The relevance of Ki-67 and COX-2 immunoexpression in right-sided versus left-sided sporadic cancer colon in Egyptian patients

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Received 03 July 2014
Accepted 21 September 2014

Journal of the Arab Society for Medical Research 2014, 9:75–80

Background and aim
Cancer colon is one of the most common malignancies in Egypt. There are growing amount of data suggesting that carcinomas of the right and left colon should be considered as different tumor entities. Difference in tumor proliferation rates has been used as a prognostic tool. Ki-67 is a proliferation-associated nuclear and nucleolar protein antigen, which is expressed in all cycling cells, and it is an important marker to determine the degree of tumor malignance and invasion ability. Cyclooxygenase-2 (COX-2) is an important key enzyme required for the synthesis of prostaglandins, with high level seen in many cancers including colon cancer.

Patients and methods
A total of 167 colectomy specimens were reviewed during the period of 1 year. Fifty cases from the originally viewed 167 cases were chosen; 25 cases from the right-side colon and 25 from the left-side colon of comparable stages and grades. Each case was stained immunohistochemically for Ki-67 and COX-2 antibodies.

Results
The results of Ki-67 immunostaining showed that the difference between the right and left cases was significant ($P < 0.05$) in addition to the results of COX-2 immunostaining. We suggest that right and left cancer colon may be two different entities with possible different risk factors and different pathogenesis, and hence each may require different treatment polices as well.

Conclusion
COX-2 expression in right-side tumors more than in left-side tumors may provide a chance for right-side cancers to benefit from COX-2 inhibitor therapy.

Keywords:
cancer colon, cyclooxygenase-2, Egyptian patients, Ki-67

J Arab Soc Med Res 9:75–80

1687-4293

Introduction

Cancer colon is one of the most common malignancies in Egypt, ranking the fourth (after breast, leukemia, and bladder) in the National Cancer Institute registry [1] and the third leading cause of cancer-related mortality worldwide [2].

There are a growing amount of data suggesting that carcinomas of the right and left colon should be considered as different tumor entities [3], with different epidemiology, risk factors, manifestations, and tumorogenesis, the observation that may lead to different follow-up and treatment policies for each [4]. A deep understanding of the disease characters, related risk factors, and associations may greatly help in highlighting possible areas of future intervention in decreasing the incidence rate and in optimizing therapeutic strategies [5].

Difference in tumor proliferation rates has been used as a prognostic tool and has helped to design cancer therapeutic regimens [6]. Ki-67 is a proliferation-associated nuclear and nucleolar protein antigen, which is expressed in all cycling cells, consistently absent in quiescent cells and is not detectable during DNA repair processes [7]. Many studies show that Ki-67 is an important reference index for cell proliferation activity and an important marker to determine the degree of tumor malignance and invasion ability [8].

Cyclooxygenase-2 (COX-2) is an important key enzyme required for the synthesis of prostaglandins; its expression is regulated by mitogenic stimuli and tumor promoters. High level of COX-2 was seen in many cancers including colon cancer [9], in which it has an established prognostic potentiality; hence, it became a target for therapeutic design [10].

Using Ki-67 and COX-2 immunoexpression, the aim of this study was to highlight the characters of right-sided and left-sided sporadic cancer colon, so as to better understand the possible different features and outcomes between right and left hemicolon cancers.

Patients and methods

Cases

In this retrospective study, 167 colectomy specimens from three different laboratories were reviewed during the period of 1 year from November 2011 to November 2012.

Inclusion criteria

Inclusion criteria included confirmed sporadic nature of the tumor, confirmed diagnosis of adenocarcinoma, and available data on the exact site of the tumor.

Exclusion criteria

Patients with intestinal polyposis, prior radiotherapy, or inflammatory bowel disease were excluded.

Clinicopathologic data

Cases of colectomy were reviewed for location of the mass, their grade, and TNM stage. The grades were determined according to Compton and Greene [11].

The tumor stage (T) was determined from sections that reached the pericolic fat; the nodal status (N) was also recorded from corresponding sections and any metastasis (M) was reported from the patient file or accompanying specimens. Other data such as sex and age were obtained from the pathology reports. Colon subsite location was defined as right sided (cecum to transverse colon, excluding appendix) versus left sided (splenic flexure to sigmoid and rectum) [12].

