

Expression of Cyclin D1 in Gastric Adenocarcinoma and its Association with Staging

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Abstract

Background: Gastric cancer is a major global health concern, ranking as the 5th most common malignancy and the 3rd leading cause of cancer-related deaths worldwide, with 783,000 deaths reported in 2018 (GLOBOCAN). Over 70% of gastric cancer cases occur in developing countries. Cyclin D1, a cell cycle regulatory protein, is implicated in various oncogenic processes, including tumor progression, invasion, and metastasis. Its overexpression has potential utility in predicting tumor behavior and guiding individualized therapy. This study aimed to assess the expression of cyclin D1 in histologically diagnosed gastric adenocarcinoma and its association with tumor stage.

Methods: A cross-sectional observational study was conducted in the Department of Pathology, Dhaka Medical College, from March 2019 to February 2022. Fifty-one cases of histologically confirmed gastric adenocarcinoma were evaluated. Routine hematoxylin and eosin staining and immunohistochemistry for cyclin D1 were performed. Clinical and demographic data were recorded, and statistical analysis was done to determine associations.

Results: The mean age of patients was 57.67±12.49 years, with a male predominance (M: F = 2.4:1). Cyclin D1 expression was positive in 33 cases (64.7%). Of these, 76.5% were in T3 stage and 93.8% in N3 stage. Cyclin D1 positivity was more frequent in advanced tumor and nodal stages.

Conclusion: Cyclin D1 expression was significantly associated with higher tumor and nodal stages in gastric adenocarcinoma. Its overexpression may serve as a prognostic biomarker and aid in risk stratification and therapeutic decision-making.

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Introduction

Gastric cancer remains one of the most common and deadly cancers worldwide, especially among older males.¹ Based on GLOBOCAN 2018 data, stomach cancer is the 5th most common neoplasm and the 3rd most deadly cancer, with an estimated 783,000 deaths in 2018. Gastric cancer incidence varies markedly with geography. In Japan, Chile, Costa Rica, and Eastern Europe, the incidence is up to 20-fold higher than in North America, northern Europe, Africa, and Southeast Asia.² Over 70% of the cases of gastric carcinoma occur in developing countries.³ The incidence of gastric carcinoma has been declining in the last few years in some areas like the USA, UK, Canada, etc, because of a reduction in chronic *H. pylori* infection and smoking and a decrease use of smoked and salted food.^{4,5} But the gastric carcinoma remains a burden for Bangladesh as the prevalence of *H. Pylori* has not substantially decreased.⁶

Gastric cancer is a curable disease provided that it is detected at early stage and treated adequately. It rarely disseminates widely before it has involved the lymph nodes, and, therefore, there is an opportunity to cure the disease before dissemination.⁷

Ninety percent of all tumors of the stomach are malignant, and gastric adenocarcinoma comprises 95% of the total number of malignancies.⁸ Therefore, numerous efforts have been made to search for novel prognostic biomarkers. The cyclin D1 is a prognostic biomarker that is currently used in clinical practice in the management of gastric cancer patients.⁹

In mammalian cells, the progression of replicating cells through the cell cycle is controlled by the sequential formation, activation, and subsequent inactivation of a series of specific cyclin-dependent kinase

(CDK) complexes.¹⁰ Mammalian cells encode three D cyclins (D1, D2, and D3) that coordinately function as allosteric regulators of cyclin-dependent kinase 4 and 6 (CDK4/CDK6) to regulate cell cycle transition from G1 to S phase. Cyclin expression, accumulation, and degradation, as well as assembly and activation of CDK4/CDK6, are governed by the growth factor stimulation. Cyclin D1 is more frequently dysregulated than cyclins D2 or D3 in human cancers, and as such, it has been more extensively characterized.¹¹ The cyclin D1 is a cell cycle regulatory proto-oncogene which is located at chromosome 11q13 and forms an active complex with cyclin-dependent kinase 4 and 6 (CDK4 and CDK6) and promotes cell cycle progression by phosphorylating and inactivating the retinoblastoma protein (RB).¹²⁻¹⁴

Some studies showed that Cyclin D1 is involved in gastric carcinoma, and its overexpression might be a useful prognostic factor and indicate poor prognosis.¹⁴⁻¹⁶

Recent clinical trials have been published where some therapeutic drugs that can arrest the cell cycle and promote apoptosis in cancer cells, and also reduce cell proliferation in a dose-dependent manner, have been published.¹⁷

A number of therapeutic agents have been shown to induce cyclin D1 degradation. The therapeutic ablation of cyclin D1 may be useful for the prevention and treatment of gastric cancer.¹⁸ The critical role of cyclin D1-CDK4 in regulating cell cycle progression and the hyperactivation of cyclin D1-CDK4 in human tumors make this complex an attractive target for cancer treatment.¹¹

Limited studies have been conducted regarding cyclin D1 expression in gastric adenocarcinoma. So far, no published data

have been found regarding cyclin D1 expression in gastric adenocarcinoma in Bangladesh. The present study was done to evaluate cyclin D1 expression in gastric adenocarcinoma and to investigate the correlation with the staging of gastric adenocarcinoma.

