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Evaluation of CDX2 expression and correlation with histopathological grading of Colorectal Carcinoma

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Abstract
Background: Colorectal cancer is the third most commonly diagnosed cancer in male and second in female and third most common cause of cancer death in the world. Only histopathological diagnosis is not always sufficient to predict the clinical outcome and prognosis of colorectal carcinoma. So, it is an emerging need to use specific immunohistochemical stains. CDX2 is a prognostic indicator of colorectal carcinoma. This study focuses on association of CDX2 with grading of colorectal carcinoma.
Materials and Methods: Fifty-one surgical specimens of large intestine were included in this cross-sectional study conducted in Sir Salimullah Medical College from July 2019 to June 2021. Specimens were processed routinely for Haematoxylin and Eosin followed by immunohistochemistry for CDX2. Histopathological and immunohistochemical results were analyzed. Statistical analysis was done using Statistical Package for Social Sciences version 22.
Observation and results: Among 51 histopathologically diagnosed colorectal carcinoma cases 11 were well differentiated adenocarcinoma, 28 were moderately differentiated adenocarcinoma and 12 were poorly differentiated adenocarcinoma. CDX2 expression is gradually decreased with increase of WHO grading. These association was statistically significant ($p<0.05$).
Conclusion: The result of this study demonstrated that decreased CDX2 expression was associated with increased grading. So, CDX2 staining is recommended as prognostic marker which is required for predicting the prognosis and choosing the treatment options in this regard.

Keywords: CDX2 expression, histopathological grading, colorectal carcinoma

Introduction
Globally, colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females, with 1.8 million new cases and almost 861,000 deaths in 2018 according to the World Health Organization GLOBOCAN database [1]. The estimated number of new cases in 2020, worldwide in males and females of all ages were 1,065,960 (10.6%) and 8,65,630 (9.4%) respectively [2]. In Bangladesh, mid age group people are the most vulnerable for colorectal carcinoma [3]. Furthermore, male is predominant than female. Per rectal bleeding, abdominal pain and altered bowel habit are the most commonly reported clinical features of the colorectal carcinoma patients. Adenocarcinoma of usual pattern is most common type; other common type is mucin secreting adenocarcinoma [3]. Adenocarcinomas, the most frequent histological type of colorectal cancer, which accounts for some 98% of all malignancies in the large bowel [4]. The histological grade is essentially an estimate of the pace of growth and the tumor is graded according to WHO (2019) grading criteria as well differentiated, moderately differentiated and poorly differentiated [5]. Traditional prognostic factors of colorectal carcinoma include the tumor size, histologic type, tumor node metastasis according to TNM and Dukes stage and potential residual disease after initial surgery [6]. Other features known to be related to survival include vascular and perineural invasion, tumor necrosis, character of invasive margin and differentiation [7]. Despite the numerous prognostic indicators currently in use or under investigation it is impossible to predict the outcome of individual patient. For this reason, there is continuous search for better or more refined biologic markers of prognosis and more effective treatment modalities [8]. CDX2 is an intestine-specific nuclear transcription factor encoded by the human homologue of the homeobox, gene, *Drosophila caudal*. The essential role of CDX2 in intestinal development and cell phenotype, it has been the focus of numerous studies concerning colorectal tumorigenesis. Site-specific expression of CDX2 in normal intestines and expression in most intestinal adenocarcinomas are preserved, making CDX2 protein a potentially useful marker for both primary and metastatic intestinal adenocarcinomas [9].

