Original Article

Expression Pattern of Bcl-2 Protein in Gastric Adenocarcinoma in Relation to Histologic Type

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Abstract

Background: *Both intestinal and diffuse types of gastric adenocarcinoma exhibit Bcl-2 protein expression, which has been shown to be overexpressed in the gastric carcinogenic sequence. The purpose of this study was to evaluate the Bcl-2 protein expression in gastric adenocarcinoma in relation to its histological type.* ***Materials and Methods****: This cross-sectional observational study was conducted at the Department of Pathology, Sylhet MAG Osmani Medical College from March 2020 to January 2022. Seventy histologically diagnosed cases of gastric adenocarcinoma were selected with approval from the Institutional Review Board. Following inclusion and exclusion criteria, corresponding paraffin blocks were collected. H&E-stained sections were reexamined microscopically to confirm diagnosis and histological type. New sections were prepared from each block for Bcl-2 immunostaining, and Bcl-2 expression was evaluated microscopically in relation to histological type. Result: Gastric adenocarcinoma was more prevalent in older adults, with Bcl-2 overexpression observed in approximately 43% of cases. Most Bcl-2-positive cases were intestinal type (28 intestinal vs. 2 diffuse) and low-grade carcinomas (22 well/moderately differentiated vs. 8 poorly differentiated). No significant association was found between Bcl-2 expression and histological type (p>0.05).* ***Conclusion:*** *There was no significant association of Bcl-2 expression with histological type (p>0.05).*

Keywords: *Bcl-2 expression, adenocarcinoma, tumor type, histologic type, Bcl-2 immunostaining*

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Introduction

Stomach cancer ranks as the fifth most prevalent malignancy and accounts for 8.2% of all cancer-related fatalities, corresponding to 1 in every 12 deaths.[1] Every year, it causes the death of about 800,000 people worldwide.[2]

95% of stomach cancers are gastric adenocarcinomas, which originate from the stomach epithelium.[3] Etiologically, H. pylori

infection, dietary factors like salt and foods preserved with high salt content, personal habits like smoking and drinking alcohol, and hereditary factors are all linked to gastric cancer.[4]

It has been established that oncogenes, tumor suppressor genes, and DNA repair genes play a role in the pathophysiology of gastric cancer. In gastric epithelial intestine metaplasia and dysplasia, diffuse homogeneous Bcl-2 expression is commonly observed, which aids in the survival of these aberrant cells by allowing them to avoid the regulatory mechanism of programmed cell death. According to these data, one of the earliest stages of neoplastic transformation may involve Bcl-2 overexpression in premalignant lesions. [2]

In follicular lymphomas, the Bcl-2 protooncogene was initially identified at the (14:18) chromosomal breakpoint.[5] The 26 kd protein produced by the Bcl-2 gene prevents programmed cell death, or apoptosis. One possible way that tumor cells evade p53-mediated apoptosis is through the production of Bcl-2. 6 Bcl-2 overexpression prolongs cellular lifespan and promotes the clonal growth of cancerous cells by inhibiting normal cell turnover.[7] It is commonly recognized that Bcl-2 inhibits apoptosis, which accelerates the growth of tumors. According to some other earlier research, Bcl-2 overexpression inhibited cellular proliferation and had an inverse relationship with lymph node metastasis, tumor grade, depth of invasion, and blood vessel invasion, suggesting that Bcl-2 overexpression is linked to less aggressive biological behavior.[5,8,9]

The Lauren classification is the most practical way to categorize gastric cancer. Intestinal type gastric carcinoma and diffuse type gastric carcinoma are the two main types of gastric adenocarcinoma in this system. The tumor most likely develops at intestinal metaplasia sites in intestinal type gastric cancer. On the other hand, diffuse type gastric cancer spreads broadly over the gastric wall yet penetrates deeper into the stomach without producing visible mass lesions.[10] Although intestinal type gastric cancer is more common than diffuse type, both can exhibit Bcl-2 protein expression.[11,12,13] According to various publications, the incidence of bcl-2 immunostaining in intestinal type adenocarcinoma varies between 48.7 and 92.86%. [11,12,14]

Developing more effective prevention, diagnosis, and treatment options requires a general understanding of gastric carcinogenesis, which can be obtained by analyzing Bcl-2 expression in relation to histologic type.

