Original Article

DOI: 10.7860/NJLM/2026/85339.2976

Pathology Section

Prognostic Significance of Bcl-2 and Ki-67 Expression in Gastric Carcinoma: A Cross-sectional Study at a Tertiary Care Centre in Chennai, Tamil Nadu, India

S MALLIGA[1], J KARTHIK[2], S BALAJI[3]

Introduction: Gastric cancer was at fifth place in both incidence and mortality worldwide in 2022. The B-cell lymphoma (Bcl-2) anti-apoptotic gene is aberrantly expressed in a sequential manner in the carcinogenesis of gastric cancer. Ki-67 is correlated well with poorer survival and high level of expression seen in the advanced stage of gastric cancer. Biological heterogeneity and variable prognosis seen in gastric cancer are not fully explained by conventional histopathological parameters. So, the routine evaluation of Bcl-2 and Ki-67 could be helpful in identifying more aggressive disease and contribute to a better therapeutic approach.

Aim: To evaluate the Immunohistochemical (IHC) expression of Bcl-2 and Ki-67 and analyse their association with clinicopathological parameters including patient's age, gender, tumour size, tumour site, gross, Lauren's classification, World Health Organisation (WHO) histological subtypes, depth of invasion, grade, lymph node metastasis and Tumour-Node-Metastasis (TNM) staging system in gastric adenocarcinoma cases.

Materials and Methods: This cross-sectional study was conducted at the Department of Pathology, Government Kilpauk Medical College, Chennai, Tamil Nadu, India, over a period of 24 months, from April 2019 to March 2021. It included 50 surgically resected gastric adenocarcinoma cases. Histopathological evaluation and IHC staining for Bcl-2 and Ki-67 were performed. Data collected were entered in MS Excel and analysed using Statistical Package for the Social Sciences (SPSS) version 20.0. Associations between marker expression and clinicopathological parameters like patient age, gender, tumour size, tumour site, gross appearance, Lauren's classification and WHO histological subtypes, other prognostic factors were analysed using Chi-square test, and Independent sample t-test, with p-value <0.05 considered statistically significant.

Results: In the present study, the age group showing peak incidence of gastric carcinoma was 51 to 60 years with a mean age of 59.7 years. Bcl-2 positivity was observed in 29 cases (58%) and showed significant association with higher tumour grade (p=0.031) and tumour stage (p=0.019). High Ki-67 Labelling Index (LI) (≥20%) was seen in 30 cases (60%) and was significantly associated with female gender (p=0.002), tumour grade (p<0.001), depth of invasion (p=0.040), lymph node metastasis (p=0.049), and tumour stage (p=0.013). The mean Ki-67 LI was significantly higher in Bcl-2-positive tumours (p=0.014).

Conclusion: Present study documented higher expression of Bcl-2 and high Ki-67 LI in advanced tumour grade and higher American Joint Committee on Cancer (AJCC) prognostic stage which may suggest a potential association with tumour aggressiveness. Bcl-2 and Ki-67 were found to be helpful in prognosis, management, and for development of newer targeted therapy.

Keywords: Apoptosis, B-cell lymphoma protein, Gastric cancer, Immunohistochemistry, Ki-67 antigen, Prognostic factors

INTRODUCTION

Gastric adenocarcinoma is the most common type of malignancy arising from the glandular lining epithelium of gastric mucosa. Gastric adenocarcinoma is the most common malignancy arising from the glandular epithelium of the gastric mucosa. Gastric cancer remains a major global health burden and accounted for approximately 968,350 newly diagnosed cases (4.9% of all cancers) and 659,853 cancer-related deaths (6.8% of all cancer-related deaths) worldwide in 2022 [1]. There is wide variation in incidence in different countries globally. It accounts for 18% of all malignancies in Asia. In Indian population, gastric cancer was the 7th most common cancer with 64,611 new cases [1]. Incidence has been observed to be higher in the southern and north-eastern parts of India [2,3].

Gastric cancer shows marked variation in age and is more frequently diagnosed in elderly patients with higher incidence around 65 years. It is more common in males than in females with the ratio of 2:1. It is more common in lower socioeconomic groups and in individuals with mucosal atrophy, intestinal metaplasia and various predisposing factors [4]. Dietary factors and infection with Gram-negative bacterium *Helicobacter pylori* have been documented as major risk factors for tumours in the distal stomach, whereas, Gastroesophageal Reflux Disease (GERD) and obesity have been identified as major risk factors for tumours in the proximal stomach [5]. There are two histological types namely, intestinal and diffuse type in Lauren's classification system of gastric cancers. The intestinal type is more common in men and in the older age groups and it has a good prognosis. Gastric atrophy and intestinal metaplasia are the common preceding lesions in the intestinal type. The diffuse type, on the other hand, is commoner in endemic areas, in women and younger patients, and shows an association with blood Group A [6,7]. Presenting at advanced stage of cancer, presence of non specific symptoms like dyspepsia in early stage of cancer and limited options in management makes the prognosis of gastric adenocarcinoma poor. Multiple alterations at gene level are known to occur in oncogenes, tumour suppressor genes, cell adhesion molecules and cell cycle regulators are implicated in the multistep oncogenesis of gastric cancer. The IHC protein expression of Bcl-2 and Ki-67 and various markers has been proposed as a potential tool for the evaluation of the biological behaviour of gastric cancer [8].

