

Morphometric analysis and immunohistochemical expression of cytochrome C oxidase in colonic adenomas and adenocarcinomas

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Background/aim

Colorectal cancer is the seventh most common cancer in Egypt, constituting 3.47% of male cancers and 3% of female cancers. The aim of the current study is to evaluate the immunohistochemical expression of cytochrome C oxidase (CcO) as well as the nuclear morphometric measurements in colonic adenomas and adenocarcinomas.

Materials and methods

Sixty cases of colonic biopsies/specimens were involved in this study. The samples were received in the Department of Pathology of Kasr El-Aini Hospital, Cairo University, Egypt, and they were divided into three groups: 20 cases of normal control, 20 cases of colonic adenoma, and 20 cases of colonic adenocarcinoma. The expression of CcO was studied by immunohistochemistry in 60 formalin-fixed paraffin blocks. Nuclear morphometric parameters were evaluated using an image analysis system. The area percentage of the marker expression was correlated with nuclear morphometric parameters.

Results

Immunohistochemical study showed that 65% of control biopsies showed a significant difference with colonic adenocarcinoma cases in contrast and adenoma cases showed a nonsignificant difference with normal control biopsies. Mean areas percentages of CcO expression were significantly different between all three studied groups. The means of nuclear area, length, and perimeter showed a significant difference while means nuclear width and roundness showed a nonsignificant difference between all three studied groups. There was significant correlation between mean area percentage of CcO expression with mean nuclear area and perimeter in control, adenoma, and adenocarcinoma cases.

Conclusion

CcO protein is significantly expressed in the cytoplasm of normal colonic mucosa. It was significantly deficient in colonic adenocarcinoma. Nuclear morphometry can be introduced as a useful morphologic feature of patients with variable colonic lesions specially in combination with CcO.

Keywords:

colonic adenocarcinomas, colonic adenomas, cytochrome C oxidase, morphometric analysis

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Introduction

Colorectal cancer is one of the most common cancers in the Western world. The 5-year survival rate, although quietly improving, is still relatively poor at 40% [1]. Majority of large bowel cancers arise from adenomas and about 5% of these adenomatous polyps progress to malignant tumors within 5–10 years [2]. Both environmental factors and genetic susceptibility are important contributors of colorectal cancer evolution [3,4]. Colorectal cancer is the seventh most common cancer in Egypt, constituting 3.47% of male cancers and 3% of female cancers [5].

The pathway of cancer development involves multiple steps in the initiation phase that make normal cells able to turn into malignant cells and lead to the promotion

stage, which results in abnormal malignant growth and invasion in the advanced stage [6]. In colorectal carcinomas, the transformation of normal colonic epithelium into carcinoma via the mediation of adenoma is known as the adenoma–carcinoma sequence. Colorectal tumorigenesis arises from genetic/epigenetic aberrations and the concurrent accumulation of histopathological changes. The accumulation of these disruptions, which are mostly related to the regulation and expression of the prominent genes of PIK3CA, PTEN, BRAF, c-

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myc, p53, APC, and K-ras, DNA mismatch repair genes, and apoptosis, promotes the clonal expansion of tumor cells [7].

Loss in apoptosis efficacy and defects in proteins necessary for apoptosis will participate in carcinogenesis [8]. Cytochrome *C* Oxidase (CcO) acts as a marker of apoptosis and has a well-defined role in triggering apoptosis [6]. CcO is the molecular switch that promotes apoptosis under energy stress conditions [9]. Active CcO oxidizes cytochrome *C*, which in turn activates procaspase 9, enhancing the apoptosis. Cells deficient in CcO are apoptosis resistant, and in turn, they have frequent genomic instability, which leads to carcinogenesis [10].

The mitochondrion is the responsible organelle for apoptosis activation due to the presence of caspases, Bcl-2 family member, and proteins to cleave the DNA. This regulates the release of proteins from the space between the inner and outer mitochondrial membrane, which activates caspase proteases that send signal for phagocytosis of dead cells [11]. The colonic crypts are likely to show detectable defects close to an adenoma with advanced neoplasia or in the mucosa adjacent to the tumor. The 'field defect,' in CcO in the carcinogenesis process, is recognized clinically in colon cancer [12].

Computerized image analysis authorizes accurate and objective evaluation of nuclear morphology and has been used to demonstrate that the increased nuclear morphometric measurements and irregularity of shape are detected mostly in carcinoma tumors than in borderline tumors. Excellent reproducibility of nuclear morphometry has been documented in breast cancer cases. Meanwhile, nuclear morphometry has been rarely applied in colonic cancer. Quantification of proteins is an important issue, since the expression of a particular protein can reflect a pathological state in a disease [13].