Because it addresses a critical aspect of cancer biology, takes into account the inherent heterogeneity of gastric cancer, and has the potential to find important biomarkers for prognosis, therapy prediction, and a deeper understanding of disease pathogenesis, examining the expression pattern of Bcl-2 protein in gastric adenocarcinoma in relation to its histologic type is important research.

Materials and methods

This was a cross-sectional observational study conducted in the Department of Pathology, Sylhet MAG Osmani Medical College with immunostaining support from the Armed Forces Institute of Pathology (AFIP), Dhaka from March 2020 to January 2022. Prior to the commencement of this study, the thesis protocol was approved by the Institutional Ethical Committee of Sylhet M.A.G Osmani Medical College, Sylhet. Purposive sampling method was used for data collection. Tissue samples of gastric adenocarcinoma that were histologically diagnosed with endoscopic biopsy were used as study population. All the formalin-fixed paraffin-embedded tissue blocks of cases of gastric adenocarcinoma diagnosed with endoscopic biopsy tissue with the presence of adequate tumor tissue (tumor cells that invade at least in lamina propria was determined as adequacy) in the paraffin blocks for immunostaining were included in the study. Inadequate tumor tissue in paraffin blocks or poorly fixed or poorly processed tissue samples in paraffin blocks was excluded from the study. A total of 70 histologically diagnosed cases of gastric adenocarcinoma were collected for the study that fulfilled the inclusion and exclusion criteria.

As the study was conducted with formalin-fixed paraffin-embedded tissue blocks and no invasive procedure was performed in the body of the patient, so consent was not necessary. After recording relevant clinical information, a structured data collection sheet developed for the study.

Corresponding paraffin blocks and stained slides were collected from the pathology department of Sylhet MAG Osmani medical college. H&E-stained tissue sections were re-evaluated microscopically for confirmation of histological diagnosis as well as determination of histologic type and histologic grade of gastric adenocarcinoma. New sections were made from each paraffin block for immunostaining with Bcl-2.

Routine histopathological examination

H&E-stained tissue sections of each case were reviewed thoroughly to confirm the histological diagnosis of gastric adenocarcinoma. Histological type of each case was determined according to Lauren's classification. Histologic grading was performed in accordance with three tier grading systems.

Immunostaining with Bcl-2

From each paraffin block, one new section was made and stained with Bcl-2 antibody according to the Ventana Benchmark XT protocol at the immunohistochemistry laboratory of Armed Forces Institute of Pathology (AFIP), Dhaka. FLEX Monoclonal Mouse Anti-Human Bcl-2 oncoprotein Clone 124 Ready-to-use (Link) was used as primary antibody. Sections of normal Lymph node were taken as positive control.

Specimen preparation for Bcl-2 immunostaining:

From each paraffin block, 4 micrometer thick sections were cut, deparaffinized with EZ prep solution, and cell conditioning was done by using standard cell conditioner. Antigen retrieval was done by Tris-EDTA buffer.

Staining procedure of immunohistochemistry:

After antigen-retrieval, the tissue sections were stained with Bcl-2 antibody according to the Ventana Benchmark XT protocol.

Analysis of immunohistochemical staining with Bcl-2:

The expression of Bcl-2 was determined microscopically in the neoplastic cells of each case of gastric adenocarcinoma according to scoring method described by Uesugi et al., 1996. The immunoreactivity for Bcl-2 was assessed and classified into three groups as the percentage of the total number of tumor cells (Uesugi et al., 1996) as follows- None and sporadic or weak with less than 5% positive cells (-); More than5% but less than 30% strongly positive cells (1+) and more than 30% strongly positive cells (2+).