Immunohistochemical studies

Fifty cases from the originally viewed 167 cases were chosen, 25 cases from the right-side colon and 25 from the left-side colon of comparable stages and grades. For each case, two sections were put on positively charged slides and stained immunohistochemically for Ki-67 and COX-2 antibodies using a streptavidin–biotin immunoenzymatic method. This included the following steps:

(1) Deparaffinization at 60°C (20 min).
(2) Deparaffinization at room temperature (30 min).
(3) Hydration with alcohol and water.
(4) Antigen retrieval: heating the slides in a solution of 0.01 mol/l citric acid (pH 6.0) in a microwave oven (maximum power) for COX-2 (10 min) and in a pressure cooker for Ki-67 (5 min).
(5) Blockage of endogenous peroxidase in two baths of 3% (v/v) H_2O_2 (10 min each) and rinsing with water and PBS.
(6) Incubation in a moist chamber at 4°C (18 h) with monoclonal mouse antibody-specific primary antihuman MIB-1 (Dako, Carpinteria, California, USA) at a titration of 1 : 100 for Ki-67.
(7) Incubation with secondary biotinylated antibody (LSAB kit; Dako) in a moist chamber at 25°C (30 min) followed by rinsing in PBS.
(8) Incubation with the streptavidin–biotin–peroxidase complex in a moist chamber (37°C) for 30 min followed by rinsing in PBS.
(9) Disclosure by immersion in a solution of diaminobenzidine in PBS containing (v/v) H_2O_2 followed by rinsing in distilled water.
(10) Counterstaining in Harris hematoxylin, dehydration in alcohol, and mounting in Canada balsam with cover slips.

Ki-67 scoring

After scanning, 500 cells were counted in random fields of the epithelial cells using the image analysis. Program index was used in the evaluation of the smears by counting the positively stained nuclei in average 500 cells counted on the screen using Leica Qwin 500 Image Analyzer (Leica Imaging Systems Ltd, Cambridge, UK) at the Pathology Department, National Research Centre (Giza, Egypt). Fairly obvious dark-brown staining of cell nuclei was considered positive.

The calculation of the index of expression was performed using the following formula [13]:

$$\text{Positivity index} = \frac{\text{Number of immunostained nuclei}}{\text{Number of nuclei counted}}.$$

Cyclooxygenase-2 scoring

Cytoplasmic COX-2 immunoreactivity was estimated using an arbitrary semiquantitative four-step scoring system (from 0 to +++), on the basis of the intensity of cytoplasmic COX-2 staining as follows: 0 (negative), no cytoplasmic COX-2 staining; +, weak COX-2 staining; ++, moderate COX-2 staining; and +++, strong COX-2 staining.

Causes of noninterpretable results included lack of tumor tissue and presence of necrosis or crush artifact [14].

Results

Distribution of cases with respect to the location

Seventy-nine of the included cases were located in the left side, whereas 88 cases were in the right side. For cases of the right side, 87% were located in the cecum, and the rest were located in other locations in the ascending colon. For cases of the left side, 52% were located in the rectum, 34% in the sigmoid colon,

and the rest in the other parts of the descending colon.

Demographic data of the cases

The age of the cases ranged from 25 to 91 years; 50.9% were female and 49.1% were male patients. Cases of the right side were 38 (43.2%) female patients and 50 (56.8%) male patients. The age ranged from 25 to 91 years with mean value of 52.3 years. The left-side cases were 47 (59.5%) female patients and 32 (40.5%) male patients. The age ranged from 25 to 80 years with mean value of 53.4 years.

No significant difference in the mean of age and no specific sex predilection were noticed between both colon sides; however, left-sided cancer was slightly more in female patients.

TNM staging of the cases

Tumor stage (T)

For cases in both sides, the most prevalent tumor stage at the time of the diagnosis was T3 (right 88.6%, left 79.7%). Four cases on the right side were T4 (tumor reached the peritoneum), and one case locally reached the ipsilateral ovary. One case on the left side involving other intestinal loop by adhesions contained tumor cells (Table 1).

Nodal stage (N)

In all, 35 (39.7%) of the right-side tumors showed no nodal, whereas 53 cases showed positive nodal deposits: 30 were N1 and 23 were N2. A total of 30 (37.9%) of the left-side tumors showed no nodal metastasis (N0), whereas 49 cases showed positive nodal deposits: 27 cases were N1 and 22 cases were N2.

