IMMUNOHISTOCHEMICAL EXPRESSION OF CDX2 AND ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN COLORECTAL NEOPLASMS

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ABSTRACT

Introduction*: Colorectal Carcinoma is one of the most commonly diagnosed cancers worldwide. They are known to be preceded by metaplasia and adenomas. Screening, early diagnosis and intervention have found to improve the prognosis substantially. Therefore there is a vital need to identify and stratify colonic cancers according to the degree of aggressiveness. CDX2 is a master regulator of intestinal development and oncogenesis and its expression is highly specific to the intestinal epithelium. Colon cancers without CDX2 expression are often associated with an increased likelihood of aggressive features such as advanced stage, poor differentiation, vascular invasion, BRAF mutation, and the CpG island methylator phenotype.*

Aims and objectives*: The aims and objectives of the study were to evaluate the clinicopathological features of colonic carcinoma and to analyze the expression pattern of CDX2 in various lesions of the colon.*

Materials and methods*: A total of 29 cases of colonic neoplasms were studied and their clinicopathological details were analyzed. All cases were stained with CDX2 monoclonal antibodies. Percentage and intensity of CDX2 positive cells were scored and results were correlated with the clinicopathological data.*

Results: *Out of 28 cases that contained normal colonic mucosa, 27 (96.42%) cases were positive for CDX2. 25 out of the 26 (96.15%) cases containing adenomatous tissue showed CDX2 positivity. All colon cancer cases (15 cases) showed CDX2 positivity. It was found that the intensity of CDX2 staining was greater in malignant tissues than in normal mucosa or in adenomatous polyps.*

Conclusions: *Colorectal carcinoma occurred predominantly in individuals aged 60-80 years of age and the incidence was higher in males than in female, with rectum as the commonest site. The staining intensity of CDX2 was found to be higher in colonic cancer than in adjacent normal tissue or colonic adenomas.CDX2 can be a potential target for therapy in cases with strong positivity.*

***Keywords**: Colorectal Carcinoma, CDX2, immunohistochemistry*

INTRODUCTION

Colorectal carcinoma (CRC) is one of the most commonly diagnosed cancers worldwide (1.23 million, 9.7% of all cancers)[1]. By incidence, colorectal cancer is the third most common cancer in men (663 000 cases, 10.0% of the total) and the second in women (571 000 cases, 9.4% of the total) worldwide[2]. CRC represents nearly 15% of all cancer-related deaths. It is most prevalent in the United States, Canada, Australia, New Zealand and other developed countries. The incidence of this cancer is as much as 30-fold lower in India, South America, and Africa. Colorectal cancer incidence peaks at 60 to 70 years of age[2], and fewer than 20% of cases occur before age 50. Males are affected slightly more often than females.

At least two distinct genetic pathways for colon carcinogenesis have been described, viz., the *APC/β-catenin pathway*[3] and the *microsatellite instability pathway*[4]. Both pathways involve the stepwise accumulation of multiple mutations. Further, it has been hypothesized that epigenetic events, the most common of which is methylation-induced gene silencing, may enhance progression along both pathways. The classic *adenoma-carcinoma sequence* accounts for 80% of sporadic colon tumors and typically includes bi allelic mutation of the *APC* gene early in the neoplastic process. Loss of APC function causes the transcription of proto-oncogenes. This is followed by additional mutations, which also promote growth and prevent apoptosis. One such tumor suppressor gene potentially involved in colon cancer is CDX2. CDX2 is a master regulator of intestinal development and oncogenesis, and its expression is highly specific to the intestinal epithelium. Colon cancers without CDX2 expression are often associated with an increased likelihood of aggressive features such as advanced stage, poor differentiation, vascular invasion. In patients with DNA mismatch repair deficiency, mutations accumulate in microsatellite repeats, known as *microsatellite instability*. Mutations in oncogenes and silencing of distinct groups of genes due to CpG island hypermethylation[5] are also common in cancers that develop through DNA mismatch repair defects.

The aim of this study is to evaluate the expression of CDX2 antibody and its association with clinicopathological features in colonic carcinoma to differentiate the various lesions.

MATERIALS AND METHODS

All cases were obtained from the archival files of the Department of Pathology, Sri Ramachandra Medical Centre. A total of 29 cases were evaluated. Formalin-fixed, paraffin-embedded sections of 15 colonic cancers (of which 12 were adenocarcinomas and 3 were mucinous adenocarcinomas), 7 polyps (of which 4 were hyperplastic and 3 were adenomatous) and 7 adenomas (of which 6 were tubular and 1 was villous) were obtained.The adjacent normal colonic mucosa was also analyzed.

