International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Expression of Cox-2 Immunohistochemistry in Colorectal Cancer and its Correlation with Clinicopathological Parameters

Dr Navneet Kaur[1], Dr Rupinder Kaur[2], Dr Manpreet Kaur[3]

[1]3rd year Junior Resident, Department of Pathology, MMIMSR, Mullana
[2]Professor, Department of Pathology, MMIMSR, Mullana
[3]Assistant Professor, Department of Pathology, MMIMSR, Mullana

Corresponding Author:

Dr Navneet Kaur
3rd year Junior Resident,
Department of Pathology,
MMIMSR, Mullana
Email:
bassinavneet51@gmail.com

Received: 20-06-2026
Accepted: 08-07-2026
Available online: 11-07-2026

ABSTRACT

Background: Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality worldwide. Cyclooxygenase-2 (COX-2) plays an important role in colorectal carcinogenesis by promoting tumor cell proliferation, angiogenesis, invasion, and inhibition of apoptosis. Evaluation of COX-2 expression may provide valuable prognostic information and identify potential therapeutic targets in colorectal adenocarcinoma.
Aim: To evaluate the clinicopathological profile of colorectal adenocarcinoma, assess and correlate COX-2 expression by immunohistochemistry with clinicopathological parameters.
Materials and Methods: This was a 3-year cross-sectional ambispective study conducted by Department of Pathology in a tertiary care rural institution. A total of 50 histopathologically confirmed cases of colorectal adenocarcinoma diagnosed on biopsy or resection specimens were included. Clinical and histopathological details were recorded, and immunohistochemistry for COX-2 was performed using monoclonal antibody. COX-2 expression was evaluated based on staining intensity and the percentage of positively stained tumor cells. Statistical analysis was performed using SPSS version 25.0, and a p-value <0.05 was considered statistically significant.
Results: The mean age of the patients was 53.04 ± 16.05 years, with a slight female predominance (52%). Pain abdomen (44.0%) was the most common presenting complaint. Biopsy specimens constituted majority of the cases (68.0%), with right-sided colon being the most frequently involved site (36.0%). Moderately differentiated adenocarcinoma was the predominant histological grade (92.0%). Strong COX-2 staining intensity was observed in 66.0% of cases, of which 88.0% of tumors showed positivity in 76–100% of tumor cells. COX-2 total score showed a significant positive correlation with tumor ($r_s = 0.341$, $p = 0.041$) and nodal stage ($r_s = 0.298$, $p = 0.048$), whereas no significant association was found with age, sex, or histological grade.
Conclusion: COX-2 was highly expressed in the majority of colorectal adenocarcinoma cases and demonstrated significant association with advanced tumor stage and lymph node involvement. These findings suggest that COX-2 may serve as a useful prognostic biomarker and a potential therapeutic target in colorectal adenocarcinoma.

Keywords: *Colorectal adenocarcinoma, Cyclooxygenase-2, COX-2, Immunohistochemistry, Colorectal cancer, Prognostic biomarker, Tumor stage.*

INTRODUCTION

Colorectal cancer (CRC) is the most common malignancy of the gastrointestinal tract and ranks as the third most frequently diagnosed cancer and the third leading cause of cancer-related mortality worldwide[1]. The global burden of CRC continues to increase, with new cases projected to reach approximately 3.2 million by 2040, making it a major public health concern

