perineural invasions, and involvement of surgical margins by tumor cells.

The correlations between Ki-67 expression and the clinicopathological characteristics of the patients were assessed by using chi-square test (Fisher exact), and one-way ANOVA test. Survival curves were plotted by using of Kaplan–Meier method and statistical differences were assayed using the log-rank test. A *P* value was considered statistically significant if less than 0.05.

Results

Demographic and clinico-pathological features are represented in Table 1.

Correlations between Ki-67 expression and clinicopathological parameters

The mean of Ki-67 positive cell % was higher in cases of adenocarcinoma of intestinal type (Figure 1A), grade III (Figure 1B), stage IV (Figure 1C), and with positive perineural invasion (Figure 1D) compared to other cases, yet the correlation did not reach a statistically significant value.

In T1 adenocarcinoma cases the mean of Ki-67 positive cell % was (0.83 ± 0.04) (Figure 1E) compared to (0.50 ± 0.27) in T2, T3 and T4 (Figure 1F) with a highly significant difference ($P < 0.001$). In cases with positive loco-regional recurrence the mean of Ki-67 positive cell % was (0.69 ± 0.13) compared to (0.52 ± 0.26) in negative cases with a statistically significant difference ($P = 0.02$; Table 2).

A higher Ki-67 expression (at a cutoff point of 20%) was obtained in cases with tumor located in the fundus and body of the stomach and tumor with distant metastasis compared to other cases and the correlation was statistically significant ($P = 0.027$ and $P = 0.017$, respectively) (Table 3).

Analysis for disease free survival

During the follow up period, out of 31 patients who underwent surgery, 18 patients died and 13 patients remained alive (Figure 2). According to survival analysis, disease free survival (DFS) rate was calculated as 41.9% and the median DFS time was 26.767 months with a range of (4.023–49.510) months.

Factors affecting DFS

As shown in Table 4, DFS rate was not significantly influenced by patients' age, histopathological type of tumor, degree of differentiation, depth of invasion presence or absence of lymph node metastases, positive or negative

Table 1. Demographic and clinic-pathologic features of the included cases

Clinicopathological characters		N	%
Age of patient (y)	≤50	23	46.0
	>50	27	54.0
Gender	Male	31	62.0
	Female	19	38.0
Location	Cardia	11	22.0
	Fundus & Body	25	50.0
	Pylorus	14	28.0
Intervention	Surgery	31	62.0
	Endoscopy	19	38.0
Final pathology	Intestinal type adenocarcinoma	43	86.0
	Diffuse type adenocarcinoma	7	14.0
Grade	Grade I	1	2.0
	Grade II	20	40.0
	Grade III	29	58.0
Lymphovascular invasion	Negative	20	64.5
	Positive	11	35.5
Perineural invasion	Negative	20	64.5
	Positive	11	35.5
Margins	Negative	19	61.3
	Positive	12	38.7
Pathological T	T1	2	6.5
	T2	4	13.0
	T3	14	45.0
	T4	11	35.5
Pathological N	Negative	11	35.5
	Positive	20	64.5
Metastasis	Negative	26	56.5
	Positive	20	43.5
Stage	I	3	6.5
	II	6	13.0
	III	17	37.0
	IV	20	43.5
Ki-67 expression (of positive cells)	≤20	9	18.00
	>20	41	82.00

perineural invasion, involvement of surgical margins, and Ki-67 proliferative index (Figure 3).

However, the median DFS time in patients with distant metastasis (4.8 months) was less than that in patients without distant metastasis (33.7 months) and the difference was statistically highly significant ($P=0.005$). The median DFS time in patients at stage II was (29.9 months) higher than that in patients at stage IV (4.8 months) with statistical significant difference ($P=0.021$). The median DFS time in patients without lymphovascular invasion was (33.7 months), much more than that in patients with lymphovascular invasion (6.033 months) the difference was statistically significant ($P=0.015$).