Methods

This is a cross-sectional observational study. This study was carried out at the Department of Pathology, Dhaka Medical College (DMC) over a period of three years from March 2019 to February 2022. The study included the following variables: Age, sex, socioeconomic status, chief complaints, smoking history, location of tumor, size of tumor, endoscopic features, tumor types, tumor extension, tumor stage, and cyclin D1 expression. Histomorphologically diagnosed gastric adenocarcinoma cases at the Department of Pathology, DMC, Dhaka, were collected. Adenocarcinoma cases were classified according to Lauren classification. Cases from all age groups and both sexes who had undergone surgical resection of the stomach.

Sampling Method

Non-probability, consecutive sampling method.

Sample Size

A total of 51 cases were enrolled in this study

Sample Selection Criteria

This study included cases that were histologically diagnosed as gastric adenocarcinoma based on specimens obtained through partial or total gastrectomy. Patients of all ages and both sexes were considered eligible for inclusion. However, patients with a history of receiving neoadjuvant chemotherapy or radiotherapy were excluded from the study. Additionally, cases with tumors showing extensive necrosis and hemorrhage, as well as those diagnosed with

gastric tumors other than adenocarcinoma such as lymphoma, carcinoid tumor, or gastrointestinal stromal tumor (GIST) were also excluded.

Ethical Aspects

Before the commencement of this study, the thesis protocol was submitted to the Ethical Review Committee (ERC) of Dhaka Medical College, Dhaka, for approval and was approved.

Data Collection and Recording

After getting permission from the Ethical Review Committee of DMC, a total of 51 histologically diagnosed cases of gastric adenocarcinoma were selected for the study by inclusion and exclusion criteria. The cases were diagnosed at the Department of Pathology, DMC. One section from a representative paraffin block (paraffin blocks with maximum tumor bulk were chosen) for each case was selected for immunohistochemical staining with cyclin D1. All the relevant information was collected from the patient, including clinical history, radiology and imaging findings, endoscopic findings, gastrectomy findings, etc. The information was systematically recorded in a prepared pro forma. All the cases were numbered chronologically, and the same number was given to H&E-stained and immunostained slides.

Histopathological Examination

Microscopic Analysis

Hematoxylin & Eosin (H&E) stained sections of each case were re-evaluated to confirm the histopathological diagnosis of gastric adenocarcinoma, staging, and to determine the presence of adequate tumor tissue to perform immunostaining. After histological examination, relevant points were taken from the report and included in the prescribed proforma.

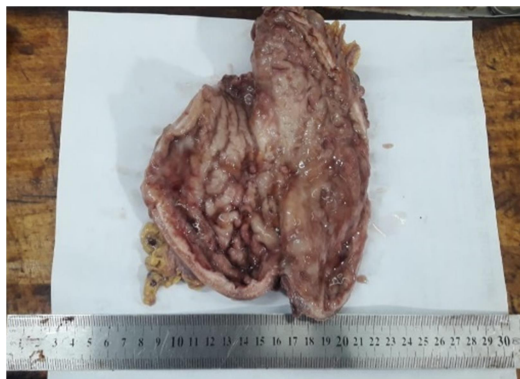


Figure 1. Photograph shows gross picture of partial gastrectomy specimen (Case no.19)

Immunohistochemical Analysis

Immunostaining for cyclin D1 was done on all 51 cases at Bangabandhu Sheikh Mujib Medical University, Dhaka. From paraffin-embedded blocks, 5-micrometer-thick sections were cut and taken on poly-L-lysine-coated slides, deparaffinized, cleared with xylene, and rehydrated through a graded series of alcohol. For antigen retrieval, the samples were treated with Dako Target Retrieval solution. Solutions were taken in a Coplin jar and pre-heated in the water bath at 65°C. Then slides were kept in this solution and heated in the water bath at 95-99 °C for 30-40 minutes.

Immunohistochemical staining for Cyclin D1 was performed using a rabbit monoclonal anti-Cyclin D1 (SP4) primary antibody (Lab Vision/Neomarkers, Fremont, CA, USA) at a dilution of 1:90. The EnVision detection system (ready-to-use, DAKO) was used as the secondary antibody. Tonsil tissue was used as a positive control. Paraffin-embedded tissue sections of 4.0 μm thickness were cut and

mounted on poly-L-lysine-coated slides. The slides were dried and incubated overnight (16 hours) at 37°C in an incubator. Deparaffinization was carried out in a hot air oven at 62°C for one hour, followed by dewaxing in three changes of xylene for five minutes each. The slides were then rehydrated through descending grades of propanol (100%, 90%, 80%, and 70%), five minutes at each step. Antigen retrieval was performed by placing the slides in preheated antigen retrieval solution in a water bath at 95°C for 30 minutes. After retrieval, the slides were allowed to cool at room temperature for 10 minutes and then placed in cooled water for another 10 minutes. The sections were washed with Tris-buffered saline (TBS) for five minutes. Immunostaining was carried out using an autostainer, following sequential steps of washing with TBS, incubation with the primary antibody for 30 minutes, washing, application of EnVision secondary antibody for 30 minutes, further washing, visualization with DAB for 5–10 minutes, and counterstaining with hematoxylin for 4–5 minutes. The slides were then washed in water, dehydrated through ascending grades of alcohol (70%, 80%, 90%, and 100%), cleared in xylene, and mounted with DPX.

Cyclin D1 immunostaining was interpreted based on the percentage of positively stained tumor cell nuclei. Cases with 0% stained cells were considered negative. Weak positivity was defined as staining in less than 10% of cells, moderate positivity as staining in 11%–50% of cells, and strong positivity as staining in more than 50% of cells.