The current study aimed to assess the immunohistochemical expression of CcO and to evaluate the nuclear morphometric measurements in Egyptian patients with colonic adenomas and adenocarcinomas. This may shed light on their potential role as early diagnostic features in colon adenocarcinoma, in an attempt to identify, in the future, patients at high risk of developing cancer.

Materials and methods

Study samples and ethical approval

A retrospective study on colonic biopsies/specimens was done. The samples were obtained as paraffin blocks,

from the Department of Pathology of Kasr El-Aini Hospital, Cairo University, Egypt. Detailed history and clinical data of patients were taken. The study was approved by the Institutional Ethics Committee of National Research Centre, Cairo (No. 18133).

Study design

Paraffin blocks of colonic biopsies/specimens (60 cases) were selected; they were divided into three groups. Group 1: 20 cases of normal control; group 2: 20 cases of colonic adenoma; and group 3: 20 cases of colonic adenocarcinoma. Biopsies were obtained from individuals undergoing colonoscopies for screening purposes or for a medically indicated reason. Tissue samples were also obtained from surgical colonic resections. The clinicopathological anonymized data were obtained from patients' charts.

Two sections of 4 µm thick each were cut from paraffin blocks using a Leica RM 2155 (Leica Microsystems Nussloch GmbH, Germany) microtome. One section was stained with hematoxylin and eosin for histopathological evaluation and grading. The other section was mounted on positively charged glass slides for immunohistochemical staining.

Immunohistochemistry

Sections of paraffin-embedded tissues were prepared and standard immunohistochemical procedures were carried out using a mouse monoclonal anti-CcO antibody, clone number (20E8C12) (1 : 250 dilution, catalog no. ab14744; Abcam Co., UK). Sections were deparaffinized, hydrated, and incubated in 3% hydrogen peroxide for 30 min to block the internal peroxidase activity. Antigen retrieval was done by microwave pretreatment for 10 min in 0.01 M citrate buffer. Subsequently, sections were incubated at 4°C overnight with the primary antibody (anti-CcO). These steps were followed by 60 min incubation with biotinylated secondary antibody (code 3024; Dako, Copenhagen, Denmark) at room temperature, avidin-biotin peroxidase complex for 50 min at room temperature and finally diaminobenzidine for 3–5 min. The slides were counterstained with hematoxylin, dehydrated, and mounted.

Positive and negative controls were stained in the same setting with the selected slides. Negative controls were used using an antibody diluent in PBS buffer instead of primary antibodies. For positive controls, rat heart was used.

Positivity for CcO was considered as cytoplasmic brown staining, as it is located in the mitochondrial

inner membrane, and its negative expression was considered with total complete loss of immunohistochemical stain [10].

Morphometric measurements

The morphometric analysis was performed at the Pathology Laboratory, at the Medical Research Centre of Excellence (MRCE) unit, National Research Centre using the image analysis system Leica Qwin DW3000 (LEICA Imaging Systems Ltd, Cambridge, England), which consists of Leica DM-LB microscope with JVC color video camera attached to a computer system.

The slide to be examined was placed on the stage of the microscope. The light source was set to the required level. Successful adjustment of illumination was checked for the video monitor. The morphometric analysis was carried out on CcO-stained slides to measure the area percentage of positive cells, and the nuclear parameters including area, length, width, perimeter, and nuclear roundness. The areas to be measured were masked by binary color, which was named a binary image. The areas of this binary images were calculated, which reflected the area of the object to be measured.

This area percentage of CcO positively stained cells was determined as an area per field in micrometer square, area fraction, and area percentage using the interactive measurement software of the system. The results appeared automatically on the monitor in the form of a table with the total, mean, standard deviation, standard error, the minimum area, and the maximum area measured.

According to the nuclear morphometric measurements, the selected nuclei with an intact wall that was outlined manually using a cursor and the shortest core axis of 100 nuclei was determined using a computer program. The nuclear results

appeared automatically on the monitor in the form of the distance measured in μm with the mean, SD, the minimum length, and the maximum length measured.

A total of 10 field measurements were taken per slide, an average mean for each slide obtained with a total magnification of 200 $\times$ (an objective lens 20 $\times$ and an eye lens with 10 $\times$) for area percentage measurement of CcO immunohistochemical expression.

A total magnification of 400 $\times$ (an objective lens 40 $\times$ and an eye lens with 10 $\times$) for nuclear parameter measurement was used.

Statistical analysis

Data was statistically described in terms of mean $\pm$ SD, median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups were done using the Mann–Whitney *U*-test for independent samples for comparing two groups and Kruskal–Wallis test for comparing more than two groups. For comparing categorical data, χ^2 -test was performed. Exact test was used instead when the expected frequency is less than 5. *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using the computer program SPSS (statistical package for the social sciences; SPSS Inc., Chicago, Illinois, USA) release 19 for Microsoft Windows (2011).