Analysis for overall survival

During the follow up period, out of 50 patients included in this study, 33 patients died and 17 patients remained alive (Figure 4). According to survival analysis, OS rate was

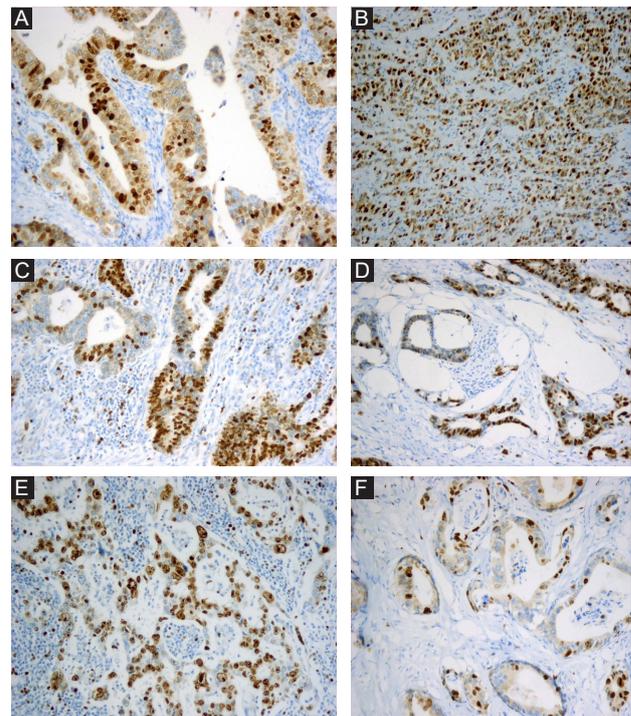


Figure 1. (A) A case of moderately differentiated gastric adenocarcinoma intestinal type with high Ki-67 proliferation index (Ki-67 x200). (B) A case of poorly differentiated gastric adenocarcinoma with high Ki-67 proliferation index (Ki-67 x200). (C) A case of moderately differentiated gastric adenocarcinoma intestinal type with high Ki-67 proliferation index (Ki-67 x200). (D) A case of moderately differentiated gastric adenocarcinoma intestinal type with perineural invasion (arrow) showing high Ki-67 proliferation index (Ki-67x200). (E) a case of moderately differentiated gastric adenocarcinoma intestinal type with invasion of submucosa PT1 with high Ki-67 proliferation index (Ki-67 x200). (F) A case of moderately differentiated gastric adenocarcinoma with infiltration of subserosal connective tissue PT3 with low Ki-67 proliferation index (Ki-67 x200).

calculated as 34.0% and the median OS time was 14.600 months with a range of (8.096-21.104) months.

Factors affecting Overall survival (OS)

OS rate was not significantly influenced by patients' age, histopathological type of tumor, depth of invasion, presence or absence of lymph node metastases, and positive or negative perineural invasion, and level of Ki-67 (Table 5, Figure 5).

However, the median OS in patients with grade I or II (30.8 months) was higher than that in patients with grade III (9.7 months) with statistical significant association ($P=0.023$).

The median OS in patients without distant metastasis (32.97 months) was higher than that in patients with distant metastasis (9.2 months) The difference was highly significant ($P<0.001$).

The median OS in patients at stage II (32.97 months) was higher than in patients at stage IV (9.2 months) with highly significant difference ($P<0.001$).

