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**Research Article**

# EVALUATION OF COX2 EXPRESSION AS A PROGNOSTIC MARKER IN COLORECTAL ADENO CARCINOMA

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## Abstract

**Introduction**: Colon adenocarcinoma (COAD), the fourth most common malignant cancer, has been the fifth leading cause of cancer-related death diseases worldwide. It was estimated that nearly 101,420 new COAD cases were diagnosed and 27,640 deaths in the United States in 2019. COX2 has an important role in colorectal tumorigenesis and the strong relationship between COX2/prostaglandin E2 (PGE2) signaling pathway and adenomatous polyposis coli gene (APC) expression in intestinal neoplasia and also plays an important role in carcinogenesis, suppression of apoptosis, angiogenesis, and metastasis of colon cancer.

**Aim of the work:** To evaluate COX2 expression in sporadic cases of colorectal adenocarcinoma in Tobruk-Libya

**Patients, Materials, and Methods:** The study group included 60 selected cases of colorectal adenocarcinoma, diagnosed at the Pathology department of Tobruk Medical Center, Libya, between 2016 and 2019. All patients were surgically treated and underwent right or left hemicolectomy according to the site of the tumor.

**Results:** The details of 60 patients selected for analyses are as follows. The mean age of the patients at initial surgery was 48.3 years (range, 38–72 years), and 42 were (70%) males and 18 (30%) were females. 18 cases of the tumors were well-differentiated adenocarcinoma (30%); 36 cases were moderately differentiated adenocarcinoma (60%) and 6 cases were poorly differentiated adenocarcinoma (10%).

**Conclusion:** COX2 could be used as useful markers to detect the invasiveness of colorectal adenocarcinoma. Aspirin can be used for the prevention of colorectal adenocarcinoma.

***Keywords:*** Immunohistochemistry (IHC), COX2, Colorectal adenocarcinoma.

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## INTRODUCTION

Colon adenocarcinoma (COAD), the fourth most commonly malignant cancer, has been the fifth leading cause of cancer-related death diseases in worldwide. It was estimated that nearly 101,420 new COAD cases were diagnosed and 27,640 deaths in the United States in 2019 [1]. Every year, more than a million patients are diagnosed with colorectal cancer (CRC) and annual mortality due to colorectal cancer is more than a half million [2]. It is estimated that approximately 25% of all colorectal tumors have a family history [3].

Inflammatory pathway considered to play a role in colorectal carcinogenesis. Inflammation and cancer have been considered to be closely linked for many years [4]. It is believed that prostaglandins are involved in the genesis of CRC. Prostaglandins are small molecules derived from arachidonic acid, which are produced from the enzyme cyclooxygenase (COX). This enzyme is subdivided into COX-1, responsible for physiological activities and therefore is called constitutive, and COX-2 expressed after stimulation of cytokines, growth factor and mitogens [5].

COX2 is dynamically expressed in the intestine and required for the maintenance of intestinal homeostasis. Colonic luminal irritation provokes rapid induction of COX2. It increases mucosal defense against injury and initiates mucosal repair [6]. Previous studies have shown the important role of COX2 in colorectal tumorigenesis and the strong relationship between COX2/prostaglandin E2 (PGE2) signaling pathway and adenomatous polyposis coli gene (APC) expression in intestinal neoplasia [7].

Numerous clinical and experimental studies have suggested that COX-2 plays an important role in carcinogenesis, suppression of apoptosis, angiogenesis, and metastasis of colon cancer [8,9]. Overexpression of COX-2 is frequently observed in colon adenoma and carcinoma [10,11]. Inhibition of COX-2 reduced colon adenoma formation in experimental animals [12,13] and patients with familial adenomatous polyposis [14]. Specific inhibitors of COX-2 reduced tumor growth in vivo and induced apoptosis in tumor cells both in vitro and in vivo [15].

The role of COX2 in colorectal carcinogenesis has not been thoroughly studied in Tobruk, Libya population. The aim of this study was to evaluate COX2 expression in sporadic CRC.

## PATIENTS, MATERIALS AND METHODS

The present study is a retrospective study. The study group included 60 selected cases of colorectal adenocarcinoma, diagnosed at Pathology department of Tobruk Medical Center, Libya, between 2016 and 2019. All patients were surgically treated and underwent right or left hemicolectomy according to the site of the tumor. The selection process was based on the histological criteria for diagnosis of colorectal adenocarcinoma and were classified into well differentiated (grade I), moderate differentiated (grade II) and poorly differentiated (grade III).

