European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

ANALYSIS OF IMMUNO HISTOCHEMICAL EXPRESSION OF Bcl2 AND Ki67 IN GASTRIC CARCINOMA AND ITS ASSOCIATION WITH HISTOPATHOLOGICAL FEATURES - A CROSS-SECTIONAL STUDY

Article History:

Name of Author:
Dr Abitha Abraham[1], Dr Supriya Papaiah[2] ,M.H.Shariff[3], Dr Prema Saldanha[4], Dr Renuka Patil[5], Dr Swaroop Raj[6]

Affiliation: *[1]Post Graduate, Department of Pathology, Yenepoya Medical College, Mangalore, Karnataka, India*
[2]Associate Professor, Department of Pathology, Yenepoya Medical College, Mangalore, Karnataka, India
[3]Professor and HOD, Department of Pathology, Yenepoya Medical College, Mangalore, Karnataka, India
[4]Professor, Department of Pathology, Yenepoya Medical College, Mangalore, Karnataka, India
[5]Associate Professor, Department of Pathology, Yenepoya Medical College, Mangalore, Karnataka, India
[6]Professor, Department of Pathology, Yenepoya Medical College, Mangalore, Karnataka, India

Corresponding Author:
Dr Supriya Papaiah

Received: 11-11-2025
Revised: 25-11-2025
Accepted: 09-12-2025
Published: 30-12-2025

Abstract: Introduction: Gastric cancer is one of the major causes of cancer-related deaths worldwide. Early diagnosis is challenging due to nonspecific symptoms, leading to late-stage detection and poor prognosis. Bcl2 (B-cell lymphoma 2) and Ki67 are key biomarkers associated with gastric carcinoma, playing crucial roles in tumor progression, apoptosis and proliferation. The combined evaluation of Bcl2 and Ki67 expression may provide insights into the biological behavior of gastric carcinoma and help guide therapeutic strategies, particularly in personalized treatment approaches. Materials and methods: A total of 42 gastric specimens, including biopsies and resection specimens, collected between September 2023 and December 2024 were included in the study. The sections were stained with routine Hematoxylin and Eosin. Immunohistochemical analysis (IHC) was performed using antibodies against Bcl2 and Ki67. Statistical analysis done to observe frequency and significance. Results: This study included 42 cases of gastric carcinoma (23 resections and 19 biopsies), with a male predominance. Moderately differentiated adenocarcinoma was the most common (43.4%), followed by poorly differentiated (30.4%) and well-differentiated tumors (26%). Bcl-2 positivity was more frequent in well- differentiated tumors (87.5%). Ki-67 expression showed no clear association with histological grade, as its distribution was relatively uniform across grades. Conclusion: This study found that Bcl2 positivity is more prevalent in well-differentiated tumors, suggesting a potential role of Bcl2 in tumor differentiation and survival. Ki67 expression did not show a clear correlation with histological grade, implying that tumor proliferation activity may not be solely dependent on differentiation status.

Keywords: Gastric carcinoma, Immunohistochemistry, Bcl2, Ki67

INTRODUCTION

Gastric cancer remains a major global health problem and is the fifth most commonly diagnosed malignancy and the fourth leading cause of cancer-related mortality worldwide [1]. Most cases are diagnosed at an advanced stage due to late clinical presentation, limiting the availability of curative treatment options [1]. The five-year survival rate for advanced gastric cancer ranges from 5% to 20%, with a median overall survival of approximately one year [2]. The incidence increases with age, peaking between 60 and 80 years, and is rare below 30 years of age [2]. Gastric cancer shows a clear male predominance, with men being two to four times more likely to develop the disease than women [2]. Dietary factors and Helicobacter pylori infection are major risk

factors for distal gastric cancers, while gastroesophageal reflux disease and obesity are more strongly associated with proximal tumors. In India, a higher incidence has been reported in the southern and north eastern regions [2,3].

According to the World Health Organization and Lauren classification, gastric carcinoma is broadly divided into intestinal and diffuse types [4]. The intestinal type is more common in older men and is often associated with precursor lesions such as chronic atrophic gastritis and intestinal metaplasia, with *H. pylori* infection, dietary factors, and obesity playing significant roles [5]. This type generally carries a better prognosis than the diffuse variant.