In the study De Lott *et al*. [9] using tissue microarrays and a monoclonal antibody against CDX2, demonstrate that CDX2 is a useful marker for intestinal-type differentiation in neoplastic epithelium by comparing tumors of various sites while expanding the quantity and types of tumors evaluated for CDX2 staining. Further correlation between tumor histology and CDX2 immunoreactivity was also assessed in colorectal adenocarcinomas to confirm the loss of CDX2 expression in poorly differentiated colorectal carcinomas [9]. CDX2 is a useful marker of intestinal-type differentiation which is relatively sensitive marker of intestinal origin in the well-differentiated, moderately differentiated, mucinous, and signet ring cell carcinomas. It has been confirmed that CDX2 may be useful in determining the site of origin for some metastatic intestinal-type tumors but may be less useful in poorly differentiated metastatic colorectal cancers [9]. One study showed lack of CDX2 expression could be a useful marker to identify high-risk patients with poor prognosis [10]. In a similar study it concluded that Low CDX2 expression in tumours with a high stromal content identified patients with a particularly poor prognosis and it also identifies patients who have a high risk of relapse and a poor outcome, and who may benefit from targeted therapy [11]. Many studies, including the work by Dalerba et al. [12] underline the unfavorable survival time in patients with a complete absence of CDX2 in the tumor, a feature that occurs in approximately 5% of patients [12, 13]. Dalerba et al. [12] concluded CDX2 as a Prognostic Biomarker in Stage II and Stage III Colon Cancer and they concluded that lack of CDX2 expression identified a subgroup of patients with high-risk stage II colon cancer who appeared to benefit from adjuvant chemotherapy. Bae et al. [14] concluded that loss of CDX2 expression is associated with aggressive clinical behavior and can be used as a prognostic marker in CRCs [14]. Shigematsu et al. [15] showed in their study that reduced expression of CDX2 indicates poor Disease-Free Survival and Overall Survival [15]. In the study by Asgari-Karchekani *et al*. [16] concluded that the downregulated CDX2 expression is associated with female gender, right-sided tumors, mucinous tumors, lymph node involvement, high-grade tumor, and advanced overall pathological staging and can be considered as a possible prognostic factor for patients follow-up [16]. The objective of the current study was to see the expression of CDX2 in colorectal carcinoma as well as the evaluation of its association with histologic grade.

Martials and Methods

This prospective, cross sectional study was conducted in Sir Salimullah Medical College from July, 2019 to June, 2021 on 51 cases of histopathologically diagnosed colorectal carcinoma patients. The sampling technique was purposive and convenient. Specimens were processed routinely for Haematoxyline and Eosin stain and analyzed under microscope. One section for CDX2 immunostaining were prepared from each case. Histological characteristics were recorded according to World Health Organization (2019) recommendation. Histology was assessed for histological grade and tumor subtypes. Immunostaining was done by using Dako Autostainer plus at the immunohistochemistry laboratory, department of Pathology, Square Hospital and results were analyzed. Interpretation, statistical analysis (Statistical Package for Social Sciences version 22 for Windows) and evaluation were done.

Immunohistochemical Study

CDX2: After taking 4 micrometer sections from each case, the sections were deparaffinized with xylene and rehydrated properly. Then antigen retrieval was done with Dako Target Retreival solution. Sections were then stained with monoclonal mouse antihuman CDX2 antibody (clone DAK). Immunostaining was done by using Dako Autostainer plus at the immunohistochemistry laboratory, department of Pathology, Square Hospital. For CDX2 immunostaining, positive control was taken from sections of appendix. To validate the stain negative control was taken from sections of normal colon tissues by omitting the primary antibody.

Interpretation of Staining

A brown nuclear stain shows a positive expression for CDX2. This positivity was analyzed.

- All immunostained sections were examined under light microscope.
- Both are nuclear antigen which is evaluated semi quantitatively.
- Cell counts positive and negative for the expression are performed under X400 magnification for the most intensely stained regions (i.e in hot spots).
- It was scored by counting 500 cells in each section.
- Every stained nucleus was considered positive irrespective of intensity.

Sections stained for CDX2 were recorded as follows [17]:
0 - (<5% cells stained);
+ (5-25% of cells stained);
++ (26-75% of cells stained); and
+++ (>75% of cells stained) [17]
0 (<5% cells stained) was counted as negative and other scores were counted as positive.

Observations and Results

This cross-sectional study was conducted on 51 histopathologically diagnosed colorectal carcinoma patients. Histopathology was done by H & E followed by immunohistochemistry for CDX2 to observe the usefulness of these two immune markers in patients with CRC for grading and staging. The following observations were made.