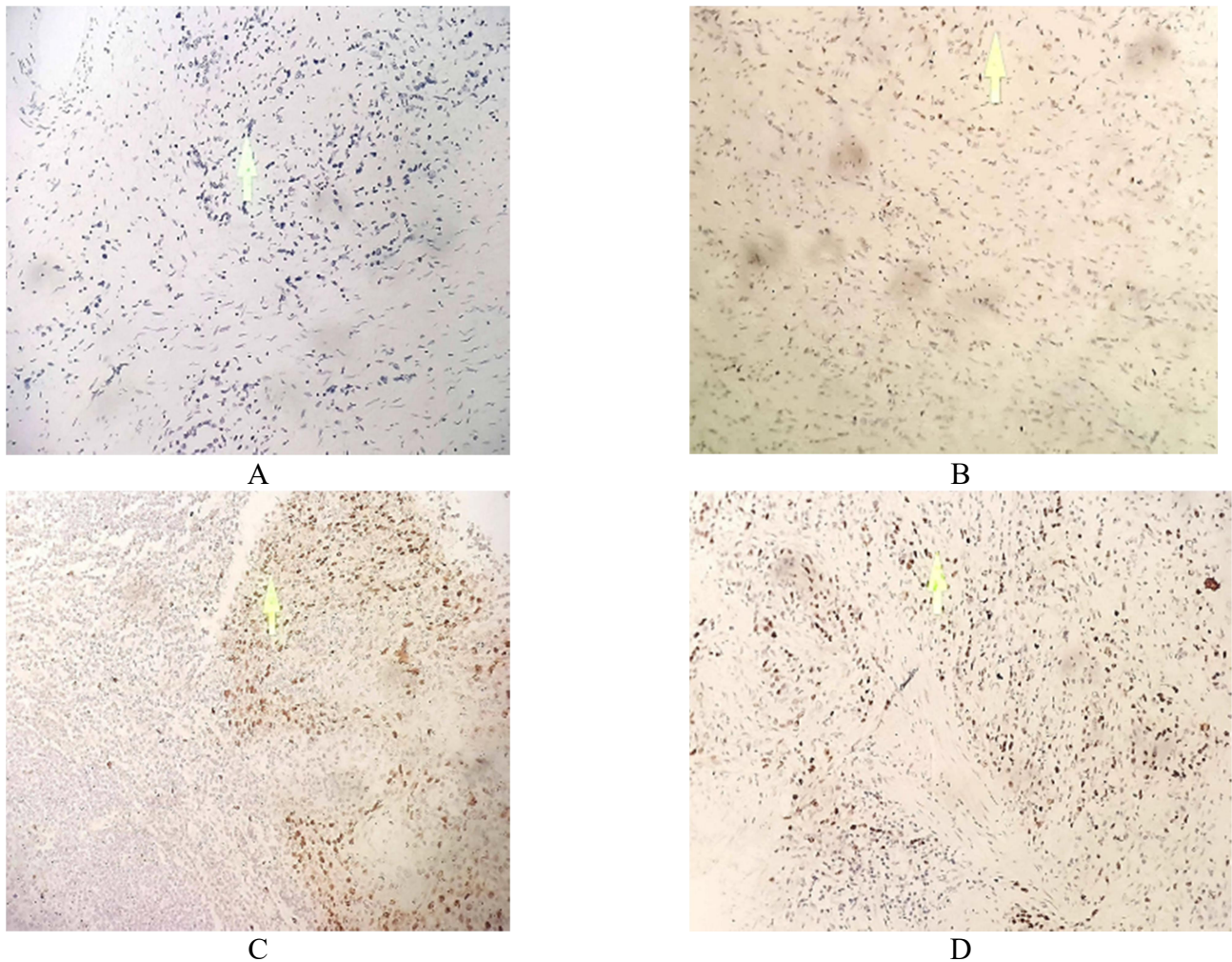


Figure 2. Photographs .(A) Photomicrograph of gastric adenocarcinoma showing negative staining of cyclin D1 (Case no.22, IHC X200), (B) Photomicrograph of gastric adenocarcinoma showing weak positive staining of cyclin D1 (Case no.17, IHC X200), (C) Photomicrograph of gastric adenocarcinoma showing moderate staining of cyclin D1 (Case no.6, IHC X200), and (D) Photomicrograph of gastric adenocarcinoma showing strong positive cyclin D1 (Case no.34, IHC X200).

Statistical Analysis of Data

Statistical analysis of the result was obtained by a window-based computer software device with Statistical Packages for Social Sciences version 23 (SPSS-23). Analysis of the association between cyclin D1 expression and stage of carcinoma. Analysis of the association between stage of carcinoma was performed by the Chi-square test. P values less than 0.05 were considered significant. The results were calculated by using statistical formulas and presented in tables, bar diagrams, and Pie charts.

Results

This cross-sectional study was carried out to evaluate the expression of cyclin D1 and its association with the stage of carcinoma. A total of 60 diagnosed cases of gastric adenocarcinoma were taken for

this study. Nine cases were excluded due to incomplete history, extensive necrosis, and haemorrhage. So, a total of 51 cases were included in this study. Forty-one cases were partial/subtotal gastrectomy specimens, and ten cases were total gastrectomy specimens. All 51 cases contain epigastric lymph nodes. Forty-three cases had lymph node metastasis, and the rest eight cases were free of tumor metastasis. After gross examination, haematoxylin and eosin-stained sections were examined under a microscope for histological examination. After assessing the stage of the tumor, an immunohistochemical study of cyclin D1 was done in all 51 cases.

