Cyclo-oxygenase-2 and vascular endothelial growth factor expression in colorectal cancer patients

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Introduction
Colonic neoplastic lesions represent a common health problem in Egypt. Colorectal carcinoma (CRC) is one of the common malignancies among Egyptians. There is considerable interest in the involvement of cyclooxygenase-2 (COX-2) in colon carcinogenesis and its progression. Vascular endothelial growth factor (VEGF) is a well-characterized tumor angiogenesis factor which has a role in the development, progression and risk of metastases of CRC
The aim of the present study was to explore the correlation between COX-2 and VEGF colon tissue expression profile in colorectal cancer patients with a special emphasis on clinicopathological features.
Patients and methods
This study was carried out on 40 patients with colorectal cancer (CRC). CT and Colonoscopy were mandatory for staging and grading. CRC classification, grading and staging was done following the American Joint Committee on Cancer (AJCC) staging system.
Results
Contralateral site control biopsies were totally negative for both biomarkers in the CRC patients. COX-2 & VEGF were over expresed intensly in the advanced stage and grade of the postive expression in the CRC samples obtaind during surgery.
Conclusion
The over-expression of COX-2 and VEGF in colorectal cancer suggests the role of both of them as a risk biomarker particularly in patients with advanced stage and grade.

Keywords:
colorectal carcinoma, cyclo-oxygenase-2, vascular endothelial growth factor

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Introduction

There is considerable interest in the involvement of cyclo-oxygenase-2 (COX-2) in colon carcinogenesis and its progression. This was initiated by clinical observations in which patients who had taken NSAIDs were associated with a 30–50% reduction in colorectal cancer (CRC) risk [1,2]. COX-2, which leads to the synthesis of prostaglandins, is one of the known targets of NSAIDs and it has been reported that tumor cells overexpressing COX-2 stimulate angiogenesis, inhibit apoptosis, and increase the metastatic potential by producing prostaglandins in colon cancer cell lines [3,4].

Although NSAIDs act through COX-2 to inhibit colon cancer growth, there also appears to be COX-2-independent actions for NSAIDs. COX-2 selective inhibitors can be the core drugs for the prevention and treatment of colon cancer [5,6].

The vascular endothelial growth factor (VEGF) expression in colon cancer tissues has not been clearly studied.

VEGF is a well-characterized tumor angiogenesis factor that has a role in the development and progression of CRC and in its risk for metastases [7].

The aim of the present study was to explore the correlation between COX-2 and VEGF colon tissue expression profile in CRC patients with special emphasis on clinicopathological features.

Patients and methods

This study was carried out on 40 patients with CRC at the Theodor Bilharze research Institute after obtaining the approval of IRB. All patients were subjected to history taking and clinical examination. Computed tomography and colonoscopy were mandatory for staging and grading. During colonoscopy two biopsies were obtained from each patient: one from the lesion for diagnosis and the other from the contralateral normal side to serve as a control. All patients were subjected to elective hemicolectomy according to the site and extent of the lesion. All biopsies and surgical specimens were investigated with immunohistochemical analysis for anti-COX-2 and anti-VEGF. Serial sections were

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cut from paraffin blocks and stained with hematoxylin and eosin for routine histological examination. CRC classification, grading, and staging were carried out following the American Joint Committee on Cancer AJCC staging system.

Immunohistochemical procedures

Four-micrometer-thick tissue sections were cut from the paraffin blocks (containing both tumor and benign tissues), mounted on charged poly-l-lysine-coated slides, and subjected to immunohistochemical analysis using the avidin–biotin detection system, following the manufacturer's instructions. The antibody used was a mouse anti-human COX-2 monoclonal antibody (Dako Cytomation Norden A/S, Glostrup, Denmark; dilution 1: 50). Immunohistochemical analysis was carried out using an automatic immunostainer (Ventana Bench Mark XT; Ventana Inc., Tucson, Arizona, USA). In each analysis, positive controls consisting of CRC samples previously shown to stain with this antibody were used. Tris-buffered saline in place of the primary antibody was used as a negative control.

Interpretation of immunohistochemical staining

Cells were considered positive for COX-2 when distinct yellow to brown staining was identified in the cytoplasm and occasionally in the nuclear envelope. The extent and intensity of the staining were recorded on a scale from 0 to +++; +++ implied strong staining that was maximally intense throughout the specimen, and 0 implied negative staining. When dichotomized for statistical risk assessment (odds ratio), negative (–) and weak (+) staining was defined as low expression, whereas moderate (++) and intense (+++) staining was included in the high expression category.