Results

The present study was performed on a total of 60 cases: 20 cases of normal control colonic mucosa, 20 cases of colonic adenoma, and 20 cases of colonic adenocarcinoma. The clinicopathological data are presented in Table 1. Highest mean of age was seen in colonic cancer cases 54.27 years, male patients were found in 70% (14 cases/20), and 55% (11 cases/20) of colonic adenomas and colonic adenocarcinomas, respectively. The mean specimen size

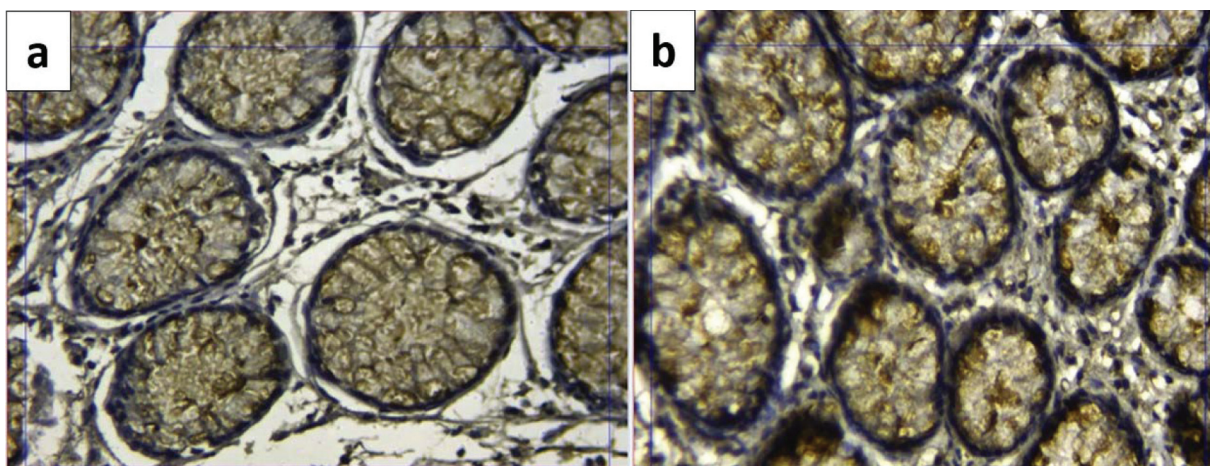
Table 1 Clinicopathological characteristics of the studied colon cases

Variables	Normal control (N=20) [n (%)]	Colonic adenomas (N=20) [n (%)]	Colonic adenocarcinomas (N=20) [n (%)]
Age (years)			
Mean $\pm$ SD	52.23 $\pm$ 1.3	50 $\pm$ 3.5	54.27 $\pm$ 2.1
Sex			
Male	10 (50)	14 (70)	11 (55)
Female	10 (50)	6 (30)	9 (45)
Size			
Mean $\pm$ SD	0.9 $\pm$ 0.05	2.5 $\pm$ 1.3	4.3 $\pm$ 1.4
Multifocal lesions			
No	20 (100)	5 (25)	8 (40)
Yes	–	15 (75)	12 (60)

Table 2 Cytochrome C oxidase immunohistochemical expression in the three colonic studied groups

Cases	Cytochrome C oxidase expression				
	Negative [n (%)]	Positive [n (%)]	P value	Area % (mean±SE)	P value
Normal control*	7 (35)	13 (65)		45.86±1.68	
Colonic adenomas*	9 (45)	11 (55)	<0.05	9.04±0.75	<0.05
Colonic carcinoma	17 (85)	3 (15)		5.45±0.35	

* $P>0.05$, nonsignificant difference.

Figure 1

(a and b) Normal control colonic tissue sections showing diffuse positive cytoplasmic immunohistochemical expression for cytochrome C oxidase (immunoperoxidase, $\times 400$).

of colonic adenoma was 2.5 cm which increased in colonic cancer lesions reaching 4.3 cm. Multifocality was present in 75% (15 cases/20) and 60% (12 cases/20) of colonic adenoma and colonic adenocarcinoma, respectively.

Immunohistochemical expression of cytochrome C oxidase

CcO was predominantly localized in the cytoplasm of colonic crypts starting at the luminal surface and extending to the base of the crypt together with evidence of stromal expression in varying degrees.

In normal control biopsies, 13 cases out of 20 (65%) were positive for CcO. On the other hand, three out of 20 (15%) of colonic cancer cases showed positivity for CcO. In colonic adenomas, nine (45%) cases were negative and 11 (55%) cases were positive for CcO which showed significant difference with colonic adenocarcinoma cases ($P<0.05$), while it showed nonsignificant difference with normal control biopsies ($P>0.05$) (Table 2).