The Bcl-2 gene was first identified in humans, in association with the t (14;18) in most cases of follicular lymphoma [9]. This gene promotes

the survival of cytokine dependent haematopoietic cells even in the absence of cytokine. It is a proto-oncogene that inhibits programmed cell death (apoptosis) and encodes a 26-kDa mitochondrial protein [10]. Apoptosis is regulated by more than 20 genes of Bcl family. The main anti-apoptotic proteins are Bcl-2, Bcl-x, and Mcl-1 and the pro-apoptotic proteins are Bax, Bak and Bid. The net result of anti-apoptotic and pro-apoptotic protein groups depends on the dominance of one of them [11]. Bcl-2 prevents the release of mitochondrial proteins by controlling the permeability. Bcl-2 protein protects the renewal potential of the gastrointestinal tract mucosa and so, expressed in the proliferative zone. In the carcinogenesis of gastric cancer, Bcl-2 is aberrantly expressed in a sequential manner. It also expressed in most cases of atrophic gastritis with intestinal metaplasia and in some cases with epithelial dysplasia [12]. Aberrant Bcl-2 expression has been identified as an important factor in the biological behaviour of gastric cancer. Its increased expression in gastric cancer might results in the development of tumour resistance to the apoptotic effect of chemoradiotherapy drugs [13]. Zhang Y et al., in a study showed that MAPK/c-Jun signaling pathway may play a role in upregulation of the anti-apoptoticproteins Bcl-2 and Bcl-xL in gastric carcinoma cells, which is induced by Epstein-Barr virus-encoded BamHI-A Rightward Frame 1 (BARF1) [14].

Uncontrolled cell proliferation not responding to the growth control mechanism is considered to be an important step in carcinogenesis. Ki-67, a nuclear protein is a marker of cell proliferation usually expressed in all active phases of the cell cycle from G1, S, G2 to M phase but not the G0 phase. Ki-67 is a large non histone protein that is coded by the gene Marker of Proliferation Ki-67 (MKI67). Ki-67 is discovered in Kiel city, Germany. Ki-67 is mainly involved in the regulation and maintenance of cell division. Mindbomb Homology-1 antibody clone (MIB-1) is the monoclonal antibody acts against different nuclear antigen epitopes of dividing cell. Proliferative activity of tumour cells is equivalent to the aggressiveness of the tumour. So, the behaviour of the tumour can be assessed by Ki-67 LI which is the fraction of Ki-67-positive tumour cells [15]. Ki-67 expression is well-correlated with poorer survival, high level of expression seen in advanced stage of the gastric cancer.

Among the studies conducted in gastric carcinoma all over the world for the expression of Bcl-2 and their prognostic significance, some results are inconclusive and some show statistically significant association [16-20]. The routine evaluation of Bcl-2 and Ki -67 could be helpful in identifying more aggressive disease and contribute to a better therapeutic approach. In the present study, of 50 cases, an attempt is made to study the prevalence of IHC expression of Bcl-2 and Ki-67% proliferative activity in gastric adenocarcinoma in Southern Indian Population, and their association with various clinicopathological variables such as age, gender, tumour size, histological type, grade, depth of infiltration, AJCC prognostic stage, lymph node status, lymphovascular invasion, perineural invasion and necrosis. Association between Bcl-2 and Ki-67 expression is assessed to evaluate the interplay between anti-apoptosis and proliferative activity in gastric carcinoma, as their combined expression may better reflect tumour aggressiveness than either marker alone.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology, Government Kilpauk Medical College, Chennai, Tamil Nadu, India, over a period of 24 months, from April 2019 to March 2021. All samples were collected with patient consent and approval of the Institutional Ethical Committee (IECNo.147/2019 dt. 22.01.2019). A total of 50 gastrectomy samples received during the study period were included in this study. Detailed history of gastric carcinoma patients regarding age, gender, clinical history and type of surgery was obtained from registers and surgical case records.

Sample size calculation: The sample size was calculated using the standard formula for cross-sectional studies:

$n = Z^2 \times p \times q / L^2$

Expected prevalence of 50% for immunohistochemical marker expression, a confidence level of 95%, and an allowable error of 14%, the minimum required sample size was calculated to be 49 cases. Accordingly, a total of 50 cases were included in the study. It is comparable to previously published single-centre IHC study evaluating Bcl-2 and Ki-67 expression in gastric carcinoma [20].

Inclusion criteria: Surgically resected tumour specimens (including total gastrectomy, subtotal gastrectomy, partial gastrectomy) of gastric adenocarcinoma patients were included in this study.

Exclusion criteria: Patients who had received neo-adjuvant therapy, endoscopic small biopsy specimens, early gastric adenocarcinomas and gastric neoplasms other than adenocarcinoma like, squamous cell carcinoma, neuroendocrine tumours, gastric lymphomas, mesenchymal neoplasms and metastatic tumours were excluded from the study.

Study Procedure

On receiving the specimen, a systematic gross examination was performed, and tumour tissues were routinely processed and 4 µm thick sections were made. For all gastrectomy cases, Haematoxylin and Eosin (H&E) stained sections were examined. The important clinicopathological parameters evaluated were: age (<55 years and ≥55 years), gender of the patient, tumour size (<5 cm and ≥5 cm), tumour site (Cardia, body, antrum or fundus), Gross appearance (Borrmann Types I to IV), Lauren's classification and WHO histological subtypes (tubular, papillary, mucinous, poorly cohesive and signet ring cell) [6,21]. Also, other prognostic factors like the depth of invasion (T1 to T4), grade of the carcinomas (G1 to G3), Lymph node metastasis and TNM staging of gastric cancer based on AJCC 8th edition and the WHO classification were evaluated [21,22]. All cases were further evaluated for the lympho-vascular invasion, perineural invasion, necrosis and lymphocytic response.