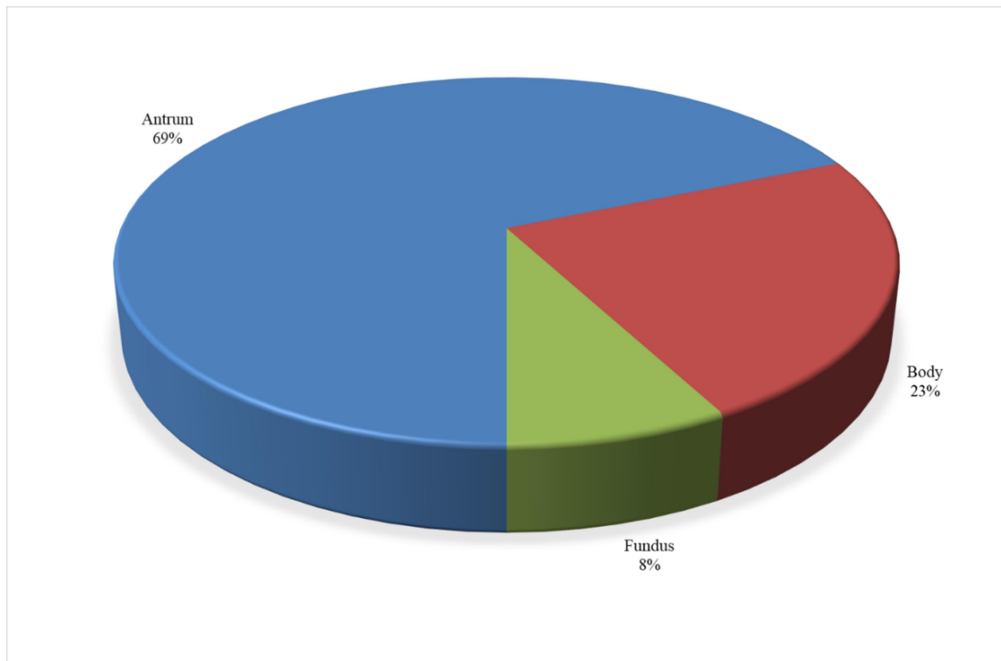


Figure 3. Pie chart of the cases according to the location of tumor (n=51)

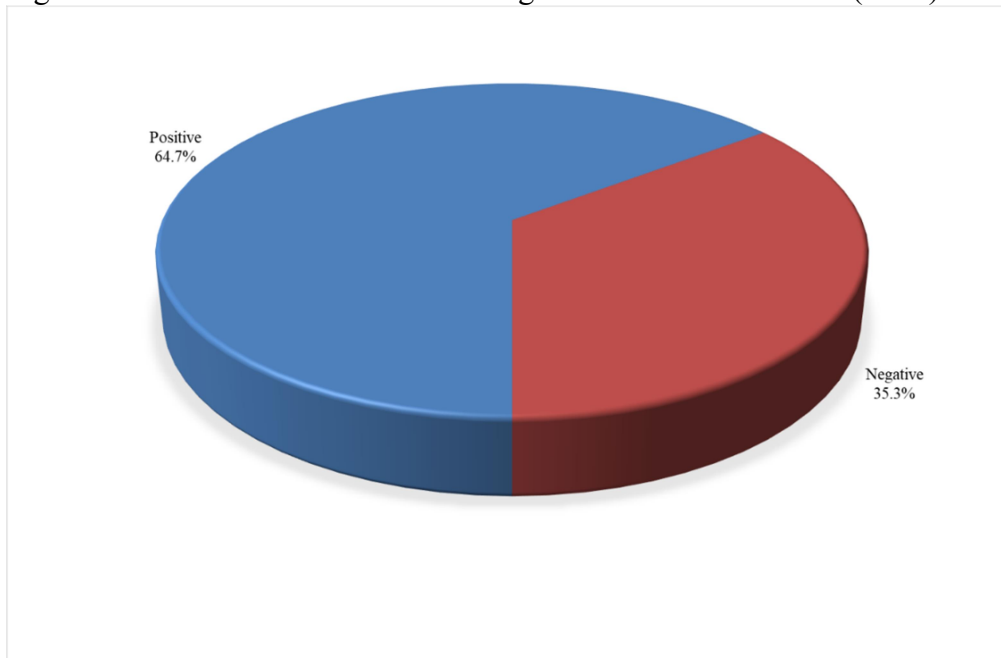


Figure 4. Pie chart of the cases according to the cyclin D1 expression (n=51)

The age distribution of the patients ranged from 32 to 83 years. Most of the patients were found to be between 61 to 70 years. The mean age (\pm SD) of the patients was 57.67 (\pm 12.49) years (Table I)

Table I: Distribution of the cases according to demographic variables (n=51)

Age (year)	Frequency	Percent
○ \leq 40	6	11.8
○ 40-50	11	21.6
○ 50-60	13	25.5
○ 60-70	14	27.5
○ $>$ 70	7	13.7
Mean \pm SD (Min-Max)	57.67 \pm 12.49 (32-83)	
Sex		
○ Male	36	70.6
○ Female	15	29.4
Socioeconomic condition		
○ Low	32	62.7
○ Middle	13	25.5
○ High	6	11.8