CcO expression in the positively immunostained cases of the normal control tissues (Fig. 1) revealed diffuse intense cytoplasmic brown immunohistochemical stain, extending along the entire crypt at the surface epithelium as well as the base of the crypt.

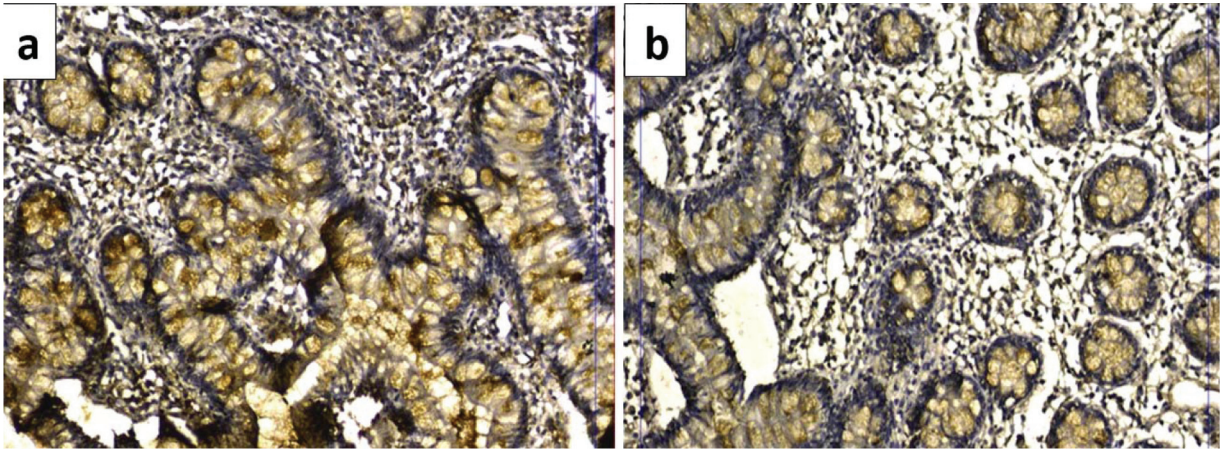
Positive immunoreactivity for CcO in colonic adenoma group was patchy and cytoplasmic. It showed less intensity than that in the normal control group, particularly in the center of cell cytoplasm, being stronger and more concentrated near the cell borders and cell membranes. The crypts were interrupted by areas of total loss of CcO (Fig. 2).

According to the third group, colonic adenocarcinoma, only 15% (three cases/20) of colonic adenocarcinoma expressed weak cytoplasmic immunohistochemical expression of CcO in the colonic crypts. On the other hand, 85% (17 cases/20) showed negative CcO immunohistochemical expression as shown in Fig. 3.

Morphometric assessment of cytochrome C oxidase immunohistochemical expression

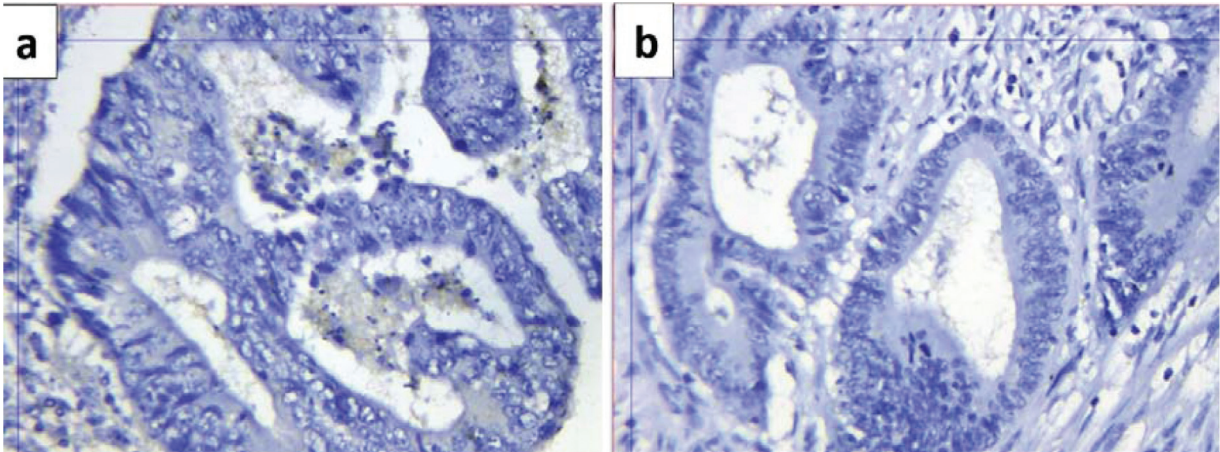
Further histopathological evaluation was done using quantitative morphometric analysis of the immunohistochemical expression of CcO. The amounts of cells showing CcO positive staining were determined using the image analysis system (Fig. 4). In the normal control colon group, 13 cases/20 showed a mean area percentage of CcO immunohistochemical expression of 45.86 ± 1.68 which decreased to 9.04 ± 0.75 in the adenoma colonic group, and further decreased to 5.45 ± 0.35 in the colonic adenocarcinoma group.