Metastasis

For the right-side cases, the liver was the most common site for metastasis (three cases), followed by the omentum (two cases). However, the left-side cases metastasized to the uterus (two cases), omentum (two cases), and liver (one case). Two cases of the right-side tumors and two cases of the left-side tumors were bifocal (within the same side area). Ten cases of the right-side tumors were of mucinous type, compared with three cases of the left side. One case of the left side showed neuroendocrine features.

Results of immunostaining

Results of Ki-67 immunostaining

The labeling index is calculated as mentioned before; the right-side cases labeling index ranged from 0.08 to 0.24 with mean 0.15 ± 0.01. Labeling index in the left-side cases ranged from 0.02 to 0.3, with mean 0.07 ± 0.01 (Table 2 and Fig. 1).

The difference between the right and left cases was significant ($P < 0.05$).

Table 1 TNM staging of the included cases

TNM stages	*n* (%)	
	Right side (*n* = 88)	Left side (*n* = 79)
T1	0 (0)	1 (1.3)
T2	6 (6.8)	14 (17.7)
T3	78 (88.6)	63 (79.7)
T4	4 (4.5)	1 (1.3)
N0	35 (39.8)	30 (38)
N1	30 (34.1)	27 (34.2)
N2	23 (26.1)	22 (27.8)
M0	82 (93.2)	74 (93.7)
M1	6 (6.8)	5 (6.3)

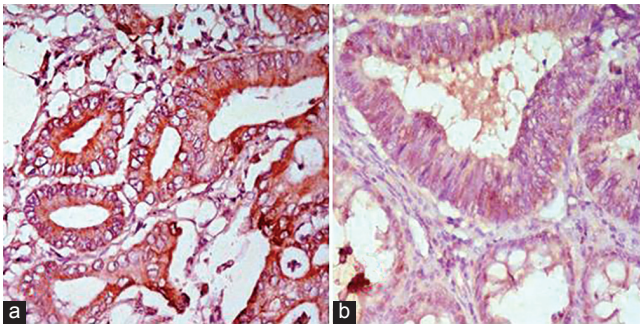
M, metastasis; N, nodal status; T, tumor stage.

Table 2 The results of cyclooxygenase-2 and Ki-67 immunostaining

Marker-Scoring	Right side	Left side
Ki-67 LI (mean ± SD)	0.15 ± 0.01	0.07 ± 0.01
COX-2 (%)		
–	0	20
+	0	80
++	40	0
+++	60	0

COX-2, cyclooxygenase-2; LI, labeling index.

Figure 1

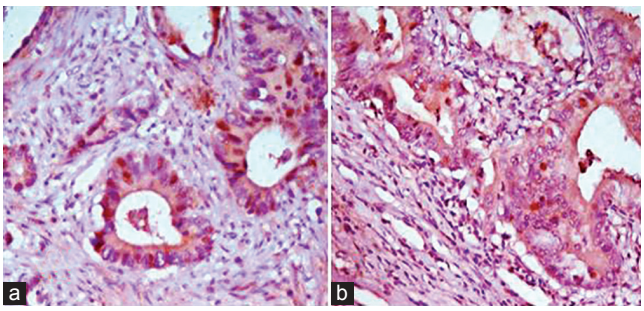


(a) Cyclooxygenase-2 (COX-2) cytoplasmic expression in right-side cancer colon case, (b) COX-2 weak cytoplasmic staining in case of left-side cancer colon (high power, COX-2).

Results of cyclooxygenase-2 immunostaining

According to the scoring system used, the right-side cases showed moderate staining (++) in 40% of the cases and strong staining (+++) in the remaining 60% of the cases. In contrast, the left-side cases showed negative staining (–) in 20% of the cases and weak staining (+) in the remaining 80% of the cases. The difference between right-side and left-side cases was as well significant ($P < 0.05$) (Fig. 2).

Figure 2



(a) Ki-67 in right-side cancer colon case; many scattered positively nuclei are seen. (b) Ki-67 in left-side cancer colon case; fewer nuclei are stained.

Discussion

According to our results, the sigmoid was the most frequent site involved in the left-side cases; likely, the cecum was the most frequently affected site on the right side. This result appears in agreement with the data of the National Cancer Institute statistics [1], in which the sigmoid and the cecum ranked as the first and second most common sites in cancer colon, respectively. The only available governmental documented registry of El Gharbia had also heighted similar data [15].