Table 1: Distribution of the study population by the grading (WHO, 2019) of the tumours, (n=51)

Grading	Number of cases	Percentage (%)
Well differentiated	11	21.6
Moderately differentiated	28	54.9
Poorly differentiated	12	23.5

Table 2: Association of CDX2 expression with histological grading (WHO, 2019), (n=51)

Grading	Frequency n (%)	CDX 2 expression Mean±SD	*p* value
Well differentiated (A)	11(21.57)	87.82±21.25	
Moderately differentiated(B)	28(54.90)	50.54±34.84	
Poorly differentiated(C)	12(23.53)	21.25±25.51	
A vs B vs C			0.001[s]
A vs B			0.023[s]
A vs C			0.004[s]
B vs C			0.001[s]

s=significant
ANOVA test followed by Bonferroni t test was done to measure the level at significance between the groups.

Table 3: Distribution of the study population by positivity index of expression of CDX 2 according to grading, (n = 51)

		Positivity Index, N (%)			
Grading	**Frequency n (%)**	**0 (<5%)**	**+ (5-25%)**	**++ (26-75%)**	**+++ (>75%)**
Well differentiated	11 (21.57)	0 (0.0)	1(9.09)	0 (0.0)	10 (90.91)
Moderately differentiated	28 (54.9)	5 (17.86)	3 (10.71)	9 (32.14)	11 (39.29)
Poorly differentiated	12 (23.5)	3 (25.0)	5 (41.67)	3 (25.0)	1 (8.33)

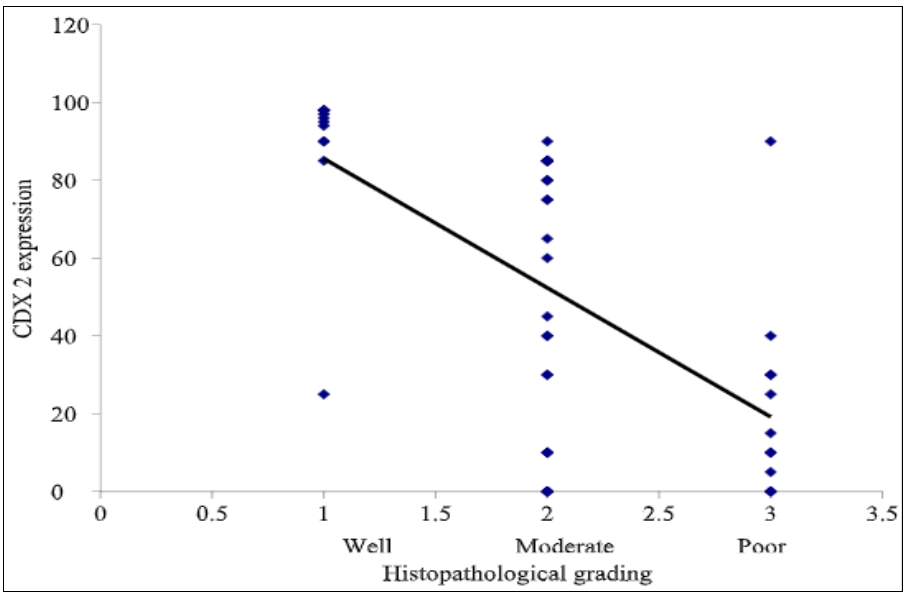

Fig 1: Correlation between histological grading and CDX2 expression.

Figure 1: Scatter diagram showing negative significant Spearman's correlation (r= -0.336; p= 0.016) between histological grading (WHO, 2019) and CDX2 expression.

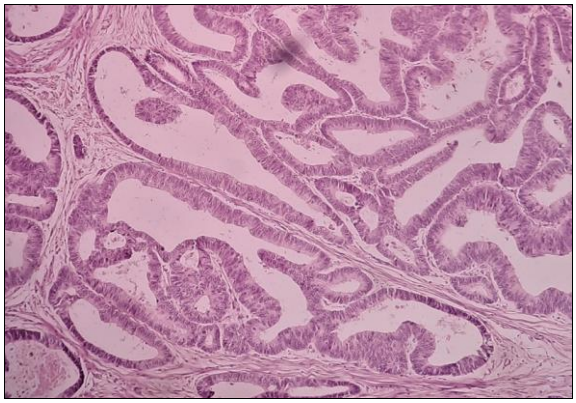

Fig 2: Photomicrograph of histopathological section of well differentiated adenocarcinoma of colon stained by H & E method (X100) (case 1).