[2]. Although the incidence and mortality of CRC among individuals older than 50 years have declined due to effective screening programs, early diagnosis, and modification of risk factors, the incidence among younger adults (<50 years) has nearly doubled since the early 1990s, emphasizing the need for improved prognostic markers and targeted therapeutic approaches [3,4].Colorectal carcinogenesis is a complex, multistep process influenced by genetic susceptibility, environmental factors, dietary habits, and chronic inflammation. Approximately 85–95% of CRC cases are sporadic and are strongly associated with diets rich in animal fat and red meat, whereas dietary fiber and calcium intake have been shown to exert protective effects [5,6].Traditionally, prognosis in CRC is based on clinicopathological parameters such as tumor size, histological grade, lymph node status, and TNM stage. However, these factors do not adequately explain the variability in clinical outcomes among patients with similar pathological features. Consequently, considerable attention has been directed toward identifying molecular biomarkers that improve prognostic assessment and guide individualized treatment strategies [8]. Among the molecular pathways implicated in colorectal carcinogenesis, the cyclooxygenase (COX) pathway (COX-1, COX-2) has attracted significant interest. Cyclooxygenase catalyzes the conversion of arachidonic acid into prostaglandins, which regulate inflammation, angiogenesis, apoptosis, cell proliferation, differentiation, and migration [9,10]. Unlike COX-1, COX-2 plays an important role in inflammation and tumor development and its overexpression has been documented in numerous malignancies, including colorectal, gastric, breast, lung, pancreatic, hepatic, and prostate cancers [13,14]. In CRC, COX-2 expression increases during the adenoma–carcinoma sequence and remains elevated throughout tumor progression. Enhanced COX-2 activity results in increased prostaglandin E2 (PGE2) production, promoting tumor cell proliferation, inhibition of apoptosis, angiogenesis through vascular endothelial growth factor (VEGF), invasion, metastasis, and suppression of antitumor immunity. Although surgery remains the cornerstone of curative treatment, chemotherapy is widely used for advanced disease, where recurrence and metastasis remain major challenges [15]. Advances in molecular oncology have facilitated the development of targeted therapies directed against specific molecular abnormalities [16]. Furthermore, epidemiological studies have demonstrated that regular use of non-steroidal anti-inflammatory drugs (NSAIDs), particularly selective COX-2 inhibitors such as celecoxib, reduces the incidence of colorectal adenomas and carcinoma, especially in patients with familial adenomatous polyposis, although their long-term use is limited by gastrointestinal and cardiovascular toxicity [17,18]. Therefore, evaluation of COX-2 expression and its association with clinicopathological parameters may provide valuable prognostic information and support the development of novel targeted therapeutic strategies for colorectal cancer.

MATERIALS AND METHODS

A 3-year cross-sectional ambispective study was conducted by Department of Pathology, at a rural tertiary care centre in north India. The study was undertaken to assess the frequency of cyclooxygenase-2 (COX-2) expression by immunohistochemistry (IHC) in 50 histologically confirmed biopsy/resected specimen cases of colorectal adenocarcinoma. A minimum sample size of 50 cases was included. The study was approved by the Institutional Ethics Committee (IEC Reference No. IEC-3255).

Inclusion Criteria

- Histologically diagnosed cases of colorectal adenocarcinoma (biopsy and/or resection specimens) were taken.

Exclusion Criteria

- Diagnosed cases where tissue blocks were unavailable.
- Known cases with prior chemotherapy/recurrent tumor.
- Cases with extensive necrosis without sufficient viable tumor cells.
- Non compliant patients or patients not willing to participate in the study.

Demographic details, relevant clinical history, imaging findings, laboratory investigations, colonoscopic findings, and histopathological data were retrieved from hospital medical records as well as histopathology requisition forms. All information was recorded using a predesigned study proforma.

For ambispective cases ,archived hematoxylin and eosin (H&E)-stained slides and formalin-fixed paraffin-embedded tissue blocks of previously diagnosed colorectal adenocarcinoma cases were retrieved from the departmental archives. All slides were reviewed independently, and relevant histopathological findings were documented. Fresh representative sections were prepared from the available paraffin blocks whenever additional routine or special staining was required.

Colonoscopic biopsy specimens and resected colorectal specimens were received in 10% neutral buffered formalin and processed according to the departmental protocol. Entire tissue was processed in small biopsy specimens whereas resection specimens were grossed as per department SOP. Representative sections were obtained and were subjected for routine tissue processing. Sections measuring 3–4 µm in thickness were prepared and stained with hematoxylin and eosin. The diagnosis of colorectal adenocarcinoma was confirmed on microscopic examination of H&E-stained sections. Special stains including Periodic Acid–Schiff (PAS), Diastase-Resistant Periodic Acid–Schiff (DPAS), and Alcian Blue–Periodic Acid–Schiff (AB-PAS) were performed whenever indicated.