Statistical analysis

Continuous variables are expressed as number and percentage. Statistical comparisons were made with the Pearson χ^2-test. Statistical significance was defined by a P value less than 0.05.

Results

The mean age of the patients was 52 years (range: 24–69 years). The associations between COX-2 and VEGF expression and clinicopathological features showed no significant differences with respect to sex, age, or tumor location. There were 22 male patients and 18 female patients; 26 patients were older than 50 years and 14 patients were younger than 50 years; and 11 patients had tumor located on the right side and 29 patients had tumor on the left side.

Clinical data of the patients with CRC, whose tissue samples were used in this study, are summarized in Table 1.

The COX-2 immunoexpression in epithelial cells and specimens is shown in Table 2. All control biopsies were negative for COX-2 expression (100%). Positive COX-2 immunoexpression showed significant increase in all surgical specimens compared with control biopsies (Figs. 1 and 2). An overall 67.5% of patients showed marked COX-2 immunoexpression with statistically significant difference ($P < 0.01$) from the rest of the patients, who showed moderate COX-2 immunoexpression (32.5%) (Table 2).

VEGF-positive immunoreactivity was detected as diffuse cytoplasmic brownish color within the epithelial cells. All control biopsies were negative for VEGF immunoreactivity. The extent of VEGF expression among the cases is shown in Table 2. CRC samples showed a higher percentage of positive cases for VEGF compared with control biopsies ($P < 0.01$) (Figs. 3 and 4).

As regards the intensity of COX-2 immunoexpression, 21 samples (75%) presented with strong COX-2 intensity, which was statistically significantly different from seven (25%) samples that presented with moderate intensity and none (0%) with mild intensity ($P < 0.01$ and $P < 0.01$, respectively). The number of samples with strong intensity (75%) was statistically significantly higher compared with controls ($P < 0.01$) (Figs 1 and 2). However, 75% of cases in the CRC group were intensely positive for VEGF, and the rest of the cases were mildly positive for VEGF (25%) with no statistically significant difference between them (Table 3).

However, tumor stage and distant metastasis were significantly associated with COX-2 expression, with

Table 1 Clinical data of the patients with colorectal cancer

Variables	Patients
Number	40
Men/women	22/18
Mean age (range) (years)	52 (24–69)
Tumor localization[a]	
Left colon	11
Right colon	29
Tumor stage (Dukes)	
A	5
B	15
C	20
Differentiation grade	
Well	6
Moderate	24
Poor	10

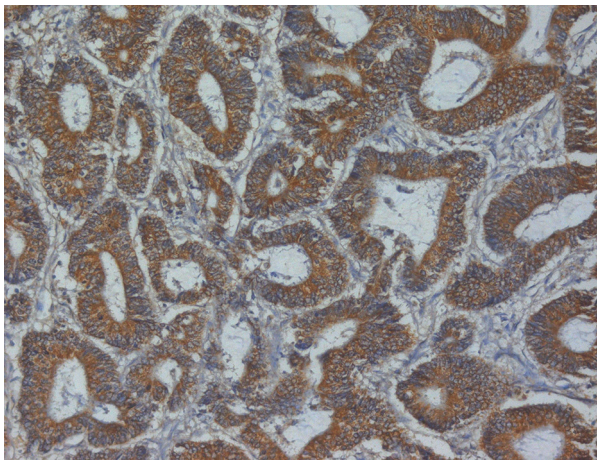
[a]Left-sided colon: descending colon, rectum and sigmoid; right-sided colon: cecum, ascending and transverse colon.

Table 2 COX-2 and VEGF immunoexpression in epithelial cells in both control and colorectal cancer patients

Pathological diagnosis	Range of COX-2[b] staining [*n* (%)]		Range of VEGF[c] staining [*n* (%)]	
	51–75 (%)	76–100 (%)	<10 (%)	>10 (%)
Control (*n* = 40)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CRC (*n* = 40)COX-2-positive cases 28/70%[a]VEGF-positivecases 30/75%[a]	11 (32.5)*	17 (67.5)*	10 (25)	30 (75)**