Table II: Distribution of the cases according to size of tumor (n=51)

Size of tumor	Frequency	Percent
\leq 5	25	49.0
$>$ 5	26	51.0
Total	51	100.0
Mean \pm SD (Min-Max)	5.67 \pm 1.56 (3-9)	

Table III: Distribution of the cases according to Lauren classification (n=51)

Lauren classification	Frequency	Percent
Intestinal	27	52.9
Diffuse	24	47.1

Table IV: Distribution of the cases according to tumor extension (n=51)

Tumor extension	Frequency	Percent
T2	7	13.7
T3	34	66.7
T4a	10	19.6

Table V: Distribution of the cases according to lymph node status (n=51)

Lymph node status	Frequency	Percent
N0	8	15.7
N1	10	19.6
N2	17	33.3
N3	16	31.4

Table VI: Distribution of the cases according to Lauren classification by cyclin D1 expression (n=51)

Lauran classification	Cyclin D1 expression		p value
	Positive	Negative	
Intestinal	18 (66.7)	9 (33.3)	0.756ns
Diffuse	15 (62.5)	9 (37.5)	

ns= Not significant

Table VII: Association of the cases according to tumor extension by cyclin D1 expression (n=51)

Tumor extension	Cyclin D1 expression		p value
	Positive	Negative	
T2	0 (.0)	7 (100.0)	0.001s
T3	26 (76.5)	8 (23.5)	
T4a	7 (70.0)	3 (30.0)	

s= Significant

Table VIII: Association of the cases according to lymph node status by cyclin D1 expression (n=51)

Lymph node status	Cyclin D1 expression		p value
	Positive	Negative	
N0	0 (.0)	8 (100.0)	<0.001s
N1	3 (30.0)	7 (70.0)	
N2	15 (88.2)	2 (11.8)	
N3	15 (93.8)	1 (6.3)	

s= Significant

Here, out of 51 cases, 36 cases (70.6%) were male and 15 cases (29.4%) were female. The male-to-female ratio was 2.4:1 (Table I).

Among 51 cases, 32 cases (62.7%) came from low socioeconomic conditions, 13 cases (25.5%) from middle, and 6 cases (11.8%) from high socioeconomic conditions (Table I).

Out of 51 cases, 22 cases (43.1%) were smokers, 15 cases (29.4%) had a habit of betel chewing, and 14 cases (27.5%) had no history.

According to the clinical presentation of the study cases, abdominal pain 39 (76.5%) was the most frequent complaint, followed by weight loss 27 (52.9%) and vomiting 26 (51.0%). The majority of the tumor involved in antral region 35 (69%), followed by the body 12 (23%) and fundus 4 (8%) (Figure 3). Among 51 cases, 26 cases (51.0%) had tumor

size >5cm and 25 cases (49.0%) had tumor size ≤5cm (Table II)

Histopathologic types of the tumor were categorized according to the Lauren classification. According to the Lauren classification, the majority of the tumor types were intestinal type, 27 cases (52.9%) (Table III).

Cases were grouped according to depth of invasion (tumor stage). 34 cases (66.7%) belonged to subserosal invasion (T3) followed by 10 cases (19.6%) that belonged to visceral peritoneal invasion without invasion of adjacent structures (T4a), and 7 cases (13.7%) belonged to muscularis propria invasion (T2) (Table IV).

Cases were grouped according to the number of lymph node metastases as N0, N1, N2, and N3. Forty-three cases had lymph node metastasis, and the rest eight cases were free

of tumor metastasis. 17 cases (33.3%) had N2 lymph node metastasis; 16 cases (31.4%) had N3 lymph node metastasis, followed by N1 10 cases (19.6%), and N0 8 cases (15.7%) (Table V).

33 cases (64.7%) showed cyclin D1 positive expression, whereas 18 cases (35.3%) showed cyclin D1 negative expression (Figure 4).

Cyclin D1 expression was positive in 66.7% (18/27) cases of intestinal type and 62.5% (15/24) cases of diffuse type according to the Lauren classification system. The difference was not statistically significant ($P < 0.05$) (Table VI).

The majority of the cases, 76.5% (26/34) with positive cyclin D1 expression, were found in T3 level. 70% (7/10) cases with positive cyclin D1 expression were found in T4a level. None of the cases, 0% (0/7), with T2 level showed positive cyclin D1 expression. The difference was statistically significant ($p < 0.05$) (Table VII).

Of the total 51 cases, 43 cases were found to have carcinoma with regional lymph node metastasis; among them, 33 cases showed positive cyclin D1 expression in their primary tumor. Cyclin D1 expression was positive in 93.8% (15/16) cases with nodal stage-3 (N3), in 88.2% (15/17) cases with nodal stage-2 (N2), and in 30% (3/10) cases with nodal stage-1 (N1). 0% (0/8) cases with nodal stage-0 (N0) showed no positive cyclin D1 expression. The difference was statistically significant ($p < 0.05$) (Table VIII).