Other clinicopathological data (gender, age, tumor size and lymph node metastasis) were extracted from medical files. All cases were studied by COX2 immuno histochemical staining monoclonal antibodies expression.

## PROCESSING PROCEDURES

For each case, a representative paraffin-embedded tissue was chosen.

The paraffin wax sections were cut at 4 microns and stained by:

a. Hematoxylin and eosin stain for routine histopathological examination.
b. Immunohistochemical staining by COX2 monoclonal antibodies.

Each case of colorectal adenocarcinoma was studied for histopathological diagnosis and was classified into well differentiated (grade I), moderate differentiated (grade II) or poorly differentiated (grade III).

Each section obtained from the blocks was placed on positive charge slides, dewaxed in xylene, rehydrated in consecutive descending concentrations of ethanol (100%, 90%, 80%, and 70%), and rinsed in distillated water.

For antigen retrieval, slides were placed in a plastic container filled with sufficient citrate buffer pH 6 and heated in a microwave oven at 100ºC for three successive times, five minutes each. The amount of fluid in the container was checked and was added if necessary to prevent slides from drying out.

The slides were immersed in 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase, and incubated with the primary antibody for COX2 (mouse monoclonal, Novocastra), at 1:100 dilution, overnight, at 40C. Chromogen application by using DAB (3,3-diaminobenzidine tetrahydrochloride). The counterstaining of the sections was done with

Mayer's Hematoxylin. Positive and internal negative controls were included for each staining procedure. Internal negative control sections were processed without the addition of primary antibodies. Fibroblast cells, known to express COX2, were used as the positive control in order to verify the accuracy of the technique. COX-2 immunostaining reactions were recognized as homogenous cytoplasmic and membranous expression.

## IMAGE ANALYZER

Image Analyzer computer system Leica Q win 500 was used for accurately measuring the area and area % as well as the intensity of reactions of COX2 monoclonal antibodies.

## MEASURING THE AREA PERCENTAGE OF REACTION

It was measured in the form of area and area percent inside a standard measuring frame of size 2927364 µm2 per 5 fields using magnification (X-200) by light microscope transferred to the monitor. Areas were masked by red binary color which could be measured using the computer system. Mean values were obtained for the whole specimens in each group.

## MEASURING THE IMMUNOSTAINING INTENSITY (OPTICAL DENSITY)

Regarding the intensity of the reaction within the cells, the optical density was measured after transforming the image into grey mode. Areas with maximum gray were masked by blue binary color and then the intensity of grey was measured.

## STATISTICAL ANALYSIS

Statistical analysis of variance (ANOVA) was used in order to explore the significant differences in the staining intensity of the positivity of COX-2 immunoreactions for each case. P-values equal to or less than 0.05 were considered statistically significant.

## RESULTS

## CLINICOPATHOLOGICAL FEATURES

The details of 60 patients selected for analyses are as follows. The mean age of the patients at initial surgery was 48.3 years (range, 38–72 years), and 42 were (70%) males and 18 (30%) were females. 18 cases of the tumours were well differentiated adenocarcinoma (30%); 36 cases were moderate differentiated adenocarcinoma(60%) and 6 cases were poorly differentiated adenocarcinoma (10%). Tumor size ranged between 32 and 95 mm, with a mean of 43.24 mm. Lymph nodes metastasis were present in 34 cases (56.7%) and 26 cases (43.3%) shows no lymph nodes metastasis.

## COX2 EXPRESSION

## [I] AREA PERCENTAGE

The expression of COX2 in moderately differentiated adenocarcinoma scored the highest levels (71.046 ± 9.897) followed by well differentiated adenocarcinoma (67.314 ± 11.944) and finally poorly differentiated adenocarcinoma (60.984 ± 8.357) as shown in (Table 01).

| Adenocarcinoma | Area% (COX2) | | ANOVA | |
|---|---|---|---|---|
| | Range | Mean ± SD | f | P-value |
| Well differentiated | 54.050 - 85.370 | 67.314 ± 11.944 | 1.25 | 0.321 |
| Moderately differentiated | 54.740 - 79.480 | 71.046 ± 9.897 | | |
| Poorly differentiated | 48.500 - 68.750 | 60.984 ± 8.357 | | |

**Table 01:** Difference in mean COX2 area percentage between adenocarcinoma grades lesions using ANOVA statistical test.