Among the studied cases, the right-side cases were slightly more than the left-side cases. Left-sided tumors were slightly more in female patients; however, there was no significant difference in the mean age between both sides. This is in accordance with results obtained by Zivković *et al.* [16], and they concluded that there were no significant differences between the right-sided and left-sided colonic carcinoma regarding both sex and age. In contrast, most of the studies highlighted the older age and female predominance in the right-side tumors [3,17,18]. In a systematic review of 17 papers, Hansen and Jess [19] have addressed the increasing age and female predominance in the right-sided cancer colon tumor when compared with the left-side cases. They proposed that the correlation between right-side cancer colon, increased age, and female sex may partly be explained by hormonal basis, genetic factors, lifestyle, and dietary habits, which may differ between women and men.

Histopathologically, in the present study, the right-side tumors showed higher rate of mucinous carcinoma. The vast majority of the cases in both sides were detected at late stage (stage T3: right side 88.6%, left 79.7% and stage T4: right side 3.52%, left side 0.79%); however, the right-sided cancer was more likely to be detected at more advanced stage (stage T1: right 0%, left 2.5% and stage T2: right 4.5%, left 11.4%; $P < 0.05$). The locally advanced tumors (T4) were significantly more in the right side; however, the metastatic cases were almost comparable in both sides. This difference may be attributed to the possible delay in occurrence of symptoms such as obstruction caused by the mass especially when developed in the relatively wide cecum or may refer to different potential biological behavior between the two sides of the colon.

Zivković *et al.* [16] noticed no significant differences between the right-sided and left-sided colonic carcinoma regarding histological grade and tumor stage. However, they agreed with us that mucinous adenocarcinomas were significantly more frequent in right-sided colon than in left-sided colon; yet, the authors could not explain this phenomenon. Many other studies screened the higher rate of mucinous adenocarcinoma among the right-side tumors [17,18]; however, none of these studies provided a possible explanation.

Although Benedix *et al.* [3] confirmed the more common comorbidities, and the worse prognosis in the right-side cases, they noticed, as we did, that the rate of synchronous distant metastases was almost comparable on both sides. Similar conclusion was achieved by Tentes *et al.* [20] and Weiss *et al.* [21]. Those authors noticed that patients with right-sided cancer were more likely to be diagnosed in a more advanced stage and to have more poorly differentiated tumors; however, they found that the recurrence rate, 5-year survival, in-hospital mortality, and morbidity were similar in both groups. Hence, they concluded that the biological behavior of right and left colon carcinomas is similar, despite minor histopathological differences that do not influence survival and development of recurrences.

The higher percentage of locally advanced tumors in the right-side cancer colon cases was observed in many studies [17,22,23]. In agreement with that Hemminki *et al.* [24] stated that patients with right-sided cancer colon had more advanced stages at diagnosis than patients with left-sided tumor and predicted a worse prognosis for these patients. Snaebjornsson *et al.* [25] also agreed with this and detailed the result by the way that the more advanced stages of right-side tumors were due to the tumor (T) and lymph node (N) stages but not due to metastases (M stage). On the basis of analysis of information from the Surveillance, Epidemiology, and End Results (SEER) database, Meguid *et al.* [12] observed that right-sided colon cancers have a worse prognosis than left-sided colon cancers. They suggest that this may be due to biological and/or environmental factors and may have particular

bearing, given the rising incidence of right-sided colon cancers.

Hansen and Jess [19] obtained the same conclusion from their systematic literature review. They proposed that, besides the hormonal and genetic factors earlier mentioned, the reason for this could be the weaker symptoms in patients with right-side cancer colon than in patients with left-side cancer, as the former is often associated with unnoticed bleeding, whereas the later is associated with changes in bowel habits, passage trouble, and other obstruction symptoms. Thus, the right-sided cancer colon patients often seek medical assistance later than left-sided cancer colon patients. The condition is worsened by the documented inferior rate of success of the colonoscopy in the detection of right-side tumors [26], which may be due to incomplete examination, the problem that is mainly pronounced and most severe in older patients and especially in women [27].