The Bcl-2 family of proteins plays a crucial role in the regulation of apoptosis, comprising both pro-apoptotic and anti-apoptotic members. Dysregulated overexpression of anti-apoptotic Bcl-2 proteins contributes to tumor development and therapeutic resistance [6]. In the gastric mucosa, Bcl-2 expression is predominantly observed in proliferative zones, suggesting a role in cellular renewal. During gastric carcinogenesis, Bcl-2 expression has been documented in chronic atrophic gastritis with intestinal metaplasia and epithelial dysplasia, while being infrequent in normal gastric mucosa. Overexpression of Bcl-2 in gastric carcinoma has been associated with resistance to chemotherapy and radiotherapy [6].

Ki-67 is a nuclear protein expressed during the active phases of the cell cycle (G1, S, G2, and M phases) but absent in the resting (G0) phase, making it a reliable marker of cellular proliferation [7]. Increased Ki-67 expression has been correlated with aggressive tumor behavior and poor prognosis in several malignancies [7].

Evaluation of apoptotic and proliferative markers such as Bcl-2 and Ki-67 may provide valuable insights into tumor biology, aggressiveness, and prognosis in gastric carcinoma. This study aimed to analyze the expression patterns of Bcl-2 and Ki-67 in gastric carcinoma and correlate them with histopathological parameters [8].

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Pathology and included prospective cases of gastric carcinoma diagnosed at our institute between September 2023 and December 2024. A total of 42 gastric specimens, including biopsies and resection specimens were included in the study. Formalin-fixed (10%) specimens were examined grossly according to standard guidelines. Paraffin-embedded tissue blocks were prepared, and 5-micron sections were cut using a microtome and stained with hematoxylin and eosin. Histopathological evaluation was performed, and relevant findings were recorded.

Representative tumor sections were subjected to immunohistochemical staining for Bcl-2 and Ki-67 using the PolyExcel HRP non-biotin, micropolymer-based DAB detection system. Immunostaining was evaluated based on staining intensity and the percentage of positive tumor cells.

Inclusion criteria: Surgical resection specimens and biopsy samples of gastric carcinoma, irrespective of histological subtypes.

Exclusion criteria:
Cases that received neoadjuvant chemotherapy or radiotherapy prior to surgery.
Specimens that were inadequate for histopathological evaluation.

Immunohistochemistry staining for bcl2 and ki67:
Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections. Three sections of 5-micron thickness were obtained from each block. Rabbit monoclonal antibody against Bcl-2 (clone EP36) and mouse monoclonal antibody against Ki-67 (clone MIB-1) (Pathnsitu Biotechnologies) were used according to the manufacturer's instructions. Appropriate positive and negative controls were included. For negative controls, the primary antibody was omitted.

Bcl-2 expression was assessed based on the percentage of positive tumor cells and categorized as shown in Table 1.

Table 1. Interpretation of Bcl2 expression

Interpretation of Bcl2 expression	
Negative	<5% tumor cells
Positive	>5% tumor cells

Ki67 :At least 1000 tumor cells (×400 magnification) from the most immune positive regions of each neoplasm would be visually counted, and the percentage of positive cells (labeling index, LI) will be calculated. Tumors were classified as Ki67-low for scores 0 and 1, and Ki67-high for scores 2 and 3 (Table 2) [9]

Table 2. Ki67 immunostaining score

Stained tumor Cells (%)	Score
< 5%	0
5% - 19%	1
20% - 49%	2
> 50%	3

By adhering to these standardized procedures and ethical guidelines, the study aimed to comprehensively analyze the expression patterns of Bcl2 and Ki67 in gastric carcinoma cases.

Statistical Analysis

Comparisons between groups of Ki67 expression levels and Bcl2 expression status were performed using the chi-square test (χ^2 test) or Fisher's exact test for categorical variables.

For continuous variables (e.g., age), comparisons were conducted using the independent t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data.

Normality was assessed using the Shapiro-Wilk test.

A p-value < 0.05 was considered statistically significant.

RESULTS

In this study, 42 gastric carcinoma cases, including biopsy and resection specimens were evaluated according to AJCC and WHO guidelines, and subjected to Bcl2 and Ki67 immunohistochemical analysis. Most patients belonged to the 61–70-year age group, with a male predominance. Of the specimens, 19 were biopsies and 23 were gastrectomies, (69% were partial and 30.4% were total gastrectomies). Adenocarcinoma NOS was the predominant subtype (54.7%), followed by poorly cohesive (28.5%), mixed (11.9%), and mucinous carcinoma (4.7%). Among the 23 adenocarcinoma NOS cases, 43.4% were Moderately differentiated, 30.4% Poorly differentiated, and 26% were Well differentiated. Lauren classification showed diffuse type in 52.3%, intestinal in 42.8%, and mixed in 4.7% (Table 3). Among resected specimens, T3 was the most frequent pathological stage (69%) and lymph node metastasis was present in 19 cases, with N0 and N1 each comprising 26.09%, N2 21.74%, and N3 17.39%. Lymphovascular invasion and perineural invasion were identified in 82.6% and 73.9% of cases, respectively.