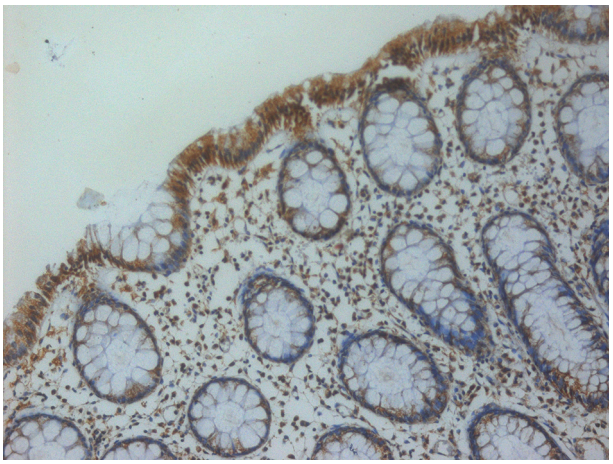
COX-2, cyclo-oxygenase-2; CRC, colorectal cancer; VEGF, vascular endothelial growth factor. [b]Range of COX-2 staining 1–25% and 26–50% equal zero for all control biopsies and CRC samples. [c]Range of VEGF<10% is considered negative for all control biopsies and CRC samples. [a]$P < 0.01$ compared with the control group, Pearson χ^2. *$P < 0.01$ compared with 26–50%, 51–75% expression respectively in the same group, Pearson χ^2. **$P < 0.01$ compared with the control group, Pearson χ^2.

Figure 1



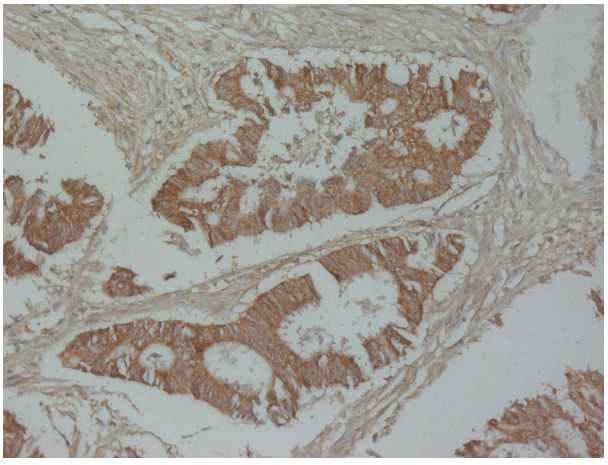
Colonic adenocarcinoma sample positive for COX-2 (IHC ×200). COX-2, cyclo-oxygenase-2.

Figure 2



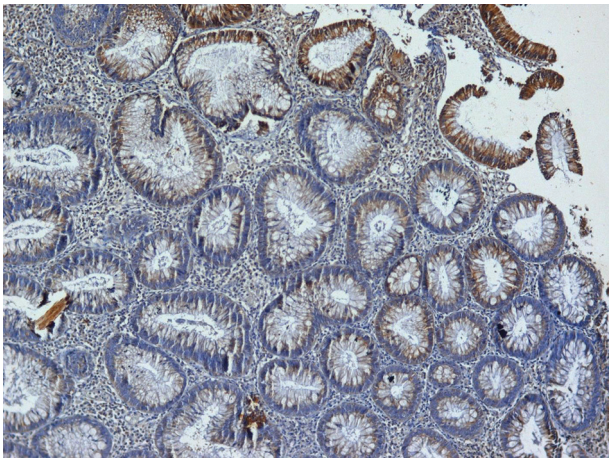
Control colonic Bx negative for COX-2 (IHC ×200). COX-2, cyclo-oxygenase-2.

Figure 3



Colonic adenocarcinoma sample positive for VEGF (IHC ×200). VEGF, vascular endothelial growth factor.

Figure 4



Control colonic Bx negative for VEGF (IHC ×200). VEGF, vascular endothelial growth factor.

higher expression being more common in advanced tumors. The percentage of Dukes C patients with positive immunoreactivity for COX-2 was statistically significantly higher compared with patients of Dukes A ($P < 0.01$) and Dukes B ($P < 0.01$). All patients with Dukes A showed moderate COX-2 immunoexpression (100%). An overall 71.4% of Dukes B patients had intense COX-2 immunoexpression, 28.6% had moderate COX-2 immunoexpression, and none (0%) had mild COX-2 expression. The results were statistically significantly different ($P < 0.01$). All patients with Dukes C showed intense COX-2 immunoexpression (100%) (Table 4).

The percentage of Dukes C patients positive for VEGF was statistically significantly higher

compared with Dukes A patients ($P < 0.01$). The percentage of Dukes B patients positive for VEGF was also statistically significantly higher compared with Dukes A patients ($P < 0.05$). All positive patients with Dukes A showed moderate VEGF immunoexpression (100%). All positive patients with Dukes B showed moderate VEGF immunoexpression (100%). All positive patients with Dukes C showed intense VEGF immunoexpression (100%) (Table 4).