Immunohistochemistry for COX-2
Following histopathological confirmation, an additional representative section measuring 3–4 µm was cut from the paraffin block and mounted on poly-L-lysine-coated slides for immunohistochemical staining. Immunohistochemistry was performed using monoclonal COX-2 antibody according to the standard laboratory protocol.

Evaluation of COX-2 Expression
COX-2 expression was evaluated as cytoplasmic staining in tumor cells. Immunoreactivity was assessed using the scoring system described by Venkatachala et al [23]. The final immunohistochemical score was calculated by adding the proportion score and intensity score.

Proportion Score

Percentage of Positive Tumor Cells	Score
No positive cells	0
1–25%	1
26–50%	2
51–75%	3
76–100%	4

Intensity Score

Staining Intensity	Score
No staining	0
Weak (yellow)	1
Moderate (yellow-brown)	2
Strong (brown)	3

Interpretation of COX-2 Expression

Total Score	Interpretation
0–2	Negative
3–4	Low positive
5–7	High positive

Statistical Analysis
The collected data was entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0. Continuous variables were expressed as mean ± standard deviation (SD), median, interquartile range (IQR), and range, whereas categorical variables were presented as frequencies and percentages. Associations between categorical variables, particularly histological grade and COX-2 expression (staining intensity and proportion of positive cells), were evaluated using Fisher's exact test owing to the relatively small subgroup sample sizes. A p-value of <0.05 was considered statistically significant.

RESULTS
The mean age of the patients was 53.04 ± 16.05 years with an age range of 17–81 years. The highest proportion of patients belonged to the 50–59 years and 60–69 years age groups (20.0% each). There was a slight female predominance, with 26 (52.0%) females and 24 (48.0%) males. Pain abdomen was the most common presenting complaint (44.0%), followed by bleeding per rectum (28.0%) and constipation (20.0%). The baseline demographic and clinical characteristics of the study population are summarized in Table 1. The clinicopathological characteristics of colorectal adenocarcinoma are presented in Figure 1. Biopsy specimens constituted the majority of cases (68.0%), and most common tumor site being the right-sided colon (36.0%). Histologically, moderately differentiated adenocarcinoma was the predominant grade (92.0%). Lymph node status was unavailable in 68.0% of cases because the majority were biopsy specimens. Among the resected specimens, T3 was the most common pathological stage (22.0%), followed by T2 (6.0%) and T4 (4.0%). Nodal staging revealed N0 and N2 disease in 12.0% of cases each, while N1 disease was present in 8.0%. Strong COX-2 staining intensity was observed in 66.0% of tumors, intermediate staining in 28.0%, and weak staining in 6.0%. Most tumors (88.0%) demonstrated COX-2 expression in 76–100% of tumor cells. Based on the combined immunohistochemical score, 98.0% of tumors showed high positive COX-2 expression. The distribution of pathological stage and COX-2 immunohistochemical expression is shown in Figure 2. None of the cases were negative for COX-2 expression. Strong staining intensity was most frequently observed in moderately differentiated tumors, while the majority of moderately differentiated tumors also demonstrated COX-2 positivity in 76–100% of tumor cells. However, neither staining intensity ($p = 0.421$) nor the percentage of tumor cells stained ($p = 0.538$) showed a statistically significant association with

histological grade based on Fisher's exact test. A significant positive correlation was observed between COX-2 expression and pathological tumor stage ($r_s = 0.341$, $p = 0.041$) as well as nodal stage ($r_s = 0.298$, $p = 0.048$), indicating increasing COX-2 expression with advancing disease stage. In contrast, patient age, gender, and histological grade did not show any statistically significant correlation with COX-2 total score ($p > 0.05$). The association between histological grade and COX-2 expression is summarized in Table 2.