Immuohistochemical examination of markers Bcl-2 and Ki-67 were done in Formalin Fixed Paraffin Embedded (FFPE) tissue samples of all cases using a technique called Super-sensitive polymer Horse Radish Peroxidase (HRP) system which is based on non biotin polymeric methods. The sections from paraffin blocks were transferred onto gelatin coated slides. Antigen retrieval by heat method was done. The retrieved antigen was bound with mouse monoclonal antibody against Bcl-2 and Ki-67 protein. Immunohistochemical staining was performed using ready-to-use mouse monoclonal antibodies against Ki-67 (clone MIB-1, IgG1; PathnSitu Biotechnologies, USA) and Bcl-2 (clone 124; PathnSitu Biotechnologies, USA), both validated for formalin-fixed paraffin-embedded tissues, strictly following the manufacturer's recommended protocol. Appropriate positive and negative controls were included in each staining run. Secondary antibody which is conjugated with HRP-polymer and diaminobenzidine used to detect antigen-antibody reaction. Immunohistochemical staining of Bcl-2 and Ki-67 was assessed based on staining intensity and the percentage of cells taking up the stain. Cytoplasmic staining in case of Bcl-2 and nuclear staining in case of Ki-67 was considered as positive. Bcl-2 immunoreactivity was categorised into two groups: positive expression - (at least 1% positive tumour cells) and negative expression- (0% positive tumour cells) [20]. The Ki-67 LI was estimated by observing positive tumour cells among 1000 tumour cells and given as percentage [23]. The Ki -67 LI of 20% was chosen as the cut off point for categorising the cases into two groups: high (LI ≥20%) and low (LI <20%) Ki-67 LI [24].

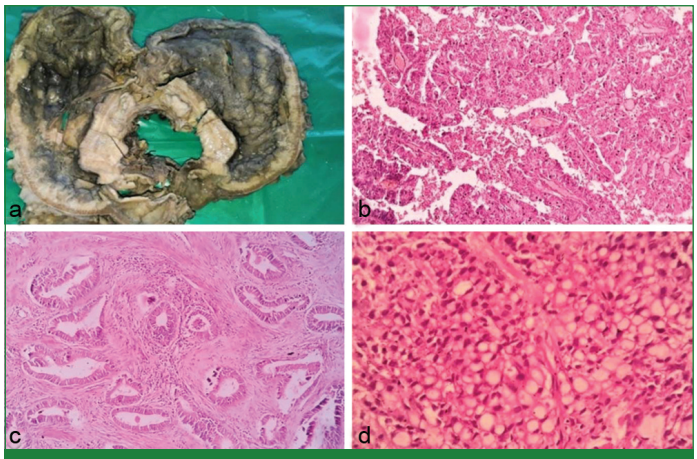
STATISTICAL ANALYSIS

The various data collected were entered in MS Excel and analysed using Statistical Package for the Social Sciences (SPSS) version 20.0. Associations between immunohistochemical expression of Bcl-2 and Ki-67 LI with clinicopathological parameters were assessed using Pearson's Chi-square test or Fisher's-exact test

where appropriate. The association between Bcl-2 expression and Ki-67 LI was assessed using Fisher's-exact test. Comparison of mean Ki-67 LI between Bcl-2 positive and negative groups was performed using an Independent samples t-test. A p-value<0.05 was considered statistically significant.

RESULTS

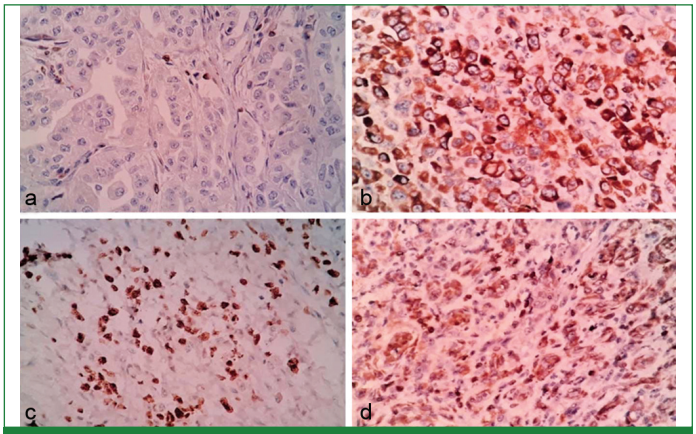
In the current study, gastric carcinoma had a peak incidence in the age group of 51-60 years with the age ranged from 30 years to 87 years. Among the 50 cases, males were 32 (64%) and females were 18 (36%). Tumour size is less than 5 cm in 22 cases (44%) and 5 cm or more in 28 cases (56%). The most common site of gastric cancer was at the pyloro-antrum which constituted about 35 (70%) cases. The most common gross type was Borrmann type-II, which accounted for 29 (58%) cases [Table/Fig-1a]. The most common Lauren's histological subtype was Intestinal carcinoma which accounted for 45 (90%) cases. The most common histological type was tubular carcinoma, which accounted for 37 (74%) cases, and there were one case of papillary adenocarcinoma and two cases of signet ring cell carcinomas [Table/Fig-1b-d]. G2 (moderately differentiated grade) was the most common grade accounting for 23 (46%) of cases. Lymphnodal metastasis was noted in 35 (70%) of cases. Most of the tumours, 23 cases (46%) were presented in stage III. Seventeen cases (34%) had lympho-vascular invasion, 10 cases (20%) of the cases had perineural invasion, 32 cases (64%) had lymphocytic infiltration response and 13 cases (26%) had necrosis.