Table 3 Comprehensive overview of Pathological findings in 42 Gastric carcinoma cases

Parameter	Category	Number of Cases	Percentage
Specimen Type	Biopsy	19	–
	Resection specimen	23	–
	• Partial gastrectomy	16	69% of resections
	• Total gastrectomy	7	30.4% of resections
Histological Subtype	Adenocarcinoma NOS	23	54.7%
	Poorly cohesive adenocarcinoma	12	28.5%
	Mixed adenocarcinoma	5	11.9%
	Mucinous adenocarcinoma	2	4.7%
Grading (Adenocarcinoma NOS only, n=23)	Well differentiated	6	26%
	Moderately differentiated	10	43.4%
	Poorly differentiated	7	30.4%
Lauren Classification	Diffuse type	22	52.3%
	Intestinal type	15	42.8%
	Mixed type	5	4.7%

Bcl2 expression showed no significant correlation with histopathological variables as seen in Table 4. Among adenocarcinoma NOS cases, Bcl2 positivity increased with differentiation-50% in poorly, 77.7% in moderately, and 83.3% in well-differentiated tumors (Figure1 and 2). By Lauren classification, Bcl2 was positive in 80% of mixed, 73% of intestinal, and 63.3% of diffuse types (p = 0.381). In resected specimens, Bcl2 positivity occurred in 57.9% of LVI-positive versus 75% of LVI-negative cases (p = 0.524), 58.8% of PNI-positive versus 66.7% of PNI-negative cases (p = 0.735), and 58.8% of node-positive versus 66.7% of node-negative cases (p = 0.735).

High Ki67 expression was observed in 54.7% of cases, with no significant association with age (p = 0.241). In adenocarcinoma NOS, high Ki67 expression tended to increase with poorer differentiation,71.4% in poorly, 66.8% in moderately, and 57.1% in well-differentiated tumors (p = 0.800). Ki67 distribution was similar across Lauren types, with high expression in 54% of diffuse, 53% of intestinal, and 40% of mixed tumors (p = 0.801). (Figure 3 and 4) Among resected specimens, high Ki67 expression was found in 52.6% of LVI-positive cases, while none of the LVI-negative cases showed high Ki67. High expression was present in 41.2% of PNI-positive and 50% of PNI-negative cases (p = 0.708) and in 41.2% of node-positive and 50% of node-negative cases (p = 0.708).
Bcl2 positivity was noted in 28 cases, of which 57.1% showed high Ki67 expression, compared with 50% among Bcl2-negative cases. This association was not statistically significant (p = 0.661).

Table 4 Association of Bcl2 and Ki67 Immunohistochemical Expression with Histological Grade, Lauren Classification, and Tumor Invasion Features

Parameter	Category	Bcl2 Positive (%)	p-value	Ki67 High (%)	p-value
Histological Grade (NOS, n=23)	Well differentiated	83.3%	0.374	57.1%	0.800
	Moderately differentiated	77.7%		66.8%	
	Poorly differentiated	50%		71.4%	
Lauren Classification (n=42)	Intestinal	73%	0.381	53%	0.801
	Diffuse	63.3%		54%	
	Mixed	80%		40%	
Lymphovascular Invasion (n=23)	Present (n=19)	57.9%	0.524	52.6%	—
	Absent (n=4)	75%		0%	
Perineural Invasion (n=23)	Present (n=17)	58.8%	0.735	41.2%	0.708
	Absent (n=6)	66.7%		50%	
Lymph Node Metastasis (n=23)	Present (n=17)	58.8%	0.735	41.2%	0.708
	Absent (n=6)	66.7%		50%	
Overall Marker Expression (n=42)	Total Bcl2 positive	28/42 (66.6%)	—	—	—
	Bcl2+ with high Ki67	57.1%	0.661	—	—
	Bcl2– with high Ki67	50%		—	
Ki67 Levels (Overall)	High Ki67	—	—	23/42 (54.7%)	—
	Low Ki67	—	—	19/42 (45.2%)	—