The median OS in patients without lymphovascular invasion (32.97 months), was higher than in patients with

Table 2. Relation between Ki-67 expression and clinicopathological characters

Clinico-pathological characters		Ki-67 expression (% of positive cells)		Test value	P value
		Mean	SD		
Final pathology	Intestinal type	0.55	0.25	0.81*	0.42
	Diffuse type	0.46	0.28		
Grade	Grade I or II	0.52	0.28	0.37*	0.72
	Grade III	0.55	0.24		
Lymphovascular invasion	Negative	0.53	0.29	0.30*	0.77
	Positive	0.50	0.28		
Perineural invasion	Negative	0.48	0.29	1.04*	0.40
	Positive	0.60	0.24		
Margins	Negative	0.55	0.26	0.60*	0.56
	Positive	0.49	0.31		
Pathological T	T1	0.83	0.04	5.81*	<0.001 HS
	T2/T3/T4	0.50	0.27		
Pathological N	Negative	0.56	0.31	0.59*	0.56
	Positive	0.50	0.26		
Metastasis	Negative	0.49	0.26	1.75*	0.09
	Positive	0.61	0.23		
Stage	I	0.47	0.35	1.33**	0.26
	II	0.55	0.34		
	III	0.48	0.24		
	IV	0.61	0.23		
Loco-regional recurrence	Negative	0.52	0.26	2.69	0.02 S
	Positive	0.69	0.13		
Systemic recurrence	Negative	0.50	0.26	1.21	0.23
	Positive	0.59	0.25		
Outcome	Alive	0.48	0.28	1.09	0.28
	Died	0.57	0.24		

* Student t test, ** One-way ANOVA test.

HS: highly significant, S: significant

lymphovascular invasion (12.2 months) and the difference reached statistical significant value ($P=0.030$).

The median OS in patients with negative surgical margins (35.8 months) was higher than that in patients with positive surgical margins (14.6 months). The correlation was statistically significant ($P=0.035$).

Discussion

Concerning the immunohistochemical expression of Ki-67 in this study, Ki-67 positively stained cells of $\leq 20\%$ were seen in 18% of patients, and $>20\%$ in 82% of patients.

The present study revealed a significant statistical association between high Ki-67 expression (at a cutoff point $>20\%$) and gastric adenocarcinoma location in fundus and body of stomach ($P=0.027$). This finding is inconsistent with the results of Ko et al (15) who stated that high Ki-67 can be used as indicator for poor prognosis in patients with well differentiated histology who developed tumors in the lower third area of the stomach not in fundus and body.

The current work showed that no significant statistical correlation was found between Ki-67 level and histologic type of the tumor ($P=0.43$). These findings are in accordance with what was obtained by Çalik et al (18) who obtained similar results. While Ko et al (15) found that

high level of Ki-67 expression was significantly associated with intestinal histological type of gastric carcinoma according to the Lauren classification.

The present study showed no significant statistical correlation between Ki-67 level and the grade of gastric carcinoma ($P=0.87$). These findings are in accordance with Zhou et al (19) who obtained the same results. In contrast El-Gendi et al (16) found that high level of Ki-67 expression was significantly associated with higher tumor grade. While Lee et al. (11) reported that high Ki-67 was associated with well differentiated tumors.

A highly significant inverse association between Ki-67 expression and depth of tumor invasion with ($P<0.001$) was detected in the current study (high Ki-67 proliferative index for pT1 tumors versus pT2, T3 and T4 tumors). This finding could be explained by the fact that the highly proliferative tumors have less invasive subclones (intratumor heterogeneity) and thus the depth of tumor invasion is less despite high proliferation (11). The other explanation is that the proliferation rate of a tumor is a temporary state that may provide no information about the history or future development of the tumor and an inverse relation exists between proliferative and invasive activities in gastric carcinoma (20).