[Table/Fig-1]: Gross and microscopic features of gastric adenocarcinoma: a) Ulceroproliferative growth in total gastrectomy specimen; b) Papillary adenocarcinoma (H&E, 40X); c) Tubular adenocarcinoma (H&E, 10X); d) Signet-ring cell carcinoma (H&E, 40x).

In the current study, 29 cases (58%) showed Bcl-2 positive expression and 21 cases (42%) were Bcl-2 negative [Table/Fig-2a,b]. Association between Bcl-2 expression and various clinicopathological characteristics of gastric carcinomas has been illustrated in [Table/Fig-3]. Bcl-2 expression showed statistically significant association with higher tumour grade (p=0.031), higher AJCC prognostic tumour stage (p=0.019), lympho-vascular invasion (p=0.002) and lymphocytic response (p=0.006). An increase in the number of cases with Bcl-2 positivity was seen with increasing depth of infiltration, mucinous carcinoma and diffuse type carcinomas.

High Ki-67 LI was seen in 30 (60%) cases and low LI in 20 (40%) cases [Table/Fig-2c,d]. The mean Ki-67 LI was 23.5%. Association between Ki-67 LI and clinicopathological variables of gastric carcinomas has been illustrated in [Table/Fig-4]. There was a direct statistically significant association between Ki-67 LI and gender with slight female predominance (p=0.002), depth of invasion (p=0.040), higher tumour grade (p<0.001), advanced lymph node metastasis (p=0.049) and higher tumour stage (p=0.013). Higher Ki-67 LI was found in poorly cohesive and signet ring cell carcinomas. A statistically significant association was observed between Bcl-2 expression and Ki-67 LI (p=0.045), with Bcl-2-positive tumours showing a significantly higher proportion of high Ki-67 LI compared to Bcl-2-negative tumours [Table/Fig-5]. The mean Ki-67 LI value was significantly higher in Bcl-2 positive tumours when compared to the LI value in Bcl-2 negative tumours and p-value is 0.014 which is statistically significant [Table/Fig-6].



[Table/Fig-2]: Immuohistochemical expression of Bcl-2 and Ki-67 in Gastric adenocarcinoma: a) Negative Bcl-2 expression (IHC, 40X); b) Positive Bcl-2 expression (IHC, 40X); c) Ki-67 low labelling index (IHC, 40X); d) Ki-67 high labelling index (IHC, 40X).

Characteristics	Presence of Bcl-2 expression (n=29)	Absence of Bcl-2 expression (n=21)	p-value
Age			
<55 years	10 (66.7%)	5 (33.3%)	p=0.416
>55 years	19 (54.3%)	16 (45.7%)	
Gender			
Male	18 (56.2%)	14 (43.8%)	p=0.738
Female	11 (61.1%)	7 (38.9%)	
Site*			
Pyloro-antrum	19 (54.3%)	16 (45.7%)	p=0.449
Body	8 (72.7%)	3 (27.3%)	
Cardia	1 (33.3%)	2 (66.7%)	
Fundus	1 (100%)	0	
Size			
<5 cm	12 (54.5%)	10 (45.5%)	p=0.661
≥5 cm	17 (60.7%)	11 (39.3%)	
Gross-borrmann classification*			
Type I	0	2 (100%)	p=0.381
Type II	18 (62.1%)	11 (37.9%)	
Type III	9 (56.2%)	7 (43.8%)	
Type IV	2 (66.7%)	1 (33.3%)	
Lauren's type			
Intestinal	26 (57.8%)	19 (42.2%)	p=0.924
Diffuse	3 (60%)	2 (40%)	
Histological type*			
Tubular	21 (56.8%)	16 (43.2%)	p=0.723
Mucinous	5 (71.4%)	2 (28.6%)	
Poorly cohesive	2 (66.7%)	1 (33.3%)	
Signet ring cell	1 (50%)	1 (50%)	
Papillary	0	1 (100%)	
Grade			
G1	5 (31.2%)	11 (68.8%)	p=0.031
G2	16 (69.6%)	7 (30.4%)	
G3	8 (72.7%)	3 (27.3%)	
T Stage			
T1	0	3 (100%)	p=0.057
T2	6 (42.9%)	8 (57.1%)	
T3	14 (66.7%)	7 (33.3%)	
T4	9 (75%)	3 (25%)	

N stage			
N0	6 (40%)	9 (60%)	p=0.138
N1	7 (50%)	7 (50%)	
N2	7 (70%)	3 (30%)	
N3	9 (81.8%)	2 (18.2%)	
AJCC prognostic stage			
I	3 (30%)	7 (70%)	p=0.019
II	8 (47.1%)	9 (52.9%)	
III	18 (78.3%)	5 (21.7%)	
Lymphovascular invasion*			
Present	15 (88.2%)	2 (11.8%)	p=0.002
Absent	14 (42.4%)	19 (57.6%)	
Perineural invasion			
Present	7 (70%)	3 (30%)	p=0.390
Absent	22 (55%)	18 (45%)	
Lymphocytic response*			
Present	14 (43.8%)	18 (56.2%)	p=0.006
Absent	15 (83.3%)	3 (16.7%)	
Necrosis			
Present	9 (69.2%)	4 (30.8%)	p=0.340
Absent	20 (54.1%)	17 (45.9%)	

[Table/Fig-3]: Association between clinicopathological characteristics of gastric carcinomas and Bcl-2 expression.
Statistical analysis: Associations were assessed using the Chi-square test or Fisher's-exact test, as appropriate. Fisher's-exact test* was applied for site, gross-borrmann classification, histological type, lymphovascular invasion, and lymphocytic response due to small expected cell counts.