Figure 2



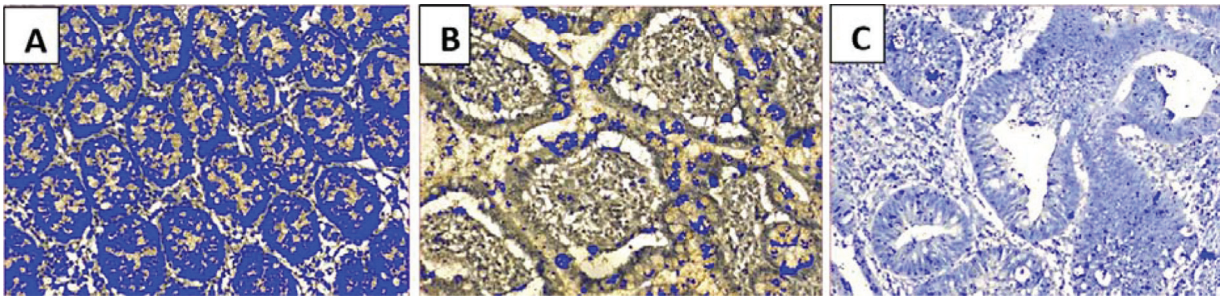
(a and b) Colonic adenoma tissue sections showing positive cytoplasmic immunohistochemical expression for cytochrome C oxidase more concentrated near the cell membrane (immunoperoxidase, $\times 200$).

Figure 3



(a and b) Colonic adenocarcinoma tissue sections showing negative immunohistochemical expression for cytochrome C oxidase (immunoperoxidase, $\times 400$).

Figure 4



Binary image using image analysis demonstrating area % cytochrome C oxidase expression (blue color) of: (a) normal control colonic sections and (b) colonic adenoma sections, and (C) colonic adenoma–carcinoma sections (immunostaining, $\times 200$).

A statistically significant difference ($P<0.05$) was present between the mean area percentages of CcO immunohistochemical expression in the three studied groups of normal colonic mucosa, colonic adenoma, and colonic adenocarcinoma (Table 2).

Nuclear morphometric results

All specimens were evaluated using image analysis for nuclear morphometric parameters, including area, width, length, perimeter, and roundness as shown in Table 3. Regarding the morphometric nuclear

features (Fig. 5), in the control group, the mean nuclear area (NA) was $15.3 \pm 2.02 \mu\text{m}^2$ while in adenoma cases it was $29.65 \pm 2.2 \mu\text{m}^2$ and increased to $56.5 \pm 3.04 \mu\text{m}^2$ in adenocarcinoma cases showing a statistically significant difference ($P < 0.05$). According to nuclear length, it measured $5.9 \pm 0.09 \mu\text{m}$ in control colonic tissue, while in adenoma cases it was $7.76 \pm 0.62 \mu\text{m}$ and it increased to $11.66 \pm 2.57 \mu\text{m}$ in adenocarcinoma cases, showing a statistically significant difference ($P < 0.05$). Also, the mean nuclear perimeter showed a statistically significant difference ($P < 0.05$) between the three studied groups as in the control group, it was $16.49 \pm 1.38 \mu\text{m}$ which increased to $21.97 \pm 1.75 \mu\text{m}$ and $32.2 \pm 2.5 \mu\text{m}$ in the adenoma and carcinoma groups, respectively. On the other hand, the mean width measurements were 3.41 ± 0.59 , 4.89 ± 0.56 , and $6.73 \pm 0.83 \mu\text{m}$, and the mean nuclear roundness measurements were 1.32 ± 0.03 , 1.39 ± 0.11 , and 1.45 ± 0.32 in normal control, colonic adenoma, and colonic adenocarcinoma groups, respectively. Thus, the last two nuclear parameters, the mean nuclear width and mean roundness, showed minor changes which did not show significant difference ($P > 0.05$) between the three studied groups.