Results

In this study, histomorphological evaluation of 70 cases of gastric carcinoma according to Lauren's classification revealed that 60 (85.7%) cases were intestinal type and 10 (14.3%) cases were diffuse type (figure 2). Immunohistochemical evaluation of 70 cases of gastric carcinoma reveals that 30 (42.9%) cases were immunoreactive for Bcl-2 and 40 (57.1%) cases were nonreactive (table 1). According to scoring system, score '0' is considered as negative, score 1+ and 2+ are considered as positive. In this study, Bcl-2 was positive in 30 (42.9%) cases and negative in 40 (57.1%) cases. Bcl-2 positivity was seen as the majority in intestinal type and only 2 in diffuse type of adenocarcinoma (table 2). According to Lauren's classification, there were 60 intestinal type & 10 diffuse type of gastric adenocarcinomas. Out of 60 cases, 28 (46.7%) were positive & 32 (53.3%) were negative for Bcl-2 status. Out of 10 diffuse types of carcinomas, 2 (20%) were positive and 8 (80%) were negative. Status of Bcl-2 between the two histogical types did not show any significant association (p=0.115) (table 3)

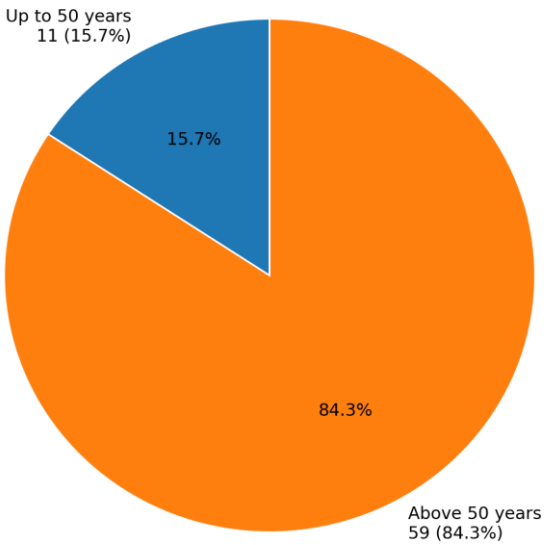

Figure 1: Distribution of study cases (n=70) by age. Results were expressed in frequency and percentage (%).

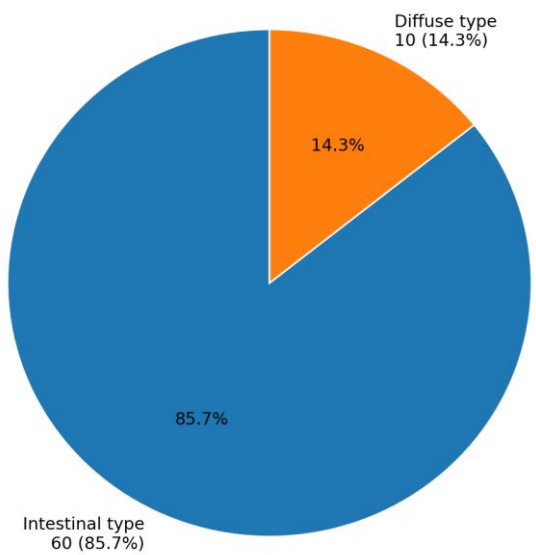

Figure 2: Distribution of the patients according to histomorphological types of gastric carcinoma (Lauren, 1965). Result were expressed in frequency and percentage (%).

Table 1: Distribution of the patients according to Bcl-2 immunoexpression

Bcl-2 immunoexpression	Frequency	Percentage (%)
Positive, ≥ 5 % Positive tumor cells	30	42.9
Negative, less than 5% Positive tumor Cells	40	57.1
Total	**70**	**100.0**

Results were expressed in frequency and percentage (%).

Table 2: Bcl-2 score of studied cases according to histologic type (Uesugi H et al., 1996)

Lauren Classification	Total number of patients	Bcl-2 IHC score 0 (-) (<5% positive stained cells)	1+ (5 to 30% positive stained cells)	2+ (>30% positive stained cells)
Intestinal type	60	32	21	7
Diffuse type	10	8	2	0
Total	**70**	**40**	**23**	**7**

Results were expressed in frequency and percentage (%).