Characteristics	Ki-67 High LI (%) (N=30)	Ki-67 Low LI (%) (N=20)	p-value
Age			
<55 years	7 (46.7%)	8 (63.3%)	p=0.577
>55 years	23 (65.7%)	12 (34.3%)	
Gender			
Male	14 (43.8%)	18 (56.2%)	p=0.002
Female	16 (88.9%)	2 (11.1%)	
Site*			
Pyloro-antrum	20 (57.1%)	15 (42.9%)	p=0.825
Body	7 (63.6%)	4 (36.4%)	
Cardia	2 (66.7%)	1 (33.3%)	
Fundus	1 (100%)	0	
Size			
<5 cm	12 (54.5%)	10 (45.5%)	p=0.485
>=5 cm	18 (64.2%)	10 (35.8%)	
Gross-borrmann classification*			
Type I	0	2 (100%)	p=0.167
Type II	17 (58.6%)	12 (41.4%)	
Type III	10 (62.5%)	6 (37.5%)	
Type IV	3 (100%)	0	
Lauren's type			
Intestinal	25 (55.6%)	20 (44.4%)	p=0.054
Diffuse	5 (100%)	0	
Histological type*			
Tubular	22 (59.5%)	15 (40.5%)	p=0.223
Mucinous	3 (42.9%)	4 (57.1%)	
Poorly cohesive	3 (100%)	0	
Signet ring cell	2 (100%)	0	
Papillary	0	1 (100%)	
Grade			
G1	2 (12.5%)	14 (87.5%)	p<0.001
G2	18 (78.3%)	5 (21.7%)	
G3	10 (90.9%)	1 (9.1%)	
T stage			
T1	2 (66.7%)	1 (33.3%)	p=0.040
T2	4 (28.6%)	10 (71.4%)	
T3	16 (76.2%)	5 (23.8%)	
T4	8 (66.7%)	4 (33.3%)	
N stage			
N0	6 (40%)	9 (60%)	p=0.049
N1	7 (50%)	7 (50%)	
N2	7 (70%)	3 (30%)	
N3	10 (90.9%)	1 (9.1%)	
AJCC prognostic stage			
I	2 (20%)	8 (80%)	p=0.013
II	11 (64.7%)	6 (35.3%)	
III	17 (73.9%)	6 (26.1%)	
Lymphovascular invasion*			
Present	12 (70.6%)	5 (29.4%)	p=0.273
Absent	18 (54.5%)	15 (45.5%)	
Perineural invasion			
Present	7 (70%)	3 (30%)	p=0.470
Absent	23 (57.5%)	17 (42.5%)	
Lymphocytic response*			
Present	17 (53.1%)	15 (46.9%)	p=0.186
Absent	13 (72.2%)	5 (27.8%)	
Necrosis			
Present	8 (61.5%)	5 (38.5%)	p=0.895
Absent	22 (59.5%)	15 (40.5%)	

[Table/Fig-4]: Association between clinicopathological characteristics of gastric carcinomas and Ki-67 LI.
Statistical analysis: Associations were assessed using the Chi-square test or Fisher's-Exact test, as appropriate. Fisher's-Exact test* was applied for site, gross-borrmann classification, histological type, lymphovascular invasion, and lymphocytic response due to small expected cell counts.

Bcl-2 protein expression	Ki-67 Labelling Index (LI) n (%)		Fisher's-exact test
	Low	High	
Negative	12 (57.1%)	9 (42.9%)	0.045
Positive	8 (27.6%)	21 (72.4%)	
Total	20 (40%)	30 (60%)	

[Table/Fig-5]: Association between Ki-67 LI and Bcl-2 expression.
Statistical analysis: Association was assessed using the Fisher's-Exact test.

Bcl-2 protein expression	No. of cases	Mean Ki-67 Labelling Index (LI)	Standard deviation	t-test of equality of means
Positive	29	27.76	14.954	p=0.014
Negative	21	17.62	12.237	

[Table/Fig-6]: Mean Ki-67 LI in Bcl-2 positive and negative gastric tumours.
Statistical analysis: Independent samples t-test was used to compare mean Ki-67 LI between Bcl-2 positive and negative groups.

DISCUSSION

Gastric cancer is one of the life-threatening non communicable diseases in both developed and developing countries. In the present study, the age group showing peak incidence of gastric carcinoma was 51 to 60 years with a mean age of 59.7 years. This is correlated with the study done by Joo YE et al., [25]. There was a significant predominance of gastric cancers seen in men (64%) than women (36%), which is similar to study done by Igarashi N et al., [26]. The most common site of gastric cancer in this study was the pyloro-antrum (70%), similar to the studies of Tzanakis NE et al., and Lazar D et al., [27,28]. The most common histological subtype of gastric cancer in the present study is tubular carcinoma (74%).