In the present study, COX-2 was used to elucidate the proposed biological behavior between the colon two sides' tumors. COX-2 is believed to be an important enzyme in the pathogenesis of colorectal cancer (CRC) through tumor promotion, apoptosis inhibition, and angiogenesis [28]. Many authors pointed to the well-established correlation between COX-2 overexpression and tumor growth and distant metastasis, and hence it becomes a well-established prognostic marker for cancer colon [29]. Moreover, studies suggested that COX-2 expression can be used to risk stratify patients into a group that may benefit from both more aggressive follow-up with treatment and from a combination treatment that includes a COX-2 inhibitor [30].

According to our results, COX-2 immunohistochemical expression was significantly higher in the right-side tumors than in the left-side tumors. As the demographic information and pathologic features of the left-side cases and right-sided cases subsets in our study were essentially matched, the findings appear to reflect true pathobiologic/genetic differences rather than a patient selection bias. In the literature, few studies handled the correlation between COX-2 expression and cancer colon location. In their meta-analysis, Peng *et al.* [29] stated that data were insufficient for them to analyze such association; however, they mentioned that some other studies found no correlation between the location of colorectal tumor and COX-2 expression. In contrast, Nasir *et al.* [31,32] noticed that COX-2 was more frequently expressed in the left-side tumors. They supposed that these data support the proposed hypothesis that COX-2 expression may be related to genetic alterations specific to tumors of each side of colon; moreover, they found that these findings may be really useful to stratify cancer colon patients into right-sided and left-sided and COX-2 expressor and nonexpressor categories, when evaluating COX-2 inhibitor and other targeted adjuvant therapies in cancer colon.

In their study, Nasir *et al.* [32] explained the results referring to the previous findings that CRCs involving the distal colon are more likely to have aneuploidy DNA, to harbor mutations in adenomatous polyposis coli (*APC*), *p53*, and *K-ras* genes, and to behave more aggressively, whereas proximal CRCs are more likely to have diploid DNA, to possess microsatellite instability, to harbor mutations in the mismatch repair genes, and to behave less aggressively as in hereditary nonpolyposis CRC; however, they did not provide a convenient explanation regarding the sporadic cancer colon cases.

In contrast, we find that the higher expression of COX-2 in the right-side cases is in agreement with our (and other authors') finding regarding the higher stage in the right-side cases and the expected poorer prognosis in this category.

Ki-67 is a nuclear and nucleolar human protein, which is present in all cycling cell and consistently absent in quiescent resting cells [7]; hence, its use as a proliferation marker has gained a wide popularity since its development in 1984 by Gerdes [33].

In cancer colon, many recent studies pointed to the correlation between higher Ki-67 immunohistochemical expression and the higher proliferation rate of the tumor, and hence poorer prognosis; Menezes *et al.* [33] confirmed the higher Ki-67 expression with tumor recurrence. Similarly, Ishida *et al.* [34] showed that, although the use of Ki-67 in cancer colon is limited, its proliferative activity was significant with respect to the appearance of lymph node and hepatic metastases. Lumachi *et al.* [35] studied the expression of Ki-67 retrospectively in cancer colon cases and concluded that its high expression was associated with a worse outcome. Similarly, Peng *et al.* [29] stated that high expression of Ki-67 in tumor tissue was a predictor of poor prognosis.

In our study, the proliferative marker Ki-67 expressed significantly higher labeling indices in the right-side cases when compared with the left-side cases, with a possible connotation of the worse outcome of the right-side cancer colon cases. To the best of our knowledge, no previous studies compared between the expressions of Ki-67 proliferative marker in both sides of the colon.

To summarize, in this study, we noticed that, in Egypt, cancer colon cases, both in the right and left sides, are generally discovered at advanced stages; moreover, the condition is worse on the right-side tumors. However, COX-2 expression in the right-side tumors more than in the left-side tumors may provide a chance for the right-side cancers to benefit from the COX-2 inhibitor therapy. The significant increased expression of both COX-2 and Ki-67 on the right-side tumors may be due to genetic differences that may suggest that different mechanisms may act during right and left colon carcinogenesis accounting for distinct biological behavior. Hence, according to our results, we suggest that right and left cancer colon may be two different entities with possible different risk factors and different pathogenesis, and hence each may require different treatment polices as well. Generally, this should have an impact on colon cancer screening and therapy in Egypt.

Acknowledgements

Conflicts of interest

None declared.

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