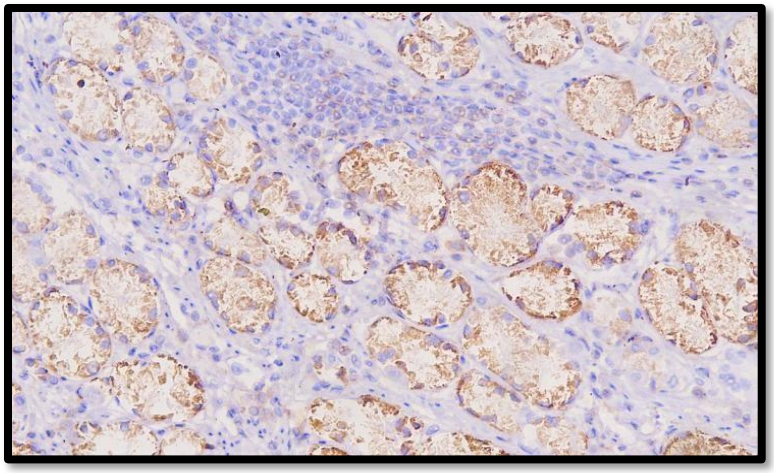

Figure. 1 Immunohistochemical Bcl-2 cytoplasmic positivity in well- differentiated gastric adenocarcinoma (200×)

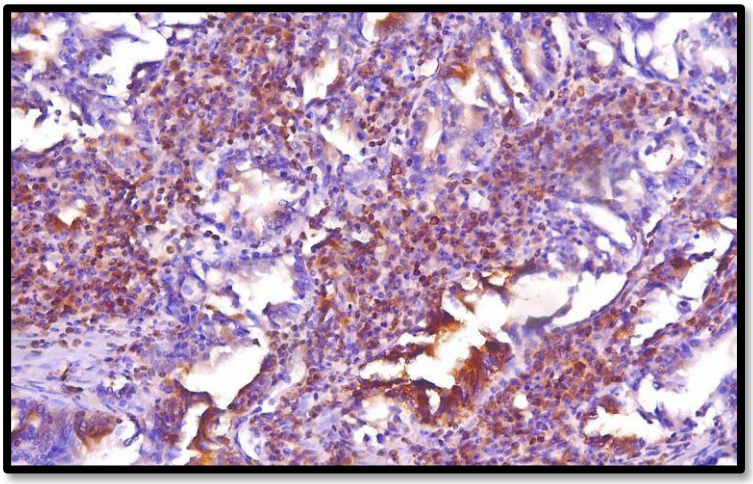

Figure. 2 Photomicrograph of IHC Bcl2 cytoplasmic staining showing positive in moderately differentiated adenocarcinoma, grade 2 (200x)

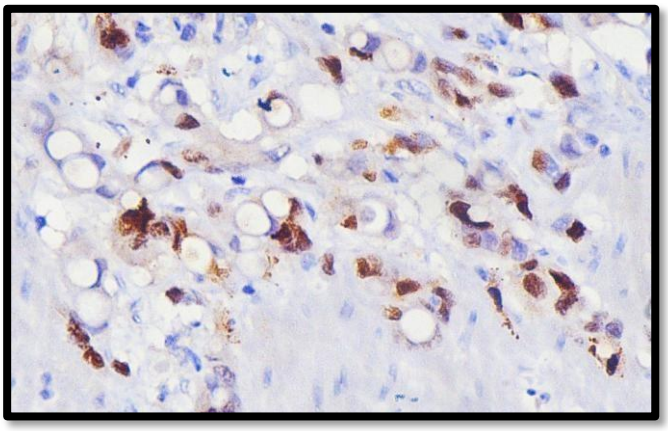

Figure. 3 Photomicrograph of IHC Ki67 nuclear staining showing positive with score 2 (400x)

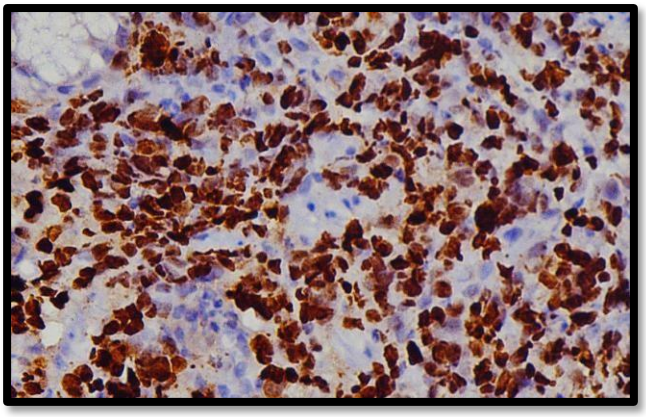

Figure. 4 Photomicrograph of IHC Ki67 nuclear staining showing positive with score 3 (400x)