This is almost similar to the study of Lazar D et al., (45.9%) [28]. The most common Lauren's type in the current study was the Intestinal type (90%). This is similar to observations made by Czyzewska J et al., (75.5%), Lazar D et al., (72.1%) and Valerdiz Casasola S et al., (81.9%) [28-30]. In the present study, the G2 tumours were more common than the other grades accounting for 46% of cases. This was in concurrence with the study conducted by Valerdiz Casasola S et al., (74.6%) [30]. A higher proportion of T3 tumours (42%), closely followed by T2 tumours (28%) was observed in the present study, similar to the studies of Joo YE et al., and de Manzoni G et al., [25,31]. Most of the cases presented in stage III (46%) followed by stage II (34%) in this study. This observation was similar to the study done by Lazar D et al., [28].

In our study, Bcl-2 expression was seen in 58% of cases. Kopp R et al., demonstrated statistically significant association between Bcl-2 immunoreactivity and the tumours of the intestinal type [16]. Zafirellis K et al., study showed Bcl-2 expression in 23.1% of gastric cancers and inverse association with lymph node metastasis and tumour grade [17]. Lauwers GY et al., documented 72% of Bcl-2 expression and it was significantly associated with the intestinal type of adenocarcinoma [18]. He observed increasing prevalence of Bcl-2 with higher histologic grades. Gryko M et al., showed 55.7% of Bcl-2 expression and significant association with higher tumour stage, with the intestinal type of Lauren's classification, Bormann's classification type 2 and with local lymph node metastases [19]. Bcl-2 expression of 67% with increased expression in low-grade and early-stage gastric adenocarcinomas is observed in study done by Tsamandas AC et al., [20]. Liu HF et al., found Bcl-2 expression to be significantly associated with tumour size, Lauren's classification and AJCC prognostic stage [32]. Banerjee R et al., found Bcl-2 expression significantly associated with gender, tumour size, Borrmann type, Lauren type, histological tumour type, tumour grade, lymphovascular invasion, pathological tumour stage and AJCC prognostic tumour stage [33].

Some studies showed higher expression of Bcl-2 in early forms of cancer, and other studies found no differences. In our study, we found significant association between tumour grade and AJCC prognostic tumour stage with Bcl-2 expression. This observation is similar to study done by Mariusz Gryko et al., [19]. The underlying reason for this is increased survival capacity of tumour cells associated with high expression of antiapoptotic Bcl-2 or by possible changes in the protein profile during the stages of cancer growth. Bcl-2 expression in association with lymphovascular invasion and lymphocytic response was found to be statistically significant. Tumour with higher T stage (depth of invasion) showed significantly increased percentage of Bcl-2 expression. The present study showed no statistically significant association between Bcl-2 expression and patient age, gender, tumour site, Borrmann gross type, tumour size, histological type, Lauren's type, lymph node metastasis, perineural infiltration and necrosis.

Proliferative activity of tumour cells can be assessed by Ki-67 LI. Ki-67 expression is correlated with a poorer survival rate. Nobuyuki Igarashi et al., found significantly higher levels of Ki-67 LI in the advanced gastric cancer group compared to the early gastric cancer group [26]. Valerdiz Casasola S et al., study showed that Ki-67 was an independent significant prognostic factor and correlated well with the length of survival [30]. Czyzewska J et al., found a statistically significant association between Ki-67 LI and histological type, presence of lymph node metastasis and tumour differentiation [29]. Joo YE et al., and de Manzoni G et al., found no association between Ki-67 LI and tumour size, depth of invasion, lymph node metastasis, stage and survival [25,31]. Tzanakis NE et al., found significant association between the Ki-67 LI and lymph node metastasis and stage [27]. Lazar D et al., demonstrated a statistically significant association between Ki-67 LI and age, tumour site, histological type and grade [28].

In the present study, there was a direct statistically significant association between Ki-67 LI and gender with slight female predominance, depth of invasion, higher tumour grade, lymph node metastasis and higher tumour stage. Higher Ki-67 LI was found in poorly cohesive and signet ring cell carcinomas, and low LI was seen in tubular and mucinous carcinomas in concurrence with the prognosis of these histological types. In comparison to the above studies, no significant association between Ki-67 LI and age, tumour site, size, gross type, Lauren's type, histological type, lymphovascular invasion, perineural infiltration, lymphocytic response and necrosis was observed.

However, the present study revealed that the mean Ki-67 LI value in Bcl-2 positive tumours (27.76) was significantly (p=0.014) higher than that of Bcl-2 negative tumours (17.62). Bcl-2-positive tumours showing a significantly (p=0.045) higher proportion of high Ki-67 LI compared to Bcl-2-negative tumours. This finding suggests that anti-apoptotic activity frequently coexists with increased proliferative activity in gastric carcinoma, supporting a combined role of apoptosis resistance and enhanced proliferation in tumour progression. Variations in the prognostic significance of Bcl-2 and Ki-67 reported across studies may be explained by differences in patient demographics, tumour behaviour, methodology, and cut-off values.

Limitation(s)

The present study has certain limitations, including a single Institutional and cross-sectional study design, which may restrict generalisability of the findings. Exclusion of patients who received neoadjuvant therapy may limit applicability to routine clinical practice. Additionally, assessment was confined to two IHC markers, and semi-quantitative scoring with fixed cut-off values may introduce observer variability and may not fully reflect tumour biological heterogeneity. Survival outcomes and treatment response were not evaluated due to lack of follow-up data.