Table 3: Distribution of the patients according to Histopathological Types of Gastric adenocarcinoma by BCL2 immunoexpression

Histopathological Types of Gastric Carcinoma	BCL2 immunoexpression		p value*
	Negative	Positive	
Diffuse	8 (80.0)%	2 (20.0)%	0.115
Intestinal	32 (53.3)%	28 (46.7)%	0.115
Total	**40 (57.1)%**	**30 (42.9)%**	

*Chi-square test was done to measure the level of significance. Figure within parenthesis indicates in percentage.

Discussion

The most prevalent type of cancer worldwide is gastric cancer. It ranks as the third most common cause of cancer-related mortality for both sexes globally. Bcl-2 overexpression has been found in solid tumors as well as hematologic malignancies. The purpose of the cross-sectional study was to assess the relationship between the histologic type and immunohistochemistry expression of the Bcl-2 protein in gastric cancer.

In the present study, the age of patients ranged from 24 to 80 years with a mean age of 58.5 years. Out of 70 cases only 11(15.7%) cases were in the age group of up to 50 years and the majority 59 (84.28%) cases were in the age group of above 50 years. It is observed that more than two third of cases were above 50 years of age. Zhou et al., (2015); Gryko et al., (2014); Azarhoush et al., (2017) observed that the patients of above 50 years age group ranging from 55% to 85.71%. This study has concordance with most of the reported studies.[2,13,14]

In the present study, distribution of the tumor by type was observed and the results showed that 60 (85.7%) cases were intestinal type and 10 (14.2%) were diffuse type. Similar study was observed by other researchers. Gryko et al., (2014) found that intestinal type was 67% and diffuse type was 31.8%.13 Zafirellis et al., (2005) found that 57.6% were intestinal type, 11.5% were mixed type and 30.7% were diffuse type. [15]

Bcl-2 status was evaluated in Lauren classification. Out of 70 cases 60 cases were intestinal type and 10 cases were diffuse type. Among 60 intestinal type carcinoma, 28 cases were Bcl-2 positive (score1+ and 2+). On the other hand, out of 10 cases of diffuse type carcinoma only 2 cases were immunoreactive (score 1+) for Bcl-2. Thus, the total positive cases were 30 out of 70 cases. In this study score 1+ and 2+ was considered as positive, score '0' was considered as negative for Bcl-2 expression. In the current study, Bcl-2 positivity was observed in 42.9%. Tsamandas et al., (2009) and Azarhoush et al., (2017) observed Bcl-2 positivity ranging from 82% to 32.6%.[14,15] The findings of the present study are not absolutely identical with the outcomes of the previous studies. The differences in Bcl-2 positive rates between studies could have been caused by variances in scoring systems, tumor heterogeneity, and geographic variation.

In the current study, Bcl-2 positivity was observed in 28 (46.6%) intestinal type out of 60 intestinal type carcinoma and 2 (20%) was found in the diffuse type out of 10 diffuse type adenocarcinoma. However, no significant association was found between Bcl-2 status and tumor type. Zafirellis et al., (2005) reported Bcl-2 positivity in intestinal type of gastric adenocarcinoma 23.3%, diffuse type 25% and mixed type 16.7%.[15] Gryko et al., (2014) found that Bcl-2 expression was significantly higher in the intestinal type, compared to the diffuse type (71.2% versus 25.0%, $p<0.001$).[13] Lauwers et al., (1995) also found significant association between Bcl-2 expressions with intestinal type adenocarcinoma.[11] On the other hand, Hai- Feng et al., (1995) and Lee et al., (2003) did not find any significant association between Bcl-2 positivity and tumor type.[12,17] Their study supports our findings.

Conclusion

Adenocarcinoma of the stomach is more common in older individuals, and that overexpression of Bcl-2 protein is found in~43% of cases. Most gastric adenocarcinoma patients that test positive for Bcl-2 are intestinal in nature (28 intestinal instances compared to 2 diffuse cases). The expression of the Bcl-2 protein did not significantly correlate with the type of tumor.

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