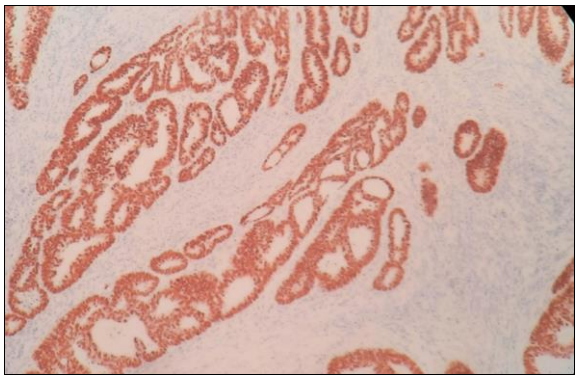

Fig 3: Photomicrograph of histopathological section of well differentiated adenocarcinoma of colon stained with CDX2 (X100) (case 1).

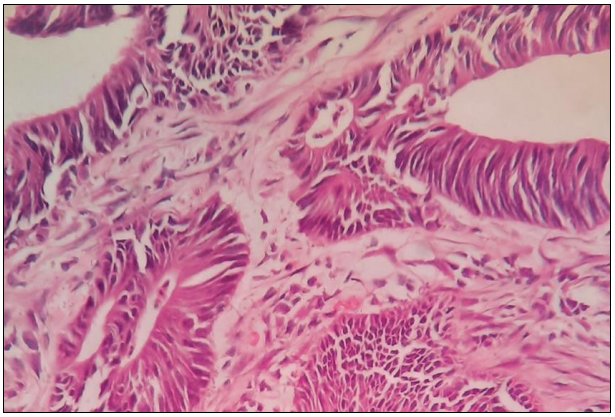

Fig 4: Photomicrograph of histopathological section of moderately differentiated adenocarcinoma of colon stained by H & E method (X400) (case 16).

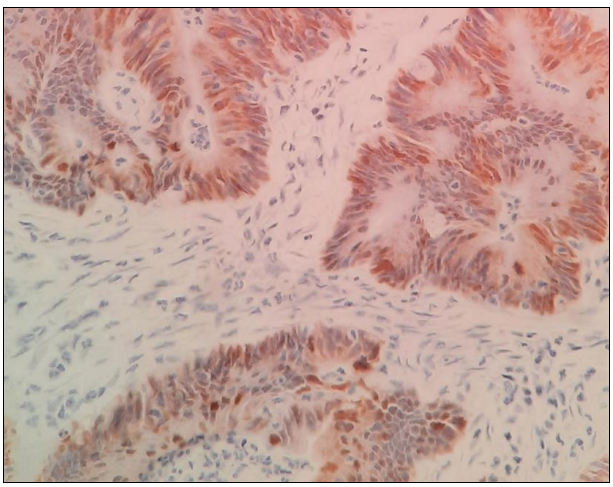

Fig 5: Photomicrograph of histopathological section of moderately differentiated adenocarcinoma of colon stained with CDX2 showing 40% expression (X400) (case 16).

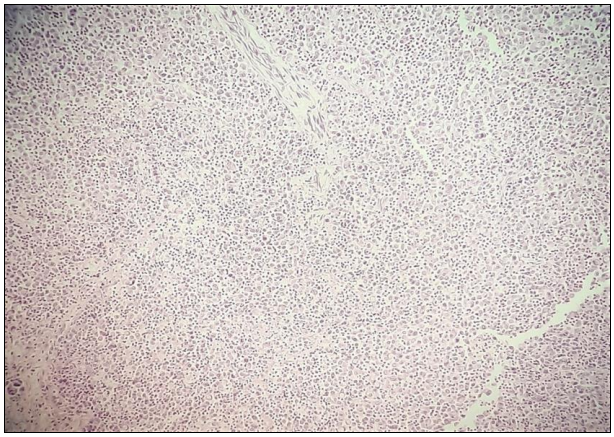

Fig 6: Photomicrograph of histopathological section of poorly differentiated adenocarcinoma of colon stained by H & E method (X100) (case 12).