## [II] OPTICAL DENSITY

The expression of COX2 in poorly differentiated adenocarcinoma scored the highest levels (66.578 ± 0.606) followed by moderately differentiated adenocarcinoma (55.730 ± 0.689) and finally well differentiated adenocarcinoma (24.990 ± 2.840) as shown in (Table 02).

| Adenocarcinoma | Optical Density (COX-2) | | ANOVA | |
|---|---|---|---|---|
| | Range | Mean ± SD | F | P-value |
| **Well differentiated** | 20.940 - 27.910 | 24.990 ± 2.840 | 783.66 | <0.001* |
| **Moderately differentiated** | 54.840 - 56.580 | 55.730 ± 0.689 | | |
| **Poorly differentiated** | 65.800 - 67.360 | 66.578 ± 0.606 | | |

**p*-value <0.05 was considered to be statistically significant.

**Table 02: Difference in mean COX2 optical density between adenocarcinoma grades lesions using ANOVA statistical test.**

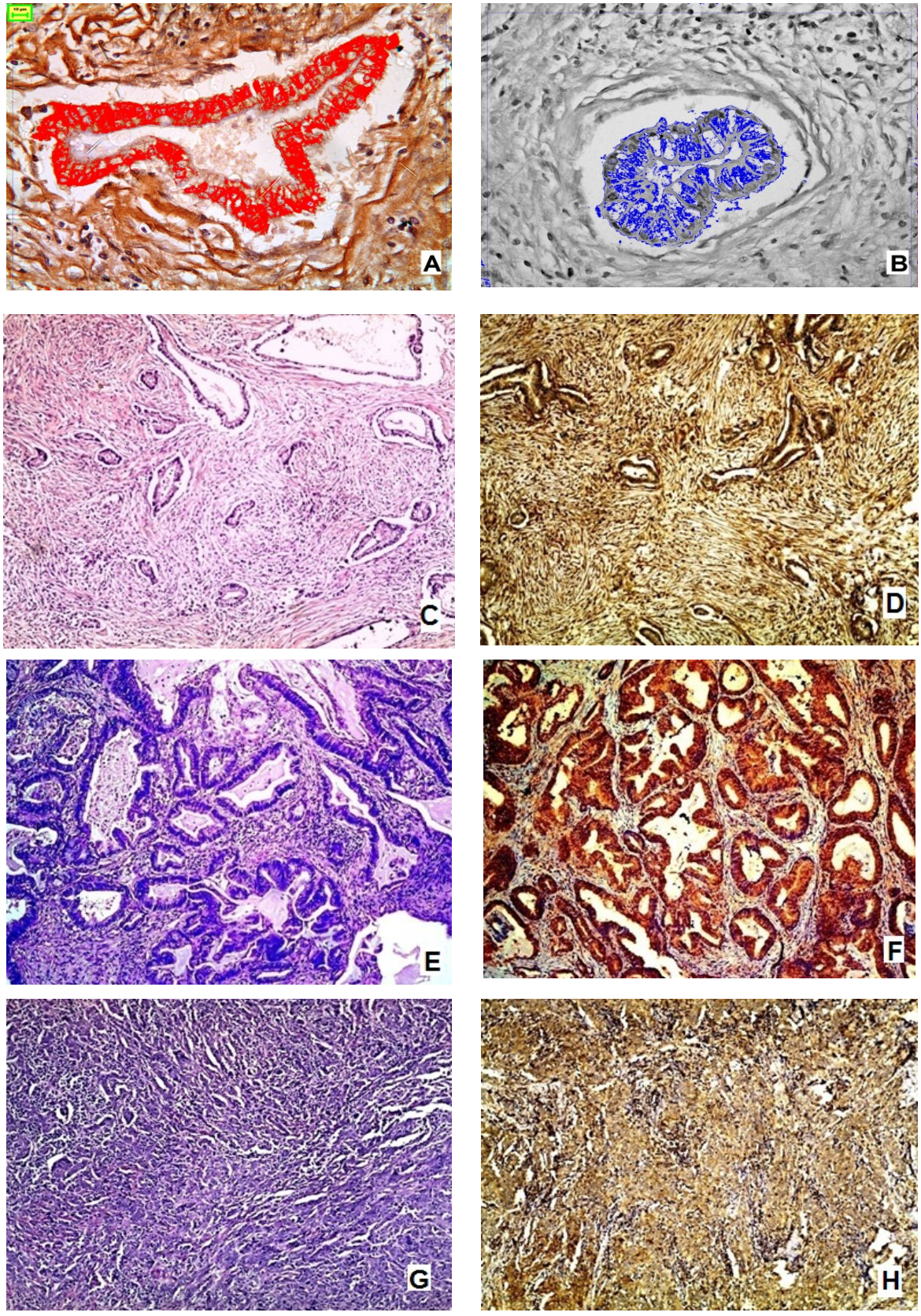


**Figure 01**

A- A copy display seen on the screen of the image analyzer system after masking the areas of positive reaction of adenocarcinoma by red binary color

showing the way of measurement the area of the reaction.

B- A copy display seen on the screen of the image analyzer system after masking the areas of positive reaction of adenocarcinoma by blue binary color to measure the intensity of reaction.

C- Well differentiated adenocarcinoma (H&E, x100).

D- Well differentiated adenocarcinoma incubated with anti COX2 antibody (Anti COX2 antibody, X100).

E- Moderately differentiated adenocarcinoma (H&E, x100).

F- Moderately differentiated adenocarcinoma incubated with anti COX2 antibody (Anti COX2 antibody, X100).

G- Poorly differentiated adenocarcinoma (H&E, x100) .

H- Poorly differentiated adenocarcinoma incubated with anti COX-2 antibody (Anti COX-2 antibody, X100).

## DISCUSSION

COX2 has been the subject of intensive investigations in cancer research in recent years. These studies collectively reported over expression in various types of cancer and suggested that COX2 may play a role in carcinogenesis [16, 17, 18].

The purpose of the present study was to demonstrate the immunohistochemical expression of COX2 among different histological grades of colorectal adenocarcinoma, and to test the hypothesis that COX2 is useful as markers for invasion and metastasis.