Our finding is compatible with that reported by Lee et

Table 3. Relation between Ki-67 expression and clinicopathological characters

Tumor characters		Ki-67 expression (% of positive cells)				Chi-square	P value
		≤ 20%		> 20%			
		N	%	N	%		
Location: Cardia	Negative	8	20.5%	31	79.5%	0.76	0.38
	Positive	1	9.1%	10	90.9%		
Fundus & Body	Negative	1	4.0%	24	96.0%	4.87	0.027 S
	Positive	8	32.0%	17	68.0%		
Pylorus	Negative	6	16.7%	30	83.3%	0.15	0.693
	Positive	3	21.4%	11	78.6%		
Final pathology	Intestinal type	7	16.3%	36	83.7%	0.62	0.43
	Diffuse type	2	28.6%	5	71.4%		
Grade	Grade I or II	4	19.0%	17	81.0%	0.03	0.87
	Grade III	5	17.2%	24	82.8%		
Lymphovascular invasion	Negative	3	15.0%	17	85.0%	1.85	0.173
	Positive	4	36.4%	7	63.6%		
Perineural invasion	Negative	5	25.0%	15	75.0%	0.18	0.66
	Positive	2	18.2%	9	81.8%		
Margins	Negative	3	15.8%	16	84.2%	1.29	0.25
	Positive	4	33.4%	8	66.6%		
Pathological T	T1	0	0.0%	2	100.0%	0.62 FE	1.00
	T2/T3/T4	7	24.1%	22	75.9%		
Pathological N	Negative	2	18.2%	9	81.8%	0.19 FE	1.00
	Positive	5	25.0%	15	75.0%		
Metastasis	Negative	0	0.0%	26	100.0%	5.6	0.017 S
	Positive	4	20.0%	16	80.0%		
Stage	I	1	33.3%	2	66.7%	0.44 FE	0.84
	II	1	16.7%	5	83.3%		
	III	3	17.6%	14	82.4%		
	IV	4	20.0%	16	80.0%		
Loco-regional Recurrence	Negative	9	100.0%	35	85.4%	1.50 FE	0.58
	Positive	0	0.0%	6	14.6%		
Systemic Recurrence	Negative	5	55.6%	26	63.4%	0.19	0.66
	Positive	4	44.4%	15	36.6%		
Outcome	Alive	3	33.3%	14	34.1%	0.002	0.96
	Died	6	66.7%	27	65.9%		

FE: Fisher Exact, S: significant.

al (11) and Badry et al (21) who reported a significant relation between high Ki-67 and early stages of invasion.

Conversely, Xiao et al (12), Pyo & Kim (22) and Amrani et al (17) reported no correlation between the Ki-67 value and level of tumor invasion.

Whereas El-Gendi et al (16) noted higher rates of high Ki-67 score among advanced T stages (91.5% and 57.1% for T3 and T4 tumors versus T1 and T2 tumors, respectively but without significant statistical correlation. These results suggest that the changes in the proliferative activity of tumor cells are related to the progression of gastric carcinoma.

Although high Ki-67 expression was observed in 75% of patients with positive lymph node metastasis, the present study showed no statistically significant association between Ki-67 expression and lymph node metastasis ($P=1.00$). This was in accordance with Pyo and Kim, (22) who reported similar results.

On the contrary, Xiong et al (23) found a significant statistical correlation between high Ki-67 expression and

lymph node metastasis and El-Gendi et al (16) reported that a high Ki-67 score was correlated significantly with greater number of regional lymph node involvement. While Lee et al (11) showed that a low Ki-67 proliferative index is related to lymph node metastasis.

The current work showed a significant statistical correlation between high Ki-67 proliferative index and distant metastasis ($P=0.017$). This result is compatible with what was obtained by Xiong et al (23), who stated that gastric carcinoma related death is mostly caused by metastasis. Predicting the presence of metastasis is helpful in improving the prognosis of patients. Association of high Ki-67 proliferative index and distant metastasis could be used to predict progression of gastric carcinoma, identify high-risk patients, and optimizing their management (23). On other hand, Lee et al (11) showed that a low Ki-67 proliferative index is related to distant metastasis.