Table 1. Baseline Demographic and Clinical Characteristics of the Study Population (n = 50)

Variable	Category	Frequency (%) / Value
Age (years)	Mean ± SD	53.04 ± 16.05
	Median (IQR)	53 (25.5)
	Range	17–81
Age Group (years)	0–19	1 (2.0)
	20–29	3 (6.0)
	30–39	8 (16.0)
	40–49	8 (16.0)
	50–59	10 (20.0)
	60–69	10 (20.0)
	70–79	9 (18.0)
	80–89	1 (2.0)
Gender	Male	24 (48.0)
	Female	26 (52.0)
Presenting Complaints*	Pain abdomen	22 (44.0)
	Bleeding per rectum	14 (28.0)
	Constipation	10 (20.0)
	Painful defecation	8 (16.0)
	Blood in stools	6 (12.0)
	Vomiting	3 (6.0)
	Diarrhoea	2 (4.0)
	Mass per rectum	2 (4.0)
	Altered bowel habits	2 (4.0)
	Others	3 (6.0)

*Multiple complaints were reported by individual patients.

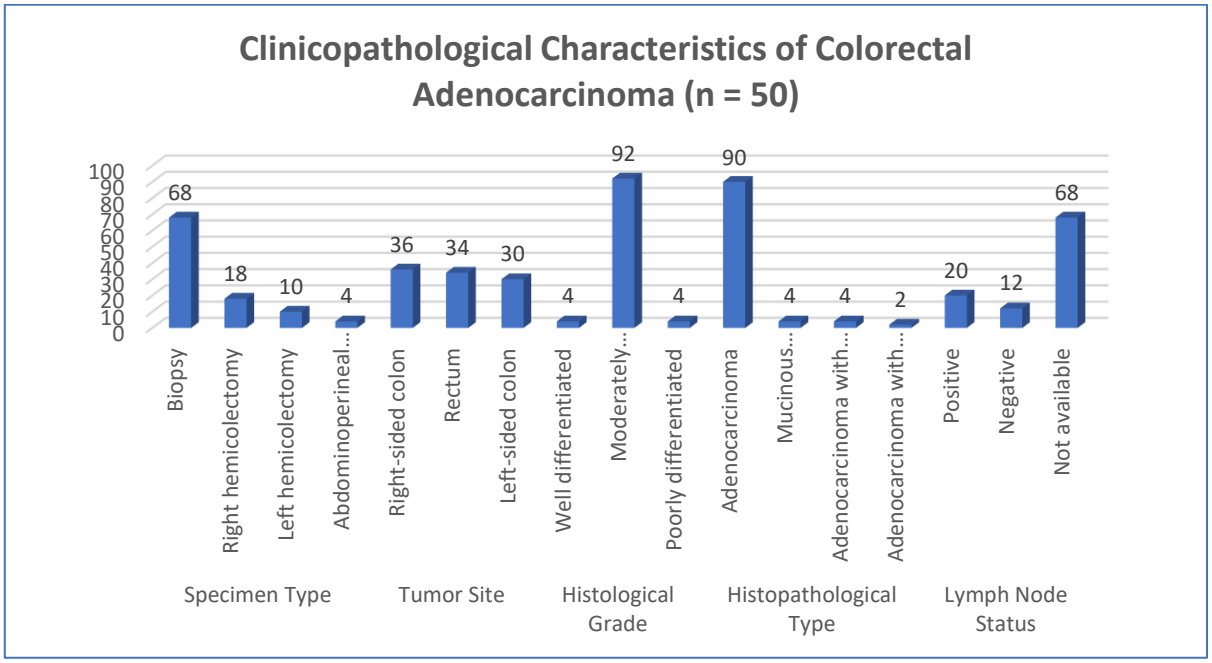

Figure 1 Clinicopathological Characteristics of Colorectal Adenocarcinoma (n = 50)