CONCLUSION(S)

The present study helped us to know the trends and expression of Bcl-2 and Ki-67 in gastric carcinoma in the Southern Indian population. Emerging markers provide us insight into the molecular basis of oncogenesis, which helps in early diagnosis, targeted therapy, reducing the disease burden and improving the outcome. The current study showed statistically significant higher expression of Bcl-2 and high Ki-67 LI in higher tumour grade and AJCC prognostic stage. This might explain the prognostic significance of these markers in gastric carcinoma. The routine evaluation of these markers could be helpful in prognosis, management and for the development of newer targeted therapy in the future.

REFERENCES

[1] Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-63. doi: 10.3322/caac.21834. Epub 2024 Apr 4. PMID: 38572751.

[2] Dikshit RP, Mathur G, Mhatre S, Yeole BB. Epidemiological review of gastric cancer in India. Indian J Med Paediatr Oncol. 2011;32(1):3-11. doi: 10.4103/0971-5851.81883. PMID: 21731209; PMCID: PMC3124986.

[3] Sharma A, Radhakrishnan V. Gastric cancer in India. Indian J Med Paediatr Oncol. 2011;32(1):12-16. doi: 10.4103/0971-5851.81884. PMID: 21731210; PMCID: PMC3124983.

[4] Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP. Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer Manag Res. 2018;10:239-48. Doi: 10.2147/CMAR.S149619. PMID: 29445300; PMCID: PMC5808709.

[5] Crew KD, Neugut AI. Epidemiology of gastric cancer. World J Gastroenterol. 2006;12(3):354-62. doi: 10.3748/wjg.v12.i3.354. PMID: 16489633; PMCID: PMC4066052.

[6] Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma: an attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965;64:31-49. doi: 10.1111/apm.1965.64.1.31. PMID: 14320675.

[7] Corso G, Seruca R, Roviello F. Gastric cancer carcinogenesis and tumor progression. Ann Ital Chir. 2012;83(3):172-76. PMID: 22595727.