Discussion

The cyclin D1 is a cell cycle regulatory proto-oncogene.¹²⁻¹⁴ High activity of cyclin D1 leads to abnormal cell proliferation.¹⁴ Cyclin D1 positive expression in gastric adenocarcinoma is associated with tumor

differentiation, TNM stage, and an increased rate of recurrence.¹⁴

The incidence of gastric carcinoma is strongly related to advanced age.^{20,21} In this study, it was observed that 66.7% of the study cases were above 50 years of age. Age distribution of the cases ranged from 32 to 83 years. The mean age (\pm SD) of the cases was 57.67 (\pm 12.49) years, which is nearly consistent with those mentioned by Du et al. (2012).²² They reported that the age of the patient ranged from 23 to 88 years, and the mean age was 58.9 \pm 11.5 years. The mean age varies from 52.6 years to 68.2 years among different countries.^{14,15,21} These variations might be due to different life expectancies in different countries.

In this study, 36 cases (70.6%) were male and 15 cases (29.4%) were female. The male-to-female ratio was 2.4:1, which is similar to some previous studies by Islam et al. (2009) and Alim et al. (2007).²³⁻²⁴ They reported that the male to female ratio was 2.36:1, and 2.05:1, respectively.

In the present study, it was observed that 32 cases (62.7%) came from low socioeconomic conditions, followed by 13 cases (25.5%) from middle, and 6 cases (11.8%) from high socioeconomic conditions. A similar observation was made by Guggenheim and Shah. (2013) and Rawla and Barsouk. (2019).^{1,25}

According to the presence of personal history, 22 cases (43.1%) were smokers, 15 cases (29.4%) had a habit of betel chewing, and 14 cases (27.5%) had no personal history. The predominance of smoking was also observed by the study done by Alim et al. (2007).²⁴

76.5% of cases in this study were presented with abdominal pain, followed by weight loss in 52.9% of cases and vomiting in 51.0% of cases. A similar observation was made by

Guggenheim and Shah. (2013) and Rawla and Barsouk. (2019).^{1,25}

In this study, 35 (69%) tumors were located in the antral region followed by body 12 (23%) tumors and fundus 4 (8%) tumors. These findings were nearly consistent with the study done by Alim et al. (2007) in Bangladesh.²⁴ They found that distal stomach was the most common site of affliction.

Tumor size is one of the strongest predictive factors for tumor aggressiveness and local recurrence. In the present study, 26 cases (51.0%) belonged to the tumor size >5 cm, and 25 cases (49.0%) belonged to the tumor size ≤5 cm. This observation was closely related to those done by Ibrahim et al. (2018).

The most frequent macroscopic type of the tumors according to Bormann classification was the ulcerated type, about 34 cases (66.7%), next in order of frequency were diffuse infiltrative, 7 cases (13.7%), polypoid, 6 cases (11.8%), and fungating, 4 cases (7.8%). These findings were nearly consistent with the observations done by previous studies by Rudi et al. (1995); Komoto et al. (1998), and Plummer et al. (2005).²⁶⁻²⁸

After histological examination, the cases were grouped according to the Lauren classification system. Of the total 51 cases, 27 cases (52.9%) were intestinal type, and 24 cases (47.1%) were diffuse type. The predominance of intestinal type can be explained by the high prevalence of *H. Pylori* infection in Bangladesh, and we know that *H. Pylori* infection is associated with intestinal type adenocarcinoma.²⁹ Several investigators also found a higher prevalence of intestinal-type gastric carcinoma in their studies: Rudi et al. (1995); Komoto et al. (1998); Polkowski et al. (1999) and Plummer et al. (2005).^{26-28,30}

In this present study, it was observed that 34 cases (66.7%) belonged to T3, followed by 10 cases (19.6%) belonging to T4a, and 7 cases (13.7%) belonged to T2. Gresta, Rodrigues, and Cabral (2014) also found, 63.5 % of cases belonged to T3 level and 19.2% of cases belonged to T2 level.³¹

According to number of lymph node (LN) metastasis, it was observed that 17 cases (33.3%) belonged to nodal stage-2 (N2) followed by 16 cases (31.4%) cases belonged to nodal stage-3 (N3), 10 cases (19.6%) belonged to nodal stage-1 (N1) and 8 cases (15.7%) belonged to nodal stage-0 (N0). This observation is nearly consistent with Ibrahim et al. (2018).¹⁴ They found that 30% of cases belonged to N3, 22.5% of cases belonged to N2, 30% of cases belonged to N1, and 17.5% of cases belonged to N0.

In this study, cyclin D1 expression was evaluated by immunohistochemistry in 51 cases. Cyclin D1 expression was found positive in 64.7% (33/51) cases, and the rest were negative. The result was nearly consistent with other reports.^{14-16,32} They found, respectively in their study that 50%, 89.3%, 50%, 72%, and 55% of cases showed positive cyclin D1 expression, and the rest of the cases showed negative cyclin D1 expression.

Of all the 51 cases, 43 cases had lymph node metastasis, and the rest eight cases were free of lymph node metastasis. Cases were grouped according to the number of lymph node metastases as N0, N1, N2, and N3. Cyclin D1 positive expression was more frequent in nodal stage N3, about 93.8%, then nodal stage N2, 88.2%, and nodal stage N1, 30.0%. Nodal stage N0 showed no cyclin D1 expression. These findings were statistically significant. So, a positive association was found between lymph node status and cyclin D1 expression in this study. Ibrahim et al.

(2018); Ru et al. (2017); Kumari et al. (2016); Bar-Sela et al. (2012) also found the similar findings.^{14,16,20,33} Ibrahim et al. (2018) found that cyclin D1 expression was positive in 100% of cases with nodal stage 3 lymph node metastasis, followed by 88.9% of cases with nodal stage 2 lymph node metastasis.¹⁴ Nodal stage N1 and N0 showed no positive cyclin D1 expression.