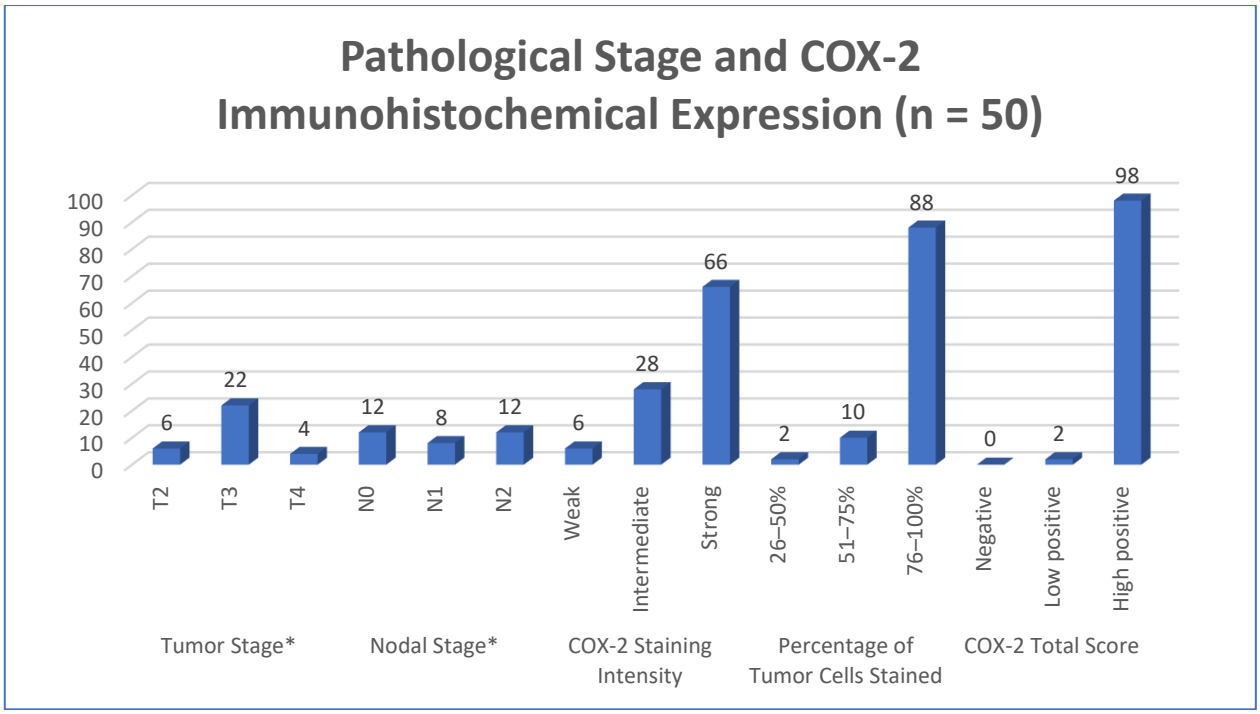

Figure 2 Pathological Stage and COX-2 Immunohistochemical Expression (n = 50)

Table 2. Association Between Histological Grade and COX-2 Expression

Histological Grade	Weak Intensity n (%)	Intermediate Intensity n (%)	Strong Intensity n (%)	26–50% Cells n (%)	51–75% Cells n (%)	76–100% Cells n (%)
Well differentiated (n=2)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (50.0)	1 (50.0)
Moderately differentiated (n=46)	2 (4.3)	12 (26.1)	32 (69.6)	1 (2.2)	4 (8.7)	41 (89.1)
Poorly differentiated (n=2)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
P-value	**0.421**			**0.538**		
Statistical test	**Fisher's exact test**			**Fisher's exact test**		

DISCUSSION

The present study was done on 50 HPE confirmed cases of colorectal carcinoma to evaluate the clinicopathological profile and COX-2 expression among them. The mean age of patients was 53.04 ± 16.05 years (range: 17–81 years), with most cases occurring in the fifth and sixth decades. Similar findings were reported by Wu and Sun [19] (59.6 ± 13.5 years), Xiong et al. [20] (58.5 ± 15.2 years), and Ibrahim et al. [21] (55.3 ± 14.7 years), confirming that colorectal carcinoma predominantly affects middle-aged and elderly individuals while in the present study, age was not significantly associated with COX-2 expression. A slight female predominance (52.0%) was observed, similar to Ezenkwa et al. [22], who reported an almost equal sex distribution (51.6% males, 48.4% females). In contrast, Wu and Sun [19], Xiong et al. [20], Venkatachala et al. [23], and Ibrahim et al. [21] reported male predominance ranging from 55.4% to 63.7%. No significant relationship between gender and COX-2 expression was identified in the present study. Pain abdomen (44.0%) was the most frequent presenting symptom, followed by bleeding per rectum (28.0%) and constipation (20.0%). Comparable observations were reported by Wu and Sun [19], who found abdominal pain in 63.0% of patients, and Xiong et al. [20], who reported abdominal pain in 50.0% and rectal bleeding in 38.0%, indicating that these remain the common clinical manifestations of colorectal carcinoma. Colon tumors (66.0%) were more frequent than rectal tumors (34.0%), with right-sided lesions slightly exceeding left-sided lesions. In contrast, Wu and Sun [19] reported rectal involvement (52.83%). Ezenkwa et al. [22] too observed rectal predominance (67.4%) in their study. Strong to intermediate COX-2 expression was noted in both colonic and rectal tumors, suggesting no significant influence of tumor location in our study. Moderately differentiated adenocarcinoma was the predominant histological grade (92.0%), in the present study which was similar to Venkatachala et al. [23] (64.6%). Wu and Sun [19] reported 57.4% moderately differentiated tumors, whereas Xiong et al. [20] and Ezenkwa et al. [22] demonstrated a wider distribution of histological grades. These findings indicate that moderately differentiated adenocarcinoma remains the commonest histological subtype. Lymph node status was assessable