[8] Zolota V, Batistatou A, Tsamandas AC, Iliopoulos G, Scopa CD, Bonikos DS. Immunohistochemical expression of TGF-beta1, p21WAF1, p53, Ki67, and angiogenesis in gastric carcinomas: a clinicopathologic study. Int J Gastrointest Cancer. 2002;32(2-3):83-89. doi: 10.1385/IJGC:32:2-3:83. PMID: 12794244.
[9] Scopa CD, Vagianos C, Kardamakis D, Kourelis TG, Kalofonos HP, Tsamandas AC. bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with rectal cancer. Appl Immunohistochem Mol Morphol. 2001;9(4):329-34. doi: 10.1097/00129039-200112000-00007. PMID: 11759059.
[10] Korsmeyer SJ. Bcl-2 initiates a new category of oncogenes: regulators of cell death. Blood. 1992;80(4):879-86. PMID: 1498330.
[11] Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell. 2005;122(6):927-39. doi: 10.1016/j.cell.2005.07.002. PMID: 16179260.
[12] Lauwers GY, Scott GV, Hendricks J. Immunohistochemical evidence of aberrant bcl-2 protein expression in gastric epithelial dysplasia. Cancer. 1994;73(12):2900-04. doi: 10.1002/1097-0142(19940615)73:12<2900::aid-cncr2820731205>3.0.co;2-0. PMID: 8199986.
[13] Lee HK, Lee HS, Yang HK, Kim WH, Lee KU, Choe KJ, et al. Prognostic significance of Bcl-2 and p53 expression in gastric cancer. Int J Colorectal Dis. 2003;18(6):518-25. doi: 10.1007/s00384-003-0491-2. Epub 2003 Jun 13. PMID: 12811476.
[14] Zhang Y, Xu M, Zhang X, Chu F, Zhou T. MAPK/c-Jun signaling pathway contributes to the upregulation of the anti-apoptotic proteins Bcl-2 and Bcl-xL induced by Epstein-Barr virus-encoded BARF1 in gastric carcinoma cells. Oncol Lett. 2018;15(5):7537-44. doi: 10.3892/ol.2018.8293. Epub 2018 Mar 19. PMID: 29725459; PMCID: PMC5920478.
[15] Krüger S, Müller H. Association of morphometry, nucleolar organizer regions, proliferating cell nuclear antigen and Ki67 antigen expression with grading and staging in urinary bladder carcinomas. Br J Urol. 1995;75(4):480-84. doi: 10.1111/j.1464-410x.1995.tb07269.x. PMID: 7788260.
[16] Kopp R, Diebold J, Dreier I, Cramer C, Glas J, Baretton G, et al. Prognostic relevance of p53 and bcl-2 immunoreactivity for early invasive pT1/pT2 gastric carcinomas: indicators for limited gastric resections? Surg Endosc. 2005;19(11):1507-12. doi: 10.1007/s00464-005-0043-7. Epub 2005 Sep 21. PMID: 16177872.
[17] Zafirellis K, Karameris A, Milingos N, Androulakis G. Molecular markers in gastric cancer: can p53 and bcl-2 protein expressions be used as prognostic factors? Anticancer Res. 2005;25(5):3629-36. PMID: 16101192.
[18] Lauwers GY, Scott GV, Karpeh MS. Immunohistochemical evaluation of bcl-2 protein expression in gastric adenocarcinomas. Cancer. 1995;75(9):2209-13. doi: 10.1002/1097-0142(19950501)75:9<2209::aid-cncr2820750904>3.0.co;2-m. PMID: 7712429.
[19] Gryko M, Pryczynicz A, Zareba K, Kędra B, Kemona A, Guzińska-Ustymowicz K. The expression of Bcl-2 and BID in gastric cancer cells. J Immunol Res. 2014;2014:953203. doi: 10.1155/2014/953203.
[20] Tsamandas AC, Kardamakis D, Tsiamalos P, Liava A, Tzelepi V, Vassiliou V, et al. The potential role of Bcl-2 expression, apoptosis and cell proliferation (Ki-67 expression) in cases of gastric carcinoma and Association with classic prognostic factors and patient outcome. Anticancer Res. 2009;29(2):703-09. PMID: 19331225.
[21] Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020;76(2):182-88. doi: 10.1111/his.13975. Epub 2019 Nov 13. PMID: 31433515; PMCID: PMC7003895.
[22] Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin. 2017;67(2):93-99. doi: 10.3322/caac.21388. Epub 2017 Jan 17. PMID: 28094848.
[23] Ko GH, Go SI, Lee WS, Lee JH, Jeong SH, Lee YJ, et al. Prognostic impact of Ki-67 in patients with gastric cancer-the importance of depth of invasion and histologic differentiation. Medicine (Baltimore). 2017;96(25):e7181. doi: 10.1097/MD.0000000000007181. PMID: 28640099; PMCID: PMC5484207.
[24] Pyo JS, Kim NY. Meta-analysis of prognostic role of Ki-67 labeling index in gastric carcinoma. Int J Biol Markers. 2017;32(4):e447-e453. doi: 10.5301/ijbm.5000277. PMID: 28561880.
[25] Joo YE, Chung IJ, Park YK, Koh YS, Lee JH, Park CH, et al. Expression of cyclooxygenase-2, p53 and Ki-67 in gastric cancer. J Korean Med Sci. 2006;21(5):871-76. doi: 10.3346/jkms.2006.21.5.871. PMID: 17043422; PMCID: PMC2721998.
[26] Igarashi N, Takahashi M, Ohkubo H, Omata K, Iida R, Fujimoto S. Predictive value of Ki-67, p53 protein, and DNA content in the diagnosis of gastric carcinoma. Cancer. 1999;86(8):1449-54. doi: 10.1002/(sici)1097-0142(19991015)86:8<1449::aid-cncr10>3.0.co;2-d. PMID: 10526272.
[27] Tzanakis NE, Peros G, Karakitsos P, Giannopoulos GA, Efstathiou SP, Rallis G, et al. Prognostic significance of p53 and Ki67 proteins expression in Greek gastric cancer patients. Acta Chir Belg. 2009;109(5):606-11. doi: 10.1080/00015458.2009.11680496. PMID: 19994803.
[28] Lazăr D, Tăban S, Sporea I, Dema A, Cornianu M, Lazăr E, et al. The immunohistochemical expression of the p53-protein in gastric carcinomas. Association with clinicopathological factors and survival of patients. Rom J Morphol Embryol. 2010;51(2):249-57. PMID: 20495739.
[29] Czyzewska J, Guzińska-Ustymowicz K, Lebelt A, Zalewski B, Kemona A. Evaluation of proliferating markers Ki-67, PCNA in gastric cancers. Rocz Akad Med Bialymst. 2004;49 Suppl 1:64-66. PMID: 15638377.
[30] Valerdiz Casasola S, Menéndez Colunga MJ, Aller Millán O, Martínez Rodríguez JM. Prognostic value of clinicopathologic factors Ki67, cyclin D1, cyclin D3 and CDK4 in gastric carcinoma. Oncología (Barc). 2004;27(9):537-43. doi: 10.4321/S0378-48352004000900004.
[31] de Manzoni G, Verlato G, Tomezzoli A, Guglielmi A, Pelosi G, Ricci F, et al. Study on Ki-67 immunoreactivity as a prognostic indicator in patients with advanced gastric cancer. Jpn J Clin Oncol. 1998;28(9):534-37. doi: 10.1093/jjco/28.9.534. PMID: 9793024.
[32] Liu HF, Liu WW, Fang DC, Men RP. Expression of bcl-2 protein in gastric carcinoma and its significance. World J Gastroenterol. 1998;4(3):228-30. Doi: 10.3748/wjg.v4.i3.228. PMID: 11819282; PMCID: PMC4723463.
[33] Banerjee R, Hajra S, Chakrabarti J, Nasare VD, Ghosh S. Expression of ERK1/2 and Bcl-2 in gastric carcinoma and their clinicopathological significance: an observational study in a tertiary care cancer hospital. Indian J Pathol Oncol. 2024;11(4):377-84. doi: 10.18231/j.ijpo.2024.081.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, Aarupadai Veedu Medical College and Hospital, Kirumampakkam, Vinayaka Mission's Research Foundation, Puducherry, India.
2. Assistant Professor, Department of Pathology, Sri Venkateshwaraa Medical College Hospital and Research Centre, Pondicherry University, Puducherry, India.
3. Assistant Professor, Department of Pathology, Sri Venkateshwaraa Medical College Hospital and Research Centre, Pondicherry University, Puducherry, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. J Karthik,
Assistant Professor, Department of Pathology, Sri Venkateshwaraa Medical College Hospital and Research Centre, Pondicherry University, Puducherry-605102, India.
E-mail: drkarthikjms@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 13, 2025
- Manual Googling: Feb 18, 2026
- iThenticate Software: Feb 23, 2026 (2%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Nov 12, 2025**
Date of Peer Review: **Dec 26, 2025**
Date of Acceptance: **Feb 24, 2026**
Date of Publishing: **Jul 01, 2026**