In our study, most of the cases (34) were found in T3 level i.e ; tumor invades up to sub serosal structures without invasion of visceral peritoneum. Cyclin D1 expression was positive in 76.5% cases that invaded T3 level and 70.0% cases that invaded T4a level and cases that invaded up to muscularis propria (T2) did not show positive cyclin D1 expression. This difference is statistically significant. So, a positive association was found between tumor extension and cyclin D1 expression in the present study. Ibrahim et al. (2018); Ru et al. (2017); Bar-Sela et al. (2012) also found a significant association between depth of invasion and cyclin D1 expression.^{14,16,33} Ibrahim et al. (2018) found, cyclin D1 expression was positive in 100% cases with T4 level, 63.6% cases with T3 level.¹⁴ The most conspicuous observation of this study was the overexpression of cyclin D1 in poorly differentiated gastric adenocarcinoma and tumor progression as well. In another study Kumari et al. (2016) stated that overexpression of cyclin D1 had been associated with early process of gastric carcinogenesis and acted as a major regulatory factor in initiation and progression of gastric carcinoma.²⁰ Ibrahim et al. (2018) also observed in their study that cyclin D1 positive expressions in GC was associated with poor survival, increased rate of recurrence and poor prognosis.¹⁴ Ru et al. (2017) stated that expression of cyclin D1 had significant correlation to clinical stage and median survival time was shorter (37 months) in patients with high mRNA expression of

cyclin D1 than in patients with low expression of cyclin D1 mRNA (41 months).³³ Shan et al. (2017) also observed that overexpression of cyclin D1 was correlated with tumor differentiation, poor survival and increased metastasis. These findings suggest that cyclin D1 can be used to determine the individual risk of disease progression, recurrence, aggressive behavior and prognosis as well.²¹ In contrast, Khabaz et al. (2016) and Amer and Eid (2019) in Egypt conducted their study separately and stated that low expression of cyclin D1 was associated with increased tumor stage.^{34,35} The reason of this difference might be attributed to the difference in the semiquantitative scoring of cyclin D1 in their study.

Although the current study showed that cyclin D1 is significantly associated with histopathological stage in gastric adenocarcinoma, it faced some limitations. It was not possible to follow up the patients, so we could not comment on the patients' outcome. It would give more appropriate information if a larger number of patients from multiple centers all over the country were included with a longer follow-up period. since several studies have observed the prognostic role of cyclin D1 in gastric adenocarcinoma, further studies are needed to establish the prognostic potential.

Conclusion

Routine use of cyclin D1 in conjunction with histopathological grading and staging in large biopsy samples may provide information about the biological behavior of the tumor, individualized risk of disease progression, as well as prognosis for categorizing the patients. This categorization may aid clinicians in further management of the patients.

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References

1. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. *Prz Gastroenterol.* 2019;14(1):26.
2. Kumar V, Abbas AK, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease. Professional ed. Philadelphia: Elsevier Health Sciences; 2014.
3. Chen J, Bu XL, Wang QY, Hu PJ, Chen MH. Decreasing seroprevalence of *Helicobacter pylori* infection during 1993–2003 in Guangzhou, southern China. *Helicobacter.* 2007;12(2):164-9.
4. Bertuccio P, Chatenoud L, Levi F, Praud D, Ferlay J, Negri E, et al. Recent patterns in gastric cancer: a global overview. *Int J Cancer.* 2009;125(3):666-73.
5. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics. *CA Cancer J Clin.* 2010;60(5):277-300.
6. Rima FA, Hussain M, Dewan RK, Haque MN, Sultana T, Chowdhury F, et al. Clinicopathologic features of gastric and gastroesophageal junction adenocarcinoma. *Mymensingh Med J.* 2020;29(1):195-201.
7. Williams NS, O'Connell PR, McCaskie AW. Bailey & Love's short practice of surgery. 27th ed. New Delhi: CRC Press; 2008. p.450-6.
8. Dicken BJ, Bigam DL, Cass C, Mackey JR, Joy AA, Hamilton SM. Gastric adenocarcinoma: review and considerations for future directions. *Ann Surg.* 2005;241(1):27-39.
9. Minarikova P, Benesova L, Halkova T, Belsanova B, Tuckova I, Belina F, et al. Prognostic importance of cell cycle regulators cyclin D1 (CCND1) and cyclin-dependent kinase inhibitor 1B (CDKN1B/p27) in sporadic gastric cancers. *Gastroenterol Res Pract.* 2016;2016:1-8.
10. Tashiro E, Tsuchiya A, Imoto M. Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. *Cancer Sci.* 2007;98(5):629-35.
11. Qie S, Diehl JA. Cyclin D1, cancer progression, and opportunities in cancer treatment. *J Mol Med.* 2016;94(12):1313-26.
12. Bizari L, Borim AA, Leite KRM, de Toledo Gonçalves F, Cury PM, Tajara EH, et al. Alterations of the CCND1 and HER-2/neu (ERBB2) proteins in esophageal and gastric cancers. *Cancer Genet Cytogenet.* 2006;165(1):41-50.
13. Arici D, Tuncer ER, Ozer HA, Simek G, Koyuncu AY. Expression of retinoblastoma and cyclin D1 in gastric carcinoma. *Neoplasma.* 2009;56(1):63-7.
14. Ibrahim HM, AbdElbary AM, Mohamed SY, Elwan A, Abdelhamid MI, Ibrahim A. Prognostic value of cyclin D1 and CD44 expression in gastric adenocarcinoma. *J Gastrointest Cancer.* 2019;50(3):370-9.
15. Feakins RM, Nickols CD, Bidd H, Walton SJ. Abnormal expression of pRb, p16, and cyclin D1 in gastric adenocarcinoma and its lymph node metastases: relationship with pathological features and survival. *Hum Pathol.* 2003;34(12):1276-82.
16. Bar-Sela G, Hershkovitz D, Haim N, Kaidar-Person O, Shulman K, Ben-Izhak O. The incidence and prognostic value of HER2 overexpression and cyclin D1 expression in patients with gastric or gastroesophageal junction adenocarcinoma in Israel. *Oncol Lett.* 2013;5(2):559-63.
17. Schettini F, De Santo I, Rea CG, De Placido P, Formisano L, Giuliano M, et al. CDK 4/6 inhibitors as single agent in advanced solid tumors. *Front Oncol.* 2018;8:608.
18. Alao JP. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Mol Cancer.* 2007;6(1):1-16.
19. Miftahussurur M, Waskito LA, Aftab H, Vilaichone RK, Subsomwong P, Nusi IA, et al. Serum pepsinogens as a gastric cancer and gastritis biomarker in South and Southeast Asian populations. *PLoS One.* 2020;15(4):e0230064.
20. Kumari S, Prasad SB, Yadav SS, Kumar M, Khanna A, Dixit VK, et al. Cyclin D1 and cyclin E2 are differentially expressed in gastric cancer. *Med Oncol.* 2016;33(5):1-10.