DISCUSSION

Gastric carcinoma is the fifth most commonly diagnosed cancer worldwide and shows a clear male predominance [1]. Diagnosis is primarily based on endoscopy and histopathological examination of biopsy specimens, with tumor grade, stage, and growth pattern being important prognostic indicators. However, as histological grading may be

subjective, immunohistochemistry serves as a valuable adjunct in tumor assessment [10]. Bcl2 is an anti-apoptotic protein localized to the mitochondrial, nuclear, and endoplasmic reticulum membranes, where it regulates caspase activation and cytochrome-c release [11]. Ki67 is a well-established proliferation marker expressed during the G1, S, G2, and M phases of the cell cycle but absent in the G0 phase [12]. The present study evaluated the expression of Bcl2 and Ki67 in gastric carcinoma and correlated their expression with histopathological parameters.

The mean age of patients in this study was 62 years (range 34–75 years), which is comparable to previous studies reporting peak incidence in the sixth to seventh decades of life [13]. A male predominance with a male-to-female ratio of 2.2:1 was observed, consistent with earlier reports. According to Lauren classification, diffuse-type carcinoma was the most frequent subtype (52%), similar to findings by Qiu et al. [14], but in contrast to studies by Park et al. [1], Lee et al. [15], Crețu et al. [16] and Chen et al. [17] ,where intestinal-type carcinoma predominated. Moderately differentiated adenocarcinoma constituted the largest proportion of cases (43%), comparable to the observations of Lee et al. [15].

Bcl2 expression was noted in 66.7% of cases, which is in agreement with Tsamandas et al. [18] and comparable to findings by Zhou et al. [19], although lower expression rates have been reported in some studies. A slightly higher frequency of Bcl2 positivity was observed in older patients, however this association was not statistically significant, consistent with study by Chakrabarthi et al. [20]. Although Bcl2 expression appeared to increase with better tumor differentiation, no statistically significant association was observed in our study. This finding was similar to the study conducted by Sameer H Fatani et al.[21].

High Ki67 expression was observed in 54.7% of cases, comparable to previously published studies by Chatpalli Pranjali et al. [22]. Ki67 expression tended to be higher in older patients and in poorly differentiated tumors; however, these associations did not reach statistical significance, consistent with study done by Chatpalli Pranjali et al. [22]. Lazar et al.[23,24,25,26], observed a close correlation between the degree of tumor differentiation and the Ki67 score,similar observations was also noted in our study.

Associations between Ki67 expression and lymph node metastasis have been variable across studies. No significant correlation was observed between Ki67 and Bcl2 expression ($p = 0.661$), consistent with the findings of Zhou et al. [19], whereas study by Kyueng-Whan Min et al[14] showed contrasting results.

Overall, the findings of this study demonstrate demographic characteristics, a predominance of diffuse-type and moderately differentiated tumors, and heterogeneous expression patterns of Bcl2 and Ki67. The absence of statistically significant associations with most clinicopathological parameters underscores the biological heterogeneity of gastric carcinoma and suggests that the prognostic significance of these biomarkers remains uncertain. These observations highlight the need for larger, multicentric studies to more clearly define the role of Bcl2 and Ki67 in gastric carcinoma.

CONCLUSION

This study assessed the association of Bcl2 and Ki67 with histopathological features of gastric carcinoma. Ki67 expression showed a heterogeneous pattern, with score 2 being most frequent (35.7%), and increased expression observed with higher tumor grade. Bcl2 was positive in 66.7% of cases, with higher expression in well-differentiated tumors. These patterns indicate that tumor progression involves dynamic changes in apoptotic and proliferative pathways. The observed expression trends suggest a potential role of Bcl2 and Ki67 in tumor differentiation and progression, contributing to a better understanding of the biological behavior of gastric carcinoma.

REFERENCES

1. Park YS, Kook MC, Kim B. A standardized pathology report for gastric cancer: 2nd edition. J Pathol Transl Med. 2023;57(1):1-27.
2. Liang YX, Deng JY, Guo HH. Characteristics and prognosis of gastric cancer in patients aged ≥ 70 years. World J Gastroenterol. 2013;19(39):6568-78.
3. Mamun TI, Younus S, Rahman MH. Gastric cancer—epidemiology, modifiable and non-modifiable risk factors, challenges and opportunities: an updated review. Cancer Treat Res Commun. 2024;41: 100845
4. Lauren P. The two histological main types of gastric carcinoma: diffuse and intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31-49.
5. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. Prz Gastroenterol. 2019;14(1):26-38.
6. Fatani SH, Mukhtar MH, Ali AS, et al.