Table 3 Morphometric nuclear features of the three colonic studied groups

Nuclear features	Normal control (mean $\pm$ SD)	Colonic adenomas (mean $\pm$ SD)	Colonic carcinomas (mean $\pm$ SD)	P value
Area	15.3 ± 2.02	29.65 ± 2.2	56.5 ± 3.04	< 0.05
Length	5.9 ± 0.09	7.76 ± 0.62	11.66 ± 2.57	< 0.05
Width	3.41 ± 0.59	4.89 ± 0.56	6.73 ± 0.83	> 0.05
Perimeter	16.49 ± 1.38	21.97 ± 1.75	32.2 ± 2.5	< 0.05
Roundness	1.32 ± 0.03	1.39 ± 0.11	1.45 ± 0.32	> 0.05

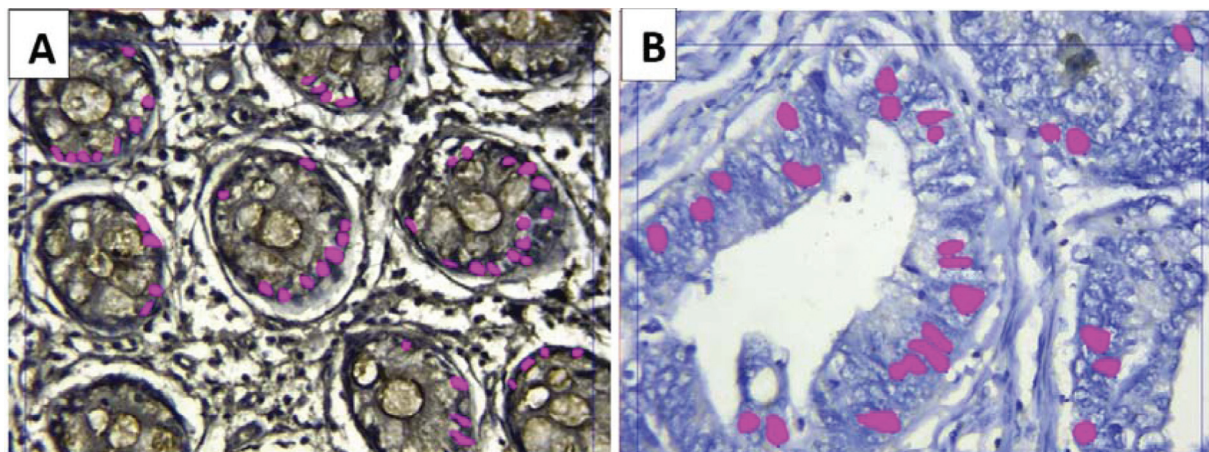
Correlation between area percentage of cytochrome C oxidase immunohistochemical expression and nuclear morphometric parameters

Regarding the correlation between area percentage of CcO immunohistochemical expression and the nuclear parameters in three studied groups, the immunohistochemical expression were classified as 0–4%, 4–10%, and greater than 10%. It was found that 65% of normal cases showed greater than 10% area percentage, while 45% of adenoma cases and 85% of carcinoma showed 0–4% area percentage revealing a significant difference between the adenoma and carcinoma groups. It was found that the increased CcO immunohistochemical expression correlated significantly with decreased mean NA and mean nuclear perimeter in the normal control group (Table 4).

Area percentage of CcO immunohistochemical expression showed statistically significant correlation ($P < 0.05$) with the mean NA and mean nuclear perimeter in this group, while it did not show significant correlation ($P > 0.05$) with the means of other nuclear parameters: length, width, and roundness. It was found that the increased area percentage of CcO immunohistochemical expression correlated significantly with decreased mean nuclear parameters (area, length, and perimeter) in the colonic adenoma group (Table 4).

Area percentage of CcO immunohistochemical expression showed statistically significant correlation ($P < 0.05$) with the mean NA, mean nuclear length, and mean nuclear perimeter in the colonic adenoma group, while it did not show significant correlation ($P > 0.05$) with the mean nuclear width and roundness. Decreased

Figure 5



Binary image (red) using image analysis demonstrating nuclear area in (a) normal control colonic sections and (b) colonic adenocarcinoma sections (immunostaining, $\times 400$).