Patients with poorly differentiated adenocarcinoma (GIII) showed significant increase in positive COX-2 immunoexpression compared with patients with well-differentiated adenocarcinoma (GI) and moderately differentiated adenocarcinoma (GII) ($P < 0.01$ and $P < 0.01$, respectively). Patients with moderately differentiated adenocarcinoma (GII) showed significant increase in positive COX-2 immunoexpression compared with patients with well-differentiated adenocarcinoma (GI) ($P < 0.01$). An overall 66.7% of patients with well-differentiated adenocarcinoma (GI) showed moderate COX-2 immunoexpression compared with the rest of the patients with intense COX-2 immunoexpression (33.3%), with statistically significant difference between them ($P < 0.01$). All patients (100%) with poorly differentiated adenocarcinoma (GIII) showed intense COX-2 immunoexpression (Table 5).

Three of six patients with well-differentiated adenocarcinoma (GI) were positive for VEGF immunoexpression (50%), 17 of 24 patients with moderately differentiated adenocarcinoma (GII) were positive for VEGF immunoexpression (70.8%), and 10 of 10 patients with poorly differentiated adenocarcinoma (GIII) were positive for VEGF immunoexpression (100%).

Table 3 Intensity of COX-2 and VEGF immunoexpression in epithelial cells in positive CRC patients

Pathological diagnosis	Intensity of COX-2 staining [*n* (%)]		Intensity of VEGF staining [*n* (%)]	
	(++) Moderate	(+++) Strong	(++) Moderate	(+++) Strong
Control ($n = 40$)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CRC ($n = 40$) COX-2-positive cases 28/70% [a]VEGF-positive cases 30/75%a	7 (25)	21 (75)*	10 (33.3)	20 (66.7)

(+) Mild intensity of COX-2 and VEGF staining equal to zero for all control biopsies and CRC samples. COX-2, cyclo-oxygenase-2; CRC, colorectal cancer; VEGF, vascular endothelial growth factor; [a]$P < 0.01$ compared with the control group, Pearson χ^2. *$P < 0.01$ compared with the control group, Pearson χ^2.

Table 4 COX-2 and VEGF immunoexpression intensity in positive CRC patients in correlation with Dukes stage

Stages of adenocarcinoma	Range of COX-2[d] staining [*n* (%)]		Range of VEGF[e] staining [*n* (%)]
	51–75 (%)	76–100 (%)	>10
Duke A (5) COX-2-positive cases 1/20%VEGF-positive cases 1/20%	1 (100)	0 (0.0)*	1 (20)*
Duke B (15) COX-2-positive cases 7/43.3% [c]VEGF-positive cases 9/60%[c]	2 (28.6)	5 (71.4)*	9 (60)*
Duke C (20) COX-2-positive cases 20/100% [a,b]VEGF-positive cases 20/100%[a,b]	0 (0.0)	20 (100)*	20 (100)*

COX-2, cyclo-oxygenase-2; CRC, colorectal cancer; VEGF, vascular endothelial growth factor; [d]Range of COX-2 staining 1–25% and 26–50% equal zero for all control biopsies and CRC samples. [e]Range of VEGF <10% is considered negative for all control biopsies and CRC samples. [a,b]$P < 0.01$ compared with Dukes stage A, B, Pearson χ^2. [c]$P < 0.01$ compared with Dukes stage A, Pearson χ^2. *$P < 0.01$ compared with 51–75% COX-2 expression in the same group, Pearson χ^2. *$P < 0.01$ compared with <10% VEGF expression in the same group, Pearson χ^2.

Table 5 Immunoexpression intensity of COX-2 and VEGF in positive CRC cases in relation to their pathological grades

Histopathological grade of adenocarcinoma	Range of COX-2[d] staining [*n* (%)]		Range of VEGF[e] staining [*n* (%)]
	51–75 (%)	76–100 (%)	51–75 (%)
Well-differentiated GI ($n = 6$) COX-2-positive cases 3/50%VEGF-positive cases 3/50%	2 (66.7)	1 (33.3)*	3 (50)
Moderately differentiated GII ($n = 24$) COX-2-positive cases 15/62.5%[c]VEGF-positive cases 17/70.8%[c]	5 (33.3)	10 (66.6)*	17 (70.8)
Poorly differentiated GIII ($n = 10$) COX-2-positive cases 10/100%[a,b]VEGF-positive cases 10/100%[a,b]	0 (0.0)	10 (100)*	10 (100)

COX-2, cyclo-oxygenase-2; CRC, colorectal cancer; VEGF, vascular endothelial growth factor; [d]Range of COX-2 staining 1–25% and 26–50% equal zero for all control biopsies and CRC samples. [e]Range of VEGF <10% is considered negative for all control biopsies and CRC samples. [a,b]$P < 0.01$ compared with GI and GII, respectively, Pearson χ^2. [c]$P < 0.01$ compared with GI, Pearson χ^2. *$P < 0.01$ compared with 51–75% COX-2 expression in the same group, Pearson χ^2.