Clinicopathological details with reference to the patient's age and sex in addition to lesion size

and configuration, histological grade and type and, in case of carcinomas, TNM staging were noted.

All cases were stained with monoclonal antibodies to CDX2 (CDX2-88; BioGenex)

Percentage of CDX2 positive cells was graded as: 0, no staining; 1+, less that 25% of the tissue stained; 2+, 25-75% of the tissue stained and 3+, more that 75% of the tissue stained.

Intensity of the stain was graded as: 0, no staining; 1+, weak staining; 2+, moderate staining; 3+ strong staining.

RESULTS

A total of twenty-nine cases were analyzed. Their ages ranged from 8 to 83 years, with a mean age of 53.86 years (Figure 1). There were 20 (73%) males and 9 (27%) females. The most common clinical presentation were altered bowel habits (47%) followed by bleeding (20%) and tiredness/fatigue (20%) (Figure 2). Out of 28 cases that contained normal colonic mucosa, 27 (96.42%) cases were positive for CDX2. 25 out of the 26 (96.15%) cases containing adenomatous tissue showed CDX2 positivity. All colon cancer cases (15 cases) showed CDX2 positivity (Figure 3).

The percentage of CDX2 staining cells was determined and it was found that in adenocarcinomas majority of the cells showed (73.4%) showed staining when compared to normal areas 71.4% and adenomas (66.6%) (Table 1)

Table 1: Percentage of CDX2 staining cells

% of cells stained	Adenocarcinoma(n- 15)	Adenoma(n- 26)	Normal Mucosa(n- 28)
0 – 10%	0	1	1
11- 50%	1	1	1
50- 75%	3	6	6
>75%	11	18	20

With respect to intensity, 71.42% of normal colonic mucosa was stained strongly (3+); 10.72% were stained moderately (2+) and 14.28% were weakly stained (1+). 3.5% were negative for CDX2. 79.16% of adenomas were strongly stained; 8.3% were stained moderately and 4.1% were weakly stained. 4.1% were negative for CDX2. 80% adenocarcinomas took up stain strongly; 13.33% were moderately stained; 6.67% were weakly stained. (Table 2)

Table 2: Intensity of CDX2 staining

Intensity	Adenocarcinoma	Adenoma	Normal Mucosa
0	0	1	1
1+	1	2	4
2+	2	2	3
3+	12	20	20

There were two cases of adenocarcinoma of high-grade dysplasia. Both these cases were positive for CDX2, but at varying intensities, with one moderately stained and the other strongly stained. Similarly, there were two cases of adenocarcinomas of low-grade dysplasia, both of which were strongly stained for CDX2.

81.8% of moderately dysplastic cancer cells were strongly stained for CDX2. It was observed that reduced extent and intensity of staining for CDX2 was associated with more advanced stages of cancer (T2 to 4a). It was also found that in 28.5% of adenomas, the stain was better picked up and was more intense in the adenoma than the surrounding normal tissue. Adenomas that did not show strong staining for CDX2 belonged to varying grades of dysplasia.

One specimen of hyperplastic polyp (33.3%) showed moderately intense staining for CDX2 whereas the others took up the stain strongly. No specific trend was observed with respect to lesion configuration and CDX2 expression by the tumor cell.