The present study revealed no significant statistical correlation between Ki-67 proliferative index and the stage of gastric carcinoma. This result concurs with the

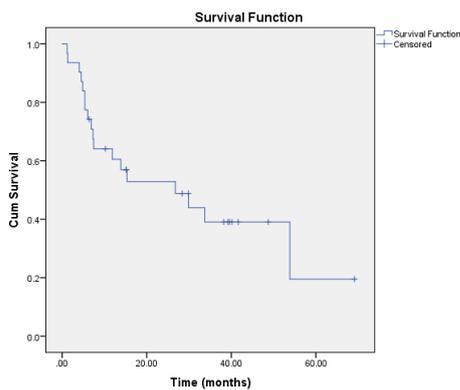


Figure 2. Kaplan-Meier analysis for disease free survival

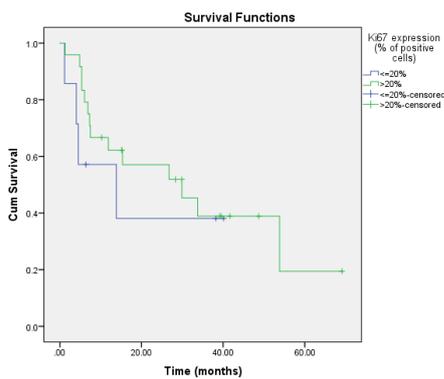


Figure 3. Correlation between DFS time and Ki-67 expression.

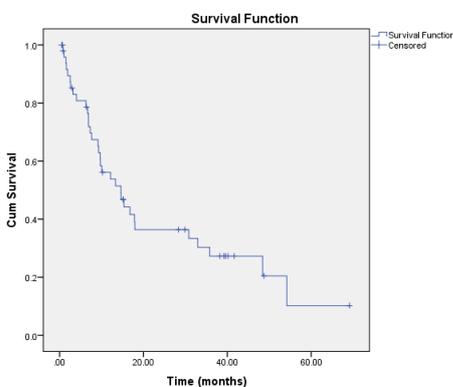


Figure 4. Correlation between DFS time and Ki-67 expression.

results obtained by Pyo and Kim (22). Whereas Xiong et al (23) reported different results, they stated that high Ki-67 levels were associated with advanced TNM stage gastric carcinoma.

No statistically significant association between Ki-67 expression and lymphovascular invasion, perineural invasion, and involvement of surgical margins in the current work. Similar results were reported by Xiao et al (12) and El Gendi et al. (16). In contrast to our study

Table 4. Overall comparisons log rank (Mantel-Cox) - (DFS)

	Chi-square	df	P value
Ki-67 expression	0.482	1	0.488
Age	0.418	1	0.518
Grade	0.821	1	0.365
Final pathology	0.027	1	0.870
PT	0.007	1	0.935
PN	2.505	1	0.113
M	7.840	1	0.005 HS
Stage	9.730	3	0.021 S
LVI	5.933	1	0.015 S
PNI	0.036	1	0.849
Margins	2.908	1	0.088

HS: highly significant, S: significant

Table 5. Overall comparisons log rank (Mantel-Cox) - (OS)

	Chi-square	df	P value
Ki-67 expression	0.154	1	0.694
Age	0.024	1	0.878
Grade	5.162	1	0.023 S
Final pathology	2.079	1	0.149
PT	0.117	1	0.733
PN	2.401	1	0.121
M	15.428	1	<0.001 HS
Stage	19.710	3	<0.001 HS
LVI	4.714	1	0.030 S
PNI	0.281	1	0.596
Margins	4.441	1	0.035 S

HS: highly significant, S: significant

Ayed et al (28) revealed a significant correlation between Ki-67 and lymphovascular invasion. This finding can be explained by small number of studied cases.

The present work detected that 12% of patients had a loco-regional lymph node recurrence, and 38% of patients had a systemic recurrence. A statistically significant relation was found between high Ki-67 expression and positive loco-regional recurrence. Regarding the relation between Ki-67 expression and locoregional recurrence in gastric carcinoma, no papers are found in the literature and this is a pilot study concerning this point.

In the current study the DFS rate was 41.9% and the median DFS time was 26.767 months. No statistically significant relation was found between the median DFS and Ki-67 expression. The same result was obtained by Badary et al (21). Inconsistent to our results Luo et al (29) observed a significant inverse relation between Ki-67 level and DFS time.