The percentage of poorly differentiated CRC patients with positive VEGF immunoexpression was statistically significantly higher compared with well-differentiated patients ($P < 0.01$). The percentage of moderately differentiated CRC patients with positive VEGF immunoexpression was also statistically significantly higher compared with well-differentiated patients ($P < 0.05$).

All positive cases of well-differentiated adenocarcinoma showed moderate VEGF immunoexpression. All positive cases of moderately and poorly differentiated adenocarcinoma showed intense VEGF immunoexpression (Table 5).

Discussion

Colonic neoplastic lesions represent a common health problem in Egypt. Colorectal carcinoma is one of the most common malignancies among Egyptians. Studies have shown that CRC patients under 30 years of age represent more than 20% of the total CRC patients [8,9].

COX-2 is an enzyme involved in the conversion of arachidonic acid to prostaglandins H2, the precursor of other prostaglandins and thromboxanes. These compounds are pivotal in the regulation of cell proliferation, angiogenesis, and the response of the human immune system to malignant tumor cells [10,11].

An important aspect in the measurement of COX-2 and VEGF expression is standardization. Standardization against control from the same patient will yield more accurate results compared with the use of a separate control group. To achieve this we obtained control biopsies from the healthy contralateral side during colonoscopy instead of from the safety margin area in the obtained pathological samples. In our study, all control biopsies with completely normal mucosa were negative for COX-2 immunoexpression, which was in agreement with previous studies [12].

We found that 70% of cases with CRC specimens were positive for COX-2 immunoexpression. This is in agreement with a study conducted by Joo *et al.* [13], which found that 62.6% of CRC patients exhibited markedly more intense positive COX-2 immunostaining compared with control colon cells.

In addition, this study showed that only 28 patients of the CRC group were positive for COX-2; and 10.7% were well-differentiated (GI), 53.5% were moderately differentiated (GII), and 35.8% were poorly differentiated (GIII). Among CRC patients with COX-2-positive immunoreactivity, 3.5% were in Dukes stage A, 25% were in Dukes stage B, and 71.5% were in Dukes stage C. These findings were in agreement with those of Sheehan and colleagues, who demonstrated that the extent of COX-2 expression in colorectal tumor epithelial cells is related to survival. He also showed a relationship between COX-2 staining and advancing Dukes tumor stage; his explanation was that colon cancer cells expressing COX-2 are more invasive, possibly because of the enhanced expression of metalloproteinase-2 [14].

The role of angiogenesis in the development and progression of human cancers has been widely studied. However, a more complete knowledge of this phenomenon is obviously required. As angiogenesis is associated with a higher risk for metastases in various types of cancer, we are interested in understanding the genetic regulation of the angiogenesis process. VEGF is a well-characterized angiogenesis factor and is known to play a crucial role in tumor angiogenesis. Moreover, a relationship between VEGF and tumor progression has recently been reported in different kinds of human cancers, including colon cancer [15,16].

In our study, 30 of 40 patients (75%) with CRC showed intense VEGF immunoexpression in the cell membrane and adjacent cytoplasm of the malignant epithelial cells. This is in agreement with a study conducted by Ochs *et al.* [17], in which 72 of 109 CRC patients (66%) were positive for VEGF immunoexpression.

In our study, 10% of CRC patients positive for VEGF had well-differentiated adenocarcinoma (GI), 56.7% of CRC patients positive for VEGF had moderately differentiated adenocarcinoma (GII), and 33.3% of CRC patients positive for VEGF had poorly differentiated adenocarcinoma (GIII). In CRC patients with VEGF immunoreactivity, 3.3% were in Dukes stage A, 30% were in Dukes stage B, and 66.7% were in Dukes stage C. These findings are in agreement with those of others, in which VEGF expression correlated significantly with stage and grade [18,19].

Conclusion

COX-2 and VEGF expression seems to provide useful prognostic information in patients with CRC. The overexpression of COX-2 and VEGF in CRC suggests the role of both as a risk biomarker particularly in patients with advanced stage and grade. Attention should be focused on these biomarker inhibitors as potential promising chemopreventive drugs against CRC.

Acknowledgements

Conflicts of interest

None declared.

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