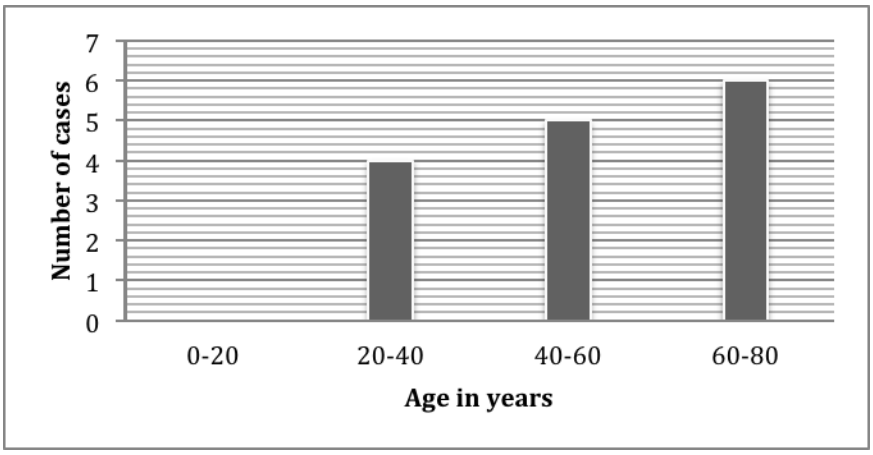

Figure 1: Age Distribution

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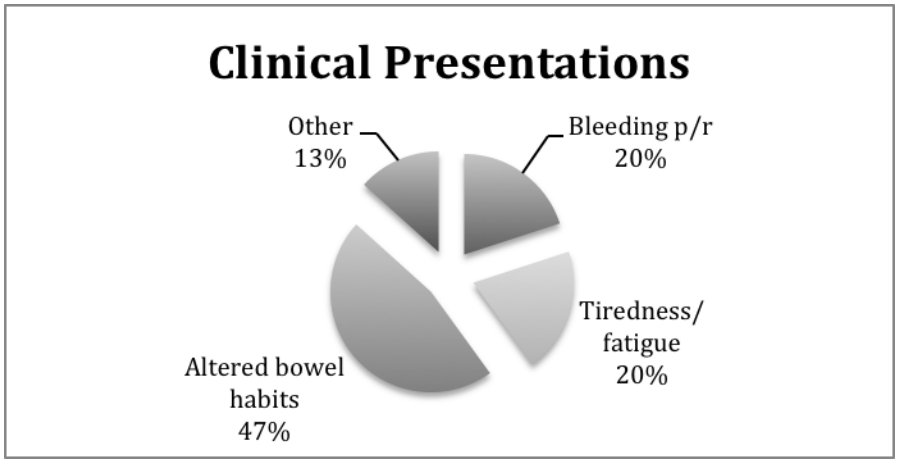