21. Shan L, Ying J, Lu N. HER2 expression and relevant clinicopathological features in gastric and gastroesophageal junction adenocarcinoma in a Chinese population. *Diagn Pathol.* 2013;8(1):1-7.
22. Du CY, Chen JG, Zhou Y, Zhao GF, Fu H, Zhou XK, et al. Impact of lymphatic and/or blood vessel invasion in stage II gastric cancer. *World J Gastroenterol.* 2012;18(27):3610-6.
23. Islam SMJ, Ali SM, Ahmed S, Afroz QD, Chowdhury R, Huda M. Histopathologic pattern of gastric cancer in Bangladesh. *J Armed Forces Med Coll Bangladesh.* 2009;5(1):21-4.
24. Alim MA, Ahad MA, Rashid MH, Islam QT, Ekram ARMS, Rassaqa MA. Endoscopic determination of location of gastric cancer in Bangladesh. *TAJ.* 2007;20(2):95-8.
25. Guggenheim DE, Shah MA. Gastric cancer epidemiology and risk factors. *J Surg Oncol.* 2013;107(3):230-6.
26. Rudi J, Müller M, Von Herbay A, Zuna I, Raedsch R, Stremmel W, et al. Lack of association of *Helicobacter pylori* seroprevalence and gastric cancer in a population with low gastric cancer incidence. *Scand J Gastroenterol.* 1995;30(10):958-63.
27. Komoto K, Haruma K, Kamada T, Tanaka S, Yoshihara M, Sumii K, et al. *Helicobacter pylori* infection and gastric neoplasia: correlations with histological gastritis and tumor histology. *Am J Gastroenterol.* 1998;93(8):1271-6.
28. Plummer JM, Gibson TN, McFarlane ME, Hanchard B, Martin A, McDonald AH. Clinicopathologic profile of gastric carcinomas at the University Hospital of the West Indies. *West Indian Med J.* 2005;54(6):364-8.
29. Rosai J. *Ackerman's surgical pathology.* 11th ed. London: Mosby; 2018. Vol. 1, p.528-67.
30. Polkowski W, van Sandick JW, Offerhaus GJA, ten Kate FJ, Mulder J, Obertop H, et al. Prognostic value of Lauren classification and c-erb B-2 oncogene overexpression in adenocarcinoma of the esophagus and gastroesophageal junction. *Ann Surg Oncol.* 1999;6(3):290-7.
31. Gresta LT, Júnior IAR, Cabral MMDA. Microvessel density quantification in gastric cancer: comparing methods for standard measures. *J Cancer Sci Ther.* 2014;6(10):401-5.
32. Begnami MD, Fregnani JHT, Nonogaki S, Soares FA. Evaluation of cell cycle protein expression in gastric cancer: cyclin B1 expression and its prognostic implication. *Hum Pathol.* 2010;41(8):1120-7.
33. Ru Y, Chen XJ, Zhao ZW, Zhang PF, Feng SH, Gao Q, et al. CyclinD1 and p57kip2 as biomarkers in differentiation, metastasis and prognosis of gastric cardia adenocarcinoma. *Oncotarget.* 2017;8(43):73860-70.
34. Khabaz MN, Buhmeida A, Ghabrah T, Qureshi IA, Butt NS, Al-Maghrabi B, et al. Cyclin D1 expression is associated with stage, grade and survival in urinary bladder carcinoma. *Int J Clin Exp Med.* 2016;9(12):23482-90.
35. Amer AI, Eid AM. Prognostic significance of Cyclin D1 in urothelial carcinoma; correlation with p53 and clinicopathological parameters. *J Am Sci.* 2019;15(1):86-91.