Table 4 Correlation between area percentage of cytochrome C oxidase immunohistochemical expression and morphometric nuclear parameters

	Area percentage of CcO immunohistochemical expression			P value
	0–4% (n=33)	4–10% (n=14)	>10% (n=13)	
Number of cases (n=60) [n (%)]				
Normal (n=20)	7 (35)	0	13 (65)	<0.05
Adenoma (n=20)	9 (45)	11 (55)	0	>0.05
Adenocarcinoma (n=20)	17 (85)	3 (15)	0	<0.05
Nuclear area (mean)				
Normal	19.8	–	10.8	<0.05
Adenoma	35.23	21.07	–	<0.05
Adenocarcinoma	69.4	43.6	–	<0.05
Nuclear length (mean)				
Normal	6.5	–	5.3	>0.05
Adenoma	9.92	5.6	–	<0.05
Adenocarcinoma	13.92	9.4	–	<0.05
Nuclear width (mean)				
Normal	3.5	–	3.2	>0.05
Adenoma	5.66	4.12	–	>0.05
Adenocarcinoma	6.68	6.6	–	>0.05
Nuclear perimeter (mean)				
Normal	20.45	–	12.45	<0.05
Adenoma	28.66	15.28	–	<0.05
Adenocarcinoma	40.87	23.53	–	<0.05
Nuclear roundness (mean)				
Normal	1.35	–	1.2	>0.05
Adenoma	1.38	1.39	–	>0.05
Adenocarcinoma	1.44	1.42	–	>0.05

area percentage of CcO immunohistochemical expression in the adenocarcinoma group was noticed with increased mean nuclear parameters (area, length, and perimeter). Area percentage of CcO immunohistochemical expression showed statistically significant correlation ($P<0.05$) with the mean NA, mean nuclear length, and mean nuclear perimeter in the colonic adenocarcinoma group, while it did not show significant correlation ($P>0.05$) with the mean nuclear width and roundness (Table 4).

Discussion

Colon cancer is one of the most common malignancies with significant morbidity and mortality [14]. Colorectal carcinogenesis arises in the mucosa with disordered cell replication and renewal [15]. Apoptosis, known as programmed cell death, is an evolutionally preserved cell suicidal process essential for managing stress and maintaining tissue homeostasis. Apoptosis serves as a safeguard mechanism against tumorigenesis. Defective apoptosis regulation drives other tumorigenic events such as accumulation of further genetic mutations, extended lifespan, growth under stress conditions, and tumor angiogenesis and metastasis; all this contribute to therapeutic resistance [16].

Loss in apoptosis capacity often results in augmented genomic instability contributing to carcinogenesis. CcO is an important mitochondrial multiprotein complex with two main substrates – oxygen and cytochrome c [17]. The CcO is known as an electron carrier; however, its biological function in the development of cancer cells have not been fully explained. As a result of ROS injury accumulation, mutations of mtDNA-encoded subunits of CcO are demonstrated in a number of tumors, including CRC [18,19], prostate cancer [20,21], hepatocellular carcinoma [22], and myelodysplastic syndrome [23].

Many studies have demonstrated that the ratio of nuclear-encoded CcO subunits to mtDNA-encoded CcO subunits increases during the carcinogenesis, suggesting that alteration of CcO subunits harmony plays an important role in the rearrangement of cancer metabolism [24,25].

Early diagnosis and identification of patients at high risk for developing colon cancer has serious importance. The identification of biomarkers that are both specific and sensitive is critically needed to target individuals at high risk for either more frequent colonoscopies, recommendations for lifestyle changes, and/or chemopreventive strategies [26].

In the present study, 60 colonic cases including 20 control cases of non-neoplastic condition, 20 cases of colonic adenoma, and 20 cases of colon adenocarcinoma were investigated to explore the role and consequences of aberrations in CcO expression in the evolution of cancer colon.

Payne *et al.* [26] who studied CcO immunohistochemical expression in 46 colonic mucosal samples from 16 patients compared the non-neoplastic patients with neoplastic ones and noticed a higher mean incidence of crypts having overall decreased expression (1.7 vs. 22.8, $P=0.03$) and a higher mean incidence having crypt-restricted loss (CRL) (0.6 vs. 3.2, $P=0.06$), respectively. CcO cellular sublocalization being at the inner membrane of the mitochondrion, the normal pattern of expression was seen in the form of an intense positive stain for CcO throughout the length of the crypt, with even heavier staining at the surface epithelium and upper portion of the crypt [26].

This goes in concordance with the results of the current study, where the immunohistochemical expression of CcO was examined in normal colonic control mucosa, adenomatous colonic cases, and adenocarcinoma cases. The normal control group showed positivity for CcO (13 cases/20; 65%) revealing intense diffuse cytoplasmic positive stain for CcO throughout the colonic crypt. Meanwhile, in adenocarcinoma cases (3 cases/20; 15%), the neoplastic colonic mucosa showed total absence of CcO immunostaining. In the adenomatous group, the CcO positive cases (11 cases/20; 55%) showed patchy cytoplasmic immunohistochemical expression of moderate intensity in some colonic crypts, being interrupted by areas of total loss of CcO.