In the current study, the OS rate was 34.0% and the median OS time was 14.600 months. The median OS time among cases with high Ki-67 expression (14.633 months)

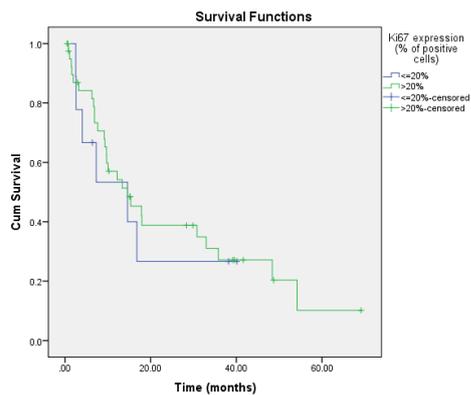


Figure 5. Correlation between OS time and Ki-67 expression.

was about equal to that in cases with Ki-67 expression (14.600 months), accordingly, there was no a statistically significant difference. This observation could be explained by the fact that most of the patients included in this study were of old age and some of them had a history of comorbidity. The same results were obtained from the studies of Calick et al (18) and Seo et al (30).

While Lee et al (11) demonstrated that early gastric cancer with higher proliferative ability may show a high expression of Ki-67, and may be correlated with higher survival rate and better prognosis. In contrast, Ko et al (15) recorded that the patients with high level of Ki-67 expression clearly showed a bad prognosis, but the differences did not reach statistical significance, which might be explained by small sample number.

The present study revealed a statistically significant association between low OS and high tumor grade ($P=0.023$). Conversely, Badary et al (21) observed no statistically significant relation between OS and tumor grade ($P=0.9$).

In the current study the correlation between mean low OS and distant metastasis and tumor stage was statistically highly significant ($P<0.001$). On the contrary, Badary et al (21) stated that no statistically significant relation was noted between mean OS and distant metastasis of gastric carcinoma.

Our work showed a significant statistical correlation between low median OS and positive surgical margins ($P=0.035$), and positive lymphovascular invasion ($P=0.030$). These findings are compatible with the results of Stahl et al. (31) who found significant statistical correlation between OS and positive surgical margins as well as lymphovascular invasion.

No statistically significant association was found in the current study between OS time and histological type of the tumor, depth of tumor invasion, and lymph node metastasis. These results are consistent with the results of Badary et al (21) regarding association of OS and lymph node metastasis. On other hand, these findings are inconsistent with the results of Xiao et al (12) in regard to the relation between OS and histological type of the tumor,

depth of tumor invasion and lymph node metastasis.

These discrepancies in the results could be explained by several factors as; this study and most of the mentioned studies were of retrospective type, and the samples or the involved populations were relatively of small size. The included cases were different regarding histological types, TNM classification, and treatment strategies. Moreover, immunohistochemical staining methods, antibody manufacturers, dilution ratios, the number of counted cells, methods of Ki-67 expression, scoring protocols for evaluation Ki-67 proliferative index, and the different cutoff values that classifying Ki-67 proliferative index into low and high groups were extremely variable. In addition to the different study regions and races of involved patients (29).

Limitations of the study

The present work included relatively limited sample size. We found the positive correlation between Ki-67 and high tumor grade, and perineural invasion indicating that Ki-67 could be a potential poor prognostic indicator, but the correlation with DFS and OS did not reach a statistically significant value. Further studies with larger sample size could reveal this positive correlation.

Authors' contribution

NMA, GRM and MMS were the principal investigators of the study. MNE and AMB, MMS were included in preparing the concept and design. MNE and AMB revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics The institutional ethical committee at Ain Shams University, Faculty of Medicine approved all study protocols. The study was based on data collection and immunohistochemical analysis of positively charged slides prepared from paraffin blocks so informed consent was not applicable to our study. This study was extracted from Master Thesis of Naema Mohamed Almabrouk at this University. Moreover, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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