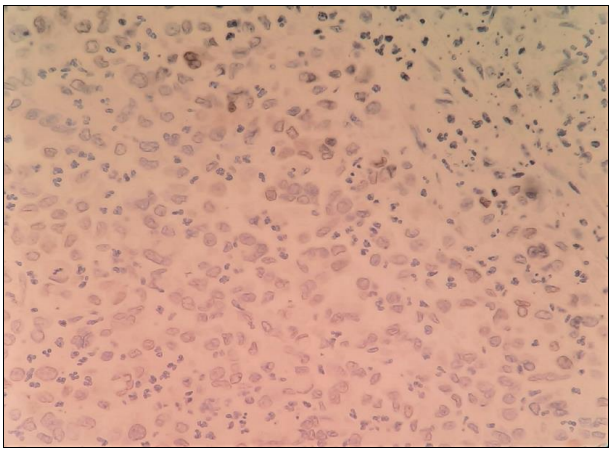

Fig 7: Photomicrograph of histopathological section of poorly differentiated adenocarcinoma of colon stained with CDX2 (X400) (case 12).

Discussion

This cross-sectional study was carried out with an aim to determine the expression of CDX2 to observe the association of this immune marker in patients with colorectal carcinoma and to evaluate their association with histopathological grading and staging. A total of 51 patients with colorectal carcinoma diagnosed histopathologically in the pathology departments of Sir Salimullah Medical College and Mitford Hospital, other teaching hospitals and private institutions in Dhaka during July 2019 to June 2021 were included in this study. The present study findings were discussed and compared with previously published relevant studies. Colorectal carcinoma is a common cause of morbidity and mortality worldwide [18]. Colorectal cancer (CRC) is already the third leading cause of cancer death in the world, and its incidence is steadily rising in developing nations. Despite recent advances in detection and treatment colorectal cancer is the third most common type of cancer and a major cause of cancer related mortality worldwide [19]. In the present study, the tumours were graded according to WHO (2019) grading system into well differentiated, moderately differentiated and poorly differentiated carcinoma. It was observed that highest 28 (54.9%) patients had moderately differentiated carcinoma followed by 12 (23.5%) patients having poorly differentiated carcinoma and 11(21.6%) patients having well differentiated carcinoma (Table I). Majority of the cases were moderately differentiated in the present study which corresponds to many other studies [20, 21]. Regarding CDX2 expression in different WHO (2019) grading of colorectal carcinoma, it was observed that 11 cases (21.57%) were well differentiated (A) and their mean CDX2 expression was 87.82±21.25, 28 cases (54.90%) were moderately differentiated (B) and their mean CDX2 expression was 50.54±34.84 and 12 cases (23.53%) were poorly differentiated (C) and their mean CDX2 expression was 21.25±25.51. The differences were statistically significant ($p<0.05$) between grading (WHO, 2019) and CDX2 expression (Table 2). It could be concluded that higher tumour grade correlates with down regulation of CDX2 which means increase of grading was associated with decrease of CDX2 expression. The table 3 shows 10 cases among 11 cases of well differentiated carcinoma belonged to score +++, 11 cases among 28 cases of moderately differentiated carcinoma belonged to score +++ and 9 cases showed score ++, and regarding poorly differentiated carcinoma 3 cases had score 0- and 5 cases had score +. Asgari-Karchekani *et al.* [16] found a significant correlation between CDX2 down regulation and high-grade tumor which corresponds to this study. The result was also similar with the study conducted by Graule *et al.* [22]. In a study by Werling *et al.* [23], CDX2 expression did not correlate with tumor grade. A statistically negative and significant Spearmans correlation was observed between histopathological grading of colorectal adenocarcinoma and CDX2 expression (r=-0.601; p=0.001) (Figure 1).

Conclusion with Recommendation

The result of this study demonstrated that decreased CDX2 expression was associated with increased grading colorectal carcinoma. CDX2 is an important prognostic factor of adenocarcinoma of colorectal origin. Its loss has been linked to more malignant phenotype and poor differentiation.

Evaluating the present study, it could be recommended that,

1. CDX2 immunostaining may improve diagnostic and prognostic accuracy of colorectal adenocarcinomas.
2. Large sample size, longer duration and multicenter studies would bring out more representative data.

Conflict of Interest

Not available

Financial Support

Not available

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