Correlation between serum anti-apoptotic Bcl2 level and its immunohistochemical expression in relation to apoptosis in gastric cancer. J Mol Biomark Diagn. 2015;6:248-50
7. Lazăr D, Tăban S, Sporea I. Ki67 expression in gastric cancer: results from a prospective study with long-term follow-up. Rom J Morphol Embryol. 2010;51(2):249-57.
8. Liu HF, Liu WW, Fang DC et al. Expression of Bcl2 protein in gastric carcinoma and its significance. World J Gastroenterol. 1998;4(3):228-30.
9. Ko GH, Go SI, Lee WS et al. Prognostic impact of Ki67 in patients with gastric cancer-the importance of depth of invasion and histologic differentiation. Medicine (Baltimore). 2017;96(25):7181-91
10. Hundahl SA. Staging, stage migration, and patterns of spread in gastric cancer. Semin Radiat Oncol. 2002;12(2):141-9
11. Tao S, Gu J, Wang Q. Translational control of Bcl2 promotes apoptosis of gastric carcinoma cells. BMC Cancer. 2021;21(1):12-8
12. Sun X, Kaufman PD. Ki67: more than a proliferation marker. Chromosoma. 2018 ;127(2):175-86.
13. El Halabi M, Horanieh R, Tamim H. The impact of age on prognosis in patients with gastric cancer: experience in a tertiary care centre. J Gastrointest Oncol. 2020;11(6):1233-41
14. Qiu MZ, Cai MY, Zhang DS. Clinicopathological characteristics and prognostic analysis of Lauren classification in gastric adenocarcinoma in China. J Transl Med. 2013;11:58-64
15. Cristescu R, Lee J, Nebozhyn M. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med. 2015;21(5):449-56
16. Crețu OI, Stepan AE, Simionescu CE et al. Classification and Grading Systems in Gastric Adenocarcinomas. Curr Health Sci J. 2022;48(3):284-91.
17. Chen YC, Fang WL, Wang RF. Clinicopathological variation of Lauren classification in gastric cancer. Pathol Oncol Res. 2016;22(1):197-202
18. Tsamandas AC, Kardamakis D, Tsiamalos P. The potential role of Bcl2 expression, apoptosis and cell proliferation (Ki67 expression) in cases of gastric carcinoma and correlation with classic prognostic factors and patient outcome. Anticancer Res. 2009;29(2):703-9.
19. Zhou Y, Li Y, Zheng J et al, Detecting of gastric cancer by Bcl2 and Ki67. Int J Clin Exp Pathol. 2015;8(6):7287-90.
20. Raya B, Hajra S, Chakrabarti J. Expression of ERK1/2 and Bcl2 in gastric carcinoma and their clinicopathological significance: an observational study in a tertiary care cancer hospital. J Can Res Ther. 2024:23472
21. Fatani SH, Mukhtar MH, Ali AS, El-Emshaty HM, Nasif WA. Correlation between serum anti-apoptotic Bcl2 level and its immunohistochemical expression in relation to apoptosis in gastric cancer. Mol Biomark Diagn. 2015;6(4):248-53.
22. Chatpalli P, Kamath S, Mysorekar V. CDX2 and Ki67 expression in gastric carcinoma and its association with clinicopathological parameters. Asian Pac J Cancer Biol. 2022;7:105-11.
23. Lazăr D, Tăban S, Sporea I Ki67 expression in gastric cancer: Results from a prospective study with long-term follow-up. Rom J Morphol Embryol. 2010;51(4):655-61.
24. Lazăr D, Tăban S, Dema A et al, Gastric cancer: the correlation between the clinicopathological factors and patients' survival (I). Rom J Morphol Embryol. 2009;50(1):41-50.
25. Lazăr D, Tăban S, Sporea I et al, Gastric cancer: correlation between clinicopathological factors and survival of patients. II. Rom J Morphol Embryol. 2009;50(2):185-94.
26. Lazăr D, Tăban S, Sporea I et al, Gastric cancer: correlation between clinicopathological factors and survival of patients (III). Rom J Morphol Embryol. 2009;50(3):369-79.