Figure 2: Clinical Presentations

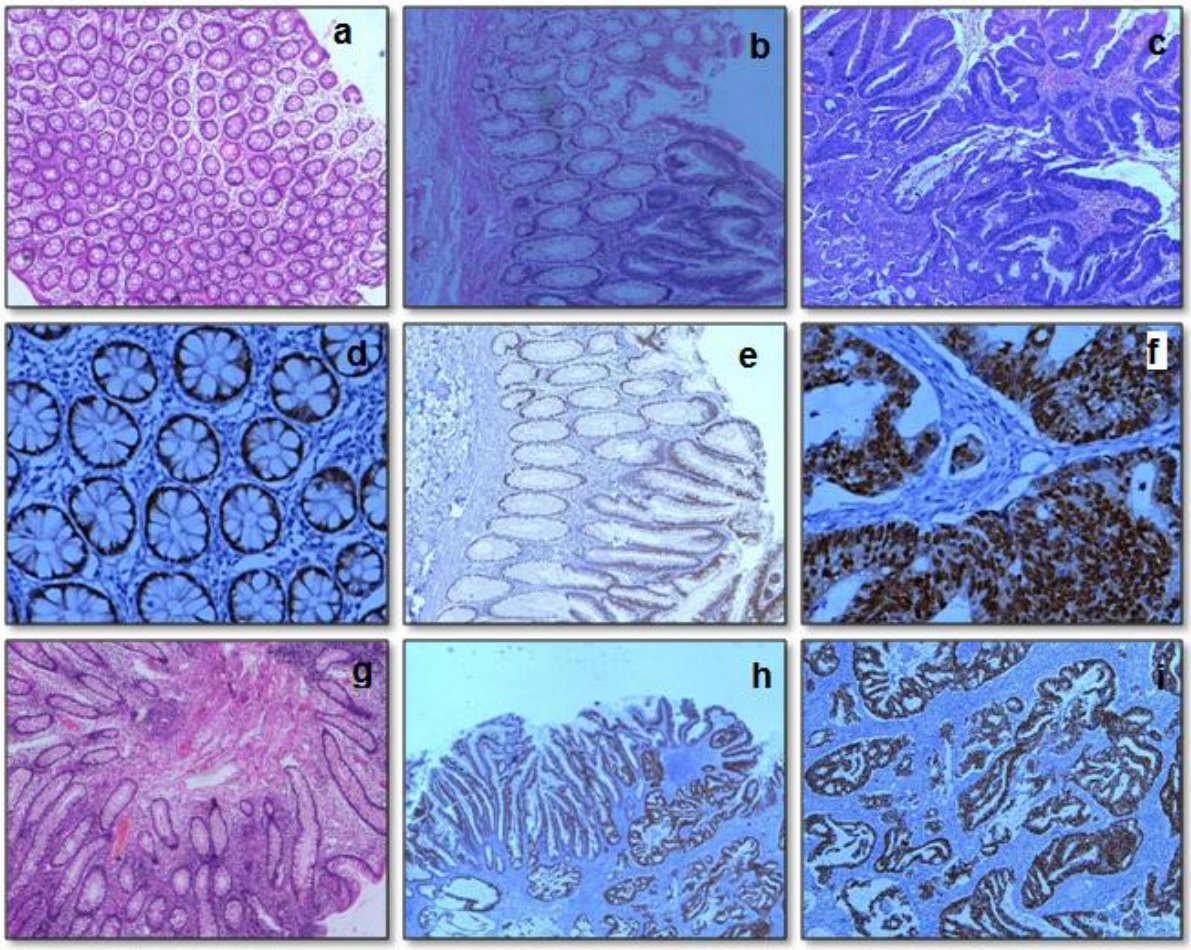

Figure 3: (a) H&E Staining of Normal Mucosa (4X); (b) H&E Staining of Adenoma adjacent to Normal Mucosa (4X); (c) H&E Staining of Adenocarcinoma (4X); (d) CDX2 expression in normal mucosa (20X); (e) CDX2 expression in adenoma adjacent to normal mucosa (4X); (f) CDX2 expression in adenocarcinoma (20X); (g) H&E Staining of Polyp (4X); (h) CDX2 expression in cancer cells adjacent to adenoma (2X); (i) CDX2 expression in adenocarcinoma (4X)

DISCUSSION

We studied a total of 29 cases including colonic adenocarcinomas, polyps and adenomas. Our findings were in accordance with a study by Sp eights VO et al[10] who found that a majority of colorectal cancers are detected from symptoms referable to the gastrointestinal tract and that patients with low stage colorectal cancer and smaller tumors were less likely to be anemic at presentation, and were more likely to have tumors located in the left side of the colon than patients with tumors at stage higher stages. Another study by Chattar-Cora et al[11] showed that adenocarcinoma was more common in the distal colon and rectum, which was similar to our findings. However, the male to female ratio was 47.6% to 52.4%, which contrasted from what we observed.

Bakaris S[12] et al conducted a study on expression of Cdx2 in colorectal adenomas as well as adenocarcinomas. They found that 100% of adenomas showed strong nuclear staining for CDX2. 30 (88.2%) of 34 colorectal adenocarcinomas, including 17(94.47%) of 18 well or moderately differentiated tumors and 13(81.2%) of 16 high-grade tumors also showed strong staining, which was similar to our findings. Another study showed that the percentage of CDX2 immunopositive cells was lower in carcinomas than in adenomas and lower in moderately or poorly differentiated tumors than in well-differentiated tumors. However, our study showed higher expression in carcinomas and we observed that higher degrees of dysplasia were associated with stronger staining. A study by Werling et al[14] showed that CDX2 was expressed uniformly in all of the evaluated colorectal and duodenal tumors. High-level expression was also seen in mucinous ovarian carcinomas and adenocarcinomas primary to the urinary bladder. This was in accordance with our findings. However, we did not evaluate adenocarcinomas of non-colorectal origin. Hooi C. E et al[13] found that expression of CDX2 in normal mucosa was localized to epithelial nuclei and carcinomas showed marked reduction in nuclear staining, with most cases negative for CDX2, which was in contrast with our findings.

CONCLUSION

From this study, it is found that CRC most commonly occurred in individuals aged between 60 – 80 years with more number of males than compared to females. The rectum was found to be the commonest site where malignancy occurred. Patients most commonly presented with complaints of altered bowel habits and a history of bleeding per rectum or with a history of tiredness / fatigue. Majority of the malignant tumors were ulceroproliferative in configuration.

All cases of cancer were positive for CDX2 while 96.15% of adenomas and 96.42% of normal mucosal tissue showed CDx2 positivity. Most cases of cancer showed strongly intense staining while adenomas and normal mucosa showed variability in intensity of staining. Adenomas of high-grade dysplasia show stronger CDX2 staining. Polyps and adenomas of milder degrees of dysplasia show variable staining. Advanced stages of cancer were associated with weaker staining.

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