only in resection specimens, with nodal metastasis identified in 10 cases. The lower nodal positivity compared with Wu and Sun [70] (85.0%), Ezenkwa et al. [22] (64.9%), Venkatachala et al. [23] (47.7%), and Ibrahim et al. [21] (73.3%) in our study was largely attributable to the predominance of biopsy specimens (68.0%) of the cases. COX-2 expression was positive in all cases, with strong staining in 66.0%. Total score showed high positivity in 98.0% of the cases. Among moderately differentiated tumors, strong staining (68.9%) was comparable to Venkatachala et al. [23] (66.6%) which was higher than that reported by Ezenkwa et al. [22] (42.0%) and Ibrahim et al. [21] (40.0%). A significant positive correlation was observed between COX-2 expression and tumor ($r_s = 0.341$, $p = 0.041$) as well as nodal stage ($r_s = 0.298$, $p = 0.048$), indicating increasing COX-2 expression with advancing disease. Similar observations were made by Venkatachala et al. [23], who reported increasing COX-2 positivity from 33.3% in stage I to 100% in stage IV tumors. Ibrahim et al. [21] demonstrated significant associations between high COX-2 expression and deeper invasion (69.1%, $p = 0.007$), nodal metastasis (73.3%, $p = 0.011$), and lymphovascular invasion (75.2%, $p = 0.005$). Likewise, Loukanov et al. [24] reported moderate-to-strong COX-2 expression with an approximately eight-fold increase in COX-2 mRNA in colorectal carcinoma. These findings support the role of COX-2 overexpression as a marker of tumor progression and aggressive biological behavior in colorectal adenocarcinoma.

CONCLUSION

The present study demonstrated that colorectal adenocarcinoma occurred predominantly in the fifth to seventh decades of life, with moderately differentiated adenocarcinoma being the most common histological subtype. COX-2 was highly expressed in the majority of cases, with 98% showing high positive expression and 66% exhibiting strong staining intensity. A significant positive correlation was observed between COX-2 expression and advanced tumor stage as well as nodal involvement, suggesting its association with tumor progression. These findings indicate that COX-2 may serve as a valuable prognostic biomarker and a potential therapeutic target in colorectal adenocarcinoma. However, larger multicentric studies with long-term follow-up are required to further validate its prognostic and therapeutic significance.

Limitations

The present study was conducted at a single tertiary care center in a rural set up with a relatively small sample size, which may limit the generalizability of the findings. A large proportion of cases were biopsy specimens, restricting complete pathological staging and lymph node assessment. In addition, long-term clinical follow-up and survival analysis were not available, precluding evaluation of the prognostic impact of COX-2 expression on patient outcomes. Multicentric studies with larger sample sizes and long-term follow-up are required to validate these findings.