Hewedi *et al.* [27] studied 50 colorectal cases including 10 control cases, 15 colonic adenoma, and 25 colorectal carcinoma cases, the CcO expression was assessed by calculating the overall decreased immunostaining (ODI) and the CRL which implied the gradual downregulation of CcO gene expression. This provided a selective advantage in the evolution and progression of the multistep process of carcinogenesis. Their results showed that colonic malignancy, ODI, and CRL were 49.1 and 2.78%, respectively, compared with 0.55 and 2.50%, respectively, for normal control mucosa and 7.32 and 47.54%, respectively, for adenomatous mucosa showing significant difference of ODI and CRL percentages. Likewise, their study concluded that CcO was frequently lost in the crypts of adenomatous polyps and this loss increased in more dysplastic adenomas, and its total absence gave rise to

colon cancers. So the deficiency of CcO expression might be considered as a reliable marker to detect at-risk patients [27].

In the present study the morphometric assessment of immunohistochemical expression of CcO indicates that the mean area percentage of positive cells showing CcO immunohistochemical expression in the normal control group was 45.86%, which decreased to 9.04% in the adenoma colonic group, and further decreased to 5.45% in the colonic adenocarcinoma group, with a statistically significant difference ($P<0.05$) between the three groups.

The CRL and/or markedly decreased overall immunohistochemical expression of CcO may prove to be reliable biomarkers of colon cancer risk, because they have been found to occur at elevated frequencies in the non-neoplastic colonic mucosa of patients with, and at high risk for, cancer. Reduction or loss of CcO activity, manifested by immunohistochemistry contributes to apoptosis resistance [27].

Data from computerized morphometry are objective, mostly accurate, and are obtained rapidly. The NA of cancer cells has a considerable role in the prediction of the ability of cancer cells to invade the microvessels in the colorectal wall and its ability to metastasize to the lymph nodes or the liver. Therefore, the nuclear morphometry has a great importance in screening and selecting patients who are at risk of hematogenic or lymph node metastatic recurrence after curative surgery for colorectal carcinoma [28].

In the present study, all specimens were evaluated using image analysis for nuclear morphometric parameters, including NA, width, length, perimeter, and roundness which revealed that the mean NAs were 15.3, 29.65, and $56.5\mu\text{m}^2$, respectively in normal, adenoma, and colonic adenocarcinoma which showed a significant difference ($P<0.05$) between all the three studied colonic groups. This was in accordance with the study of Ikeguchi *et al.* [29] who examined 343 colorectal carcinomas using quantitative nuclear structures for evaluating the metastatic potential of colorectal adenocarcinoma, by computer-assisted digital image analysis. Their results come in agreement with ours as the mean NA of the normal control was $19\mu\text{m}^2$; the mean NA for adenomas was $34\mu\text{m}^2$, and the mean NA of mucosal carcinoma was $45\mu\text{m}^2$. They demonstrated that the NA of cancer cells was determined to be one of the independent prognostic factors in malignancy, as the mean NA enlarged from normal colorectal mucosa to adenoma

and carcinoma ($P<0.001$). NAs of cancer cells in tumors with lymphatic invasion, venous invasion, lymph node metastasis, or hepatic metastasis were significantly larger than those of cancer cells in tumors without such factors [29].

In the current study, the mean NA, mean nuclear length, and mean nuclear perimeter in normal colonic mucosa, colonic adenomas, and colonic adenocarcinomas showed significant difference ($P<0.05$), while the mean nuclear width and the mean nuclear roundness in the three studied colonic groups showed minor changes with nonsignificant difference ($P>0.05$). Our results go in accordance with Deans *et al.* [30] who studied different nuclear and cellular morphometric variable parameters being measured in 312 cases of normal colorectal cases and colorectal adenocarcinoma cases. They found that all variables, except nuclear shape, differed significantly ($P<0.001$) between normal colorectal and tumor tissues [30].

Nuclear size increase and shape irregularity were more frequently detected in carcinoma, than in borderline tumors [31]. The mean NA of cells from mucosal carcinoma was significantly larger than that in cells from colorectal adenoma. Moreover, increasing abnormalities of nuclear morphometric features running in parallel with tumor progression have been reported in various cancers [32].

The NA of cancer cells was determined to be one of the independent prognostic factors in multivariate analysis. It has been considered that the nuclear morphometry provided more useful information than flow cytometry about the hematogenic metastatic potential of tumors [29].

Conclusion

The current study showed that CcO protein was expressed at a high level in the cytoplasm of colonic crypts of normal colon mucosa, while it was deficient in 85% of colonic adenocarcinoma. Nuclear morphometry as an objective and reproducible procedure showed significantly increased nuclear parameters in colonic adenocarcinoma. Such correlation may make it possible to use CcO together with nuclear morphometry as a potential early diagnostic feature of colonic adenocarcinoma.

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Nil.

Conflicts of interest

There are no conflict of interest.

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