REFERENCES

1. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 2021;14(10):101174.
2. Murphy CC, Sandler RS, Sanoff HK, Yang YC, Lund JL, Baron JA, et al. Decrease in incidence of colorectal cancer among individuals 50 years or older after recommendations for population-based screening. Clin Gastroenterol Hepatol. 2017;15(6):903-9.
3. Edwards BK, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, et al. Annual report to the nation on the status of cancer, 1975–2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. Cancer. 2010;116(3):544-73.
4. Dharwadkar P, Zaki TA, Murphy CC. Colorectal cancer in younger adults. Hematol Oncol Clin North Am. 2022;36(3):449-70.
5. Lipkin M, Newmark H. Effect of added dietary calcium on colonic epithelial-cell proliferation in subjects at high risk for familial colonic cancer. N Engl J Med. 1985;313(22):1381-4.
6. Reddy B, Engle A, Katsifis S, Simi B, Bartram HP, Perrino P, et al. Biochemical epidemiology of colon cancer: Effect of types of dietary fiber on fecal mutagens, acid, and neutral sterols in healthy subjects. Cancer Res. 1989;49(16):4629-35.
7. Wang D, DuBois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. Oncogene. 2010;29(6):781-8.
8. Peng L, Zhou Y, Wang Y, Mou H, Zhao Q. Prognostic significance of COX-2 immunohistochemical expression in colorectal cancer: A meta-analysis of the literature. PLoS One. 2013;8(3):e58891.
9. Sheng J, Sun H, Yu FB, Li B, Zhang Y, Zhu YT, et al. The role of cyclooxygenase-2 in colorectal cancer. Int J Med Sci. 2020;17(8):1095-101.
10. Xiong B, Sun TJ, Hu WD, Cheng FL, Mao M, Zhou YF, et al. Expression of cyclooxygenase-2 in colorectal cancer and its clinical significance. World J Gastroenterol. 2005;11(8):1105-8.
11. Cao Y, Prescott SM. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J Cell Physiol. 2002;190(3):279-86.
12. Koki AT, Masferrer JL. Celecoxib: A specific COX-2 inhibitor with anticancer properties. Cancer Control. 2002;9(2 Suppl):28-35.
13. Grover J, Yadav S, Vats V, Joshi Y. Cyclo-oxygenase-2 inhibitors: Emerging roles in the gut. Int J Colorectal Dis. 2003;18(4):279-91.
14. Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rödel CD, Cervantes A, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28(Suppl 4):iv22-40.

15. Ke X, Shen L. Molecular targeted therapy of cancer: The progress and future prospect. Front Lab Med. 2017;1(2):69-75.
16. DuBois RN. Nonsteroidal anti-inflammatory drug use and sporadic colorectal adenomas. Gastroenterology. 1995;108(4):1310-4.
17. Brown JR, DuBois RN. COX-2: A molecular target for colorectal cancer prevention. J Clin Oncol. 2005;23(12):2840-55.
18. Hu Z, Hu Y, Jiang H. Overexpression of COX-2 and clinicopathological features of gastric cancer: A meta-analysis. Transl Cancer Res. 2020;9(4):2200-10.
19. Wu QB, Sun GP. Expression of COX-2 and HER-2 in colorectal cancer and their correlation. World J Gastroenterol. 2015;21(20):6206-14.
20. Xiong B, Sun TJ, Hu WD, Cheng FL, Mao M, Zhou YF, et al. Expression of cyclooxygenase-2 in colorectal cancer and its clinical significance. World J Gastroenterol. 2005;11(8):1105-8.
21. Ibrahim NI, Shamsudin SH, Lee SK, Sharif SE. Prognostic significance of cyclooxygenase-2 expression in colorectal adenoma and adenocarcinoma: A clinicopathologic study. Hum Pathol Rep. 2023;31:300692.
22. Ezenkwa US, Okolo CA, Ogun GO, Akere A, Ogunbiyi OJ. Cyclooxygenase-2 expression in colorectal carcinoma, adenomatous polyps and non-tumour bearing margins of resection tissues in a cohort of Black Africans. PLoS One. 2021;16(7):e0255235.
23. Venkatachala S, Rajendran M. Correlation of COX-2 expression in colorectal carcinoma with clinicopathological features. Turk Patoloji Derg. 2017;33(3):228-34.
24. Loukanov T, Kirilov M, Fürstenberger G, Müller-Decker K. Localization of cyclo-oxygenase-2 in human recurrent colorectal cancer. Clin Invest Med. 2010;33(1):E22-9.