The variability of positive immunoreactivity of COX2 in colorectal adenocarcinoma agrees with Renkonen et al. [16], Shibata et al. [19] and Sakurai et al., [20]. This variability in expression patterns was attributed to mode of growth and invasion of malignant cells as reported by Shibata et al. [19] and Augusto et al. [21].

The direct correlation between the immuno expression of COX2 and the histological grade of malignancy of colorectal carcinomas is in accordance with Renkonen et al. [16], Kuroda et al. [17], Segawa et al. [21] and Tan and Putti [22]. They found that poorly differentiated cases showed the highest intensity of expression. However these results are in contradictory with Shibata et al. [19], Ratnasinghe et al. [23] and Kawata et al. [24]; where they showed inverse correlation between the intensity of expression of COX2 and the histological grade of malignancy. Whereas other studies showed that COX2 immunoexpression is not correlated with histological grade such as that of Augusto et al. [21], Itoh et al. [25] and Pannone et al. [26].

High expression of COX2 in invasive malignant cells may reflect a role of COX2 in the carcinogenesis, a finding described by Lee et al. [27], Choi et al. [28] and Corcoran et al. [29].They observed increased levels of anti-apoptotic protein Bcl-2 and COX2 and suggested that COX2 interfere with P53 - dependent apoptosis pathways. These results are in accordance with the finding of the present study in which increased expression of COX2 in poorly differentiated cases was detected.

Expression of COX2 in endothelial lining of blood vessels of most of the studied cases agrees with Jackel et al. [30], Kyzas et al. [31] and Kraisorn et al. [32].

Increased intensity of expression in endothelial lining of blood vessels confirmed the role of COX2 in promotion of angiogenesis as described by Seno et al. [33], Cohen et al. [34] and Castellone et al. [35]. They found that COX2 induce production of several angiogenic factors including vascular endothelial growth factors (VEGF) and transforming growth factor b (TGF-b) via induction of the COX2/PGE2 system [36, 37].

The positive expression of COX2 in inflammatory cells in most of the studied cases agrees with Gallo et al., [38] and Ogata et al. [39] who described the role of COX2 in inflammation and activation of inflammatory mediators.

The findings that the intensity of COX2 expression directly correlated with histological grade are in agreement with Eberhart et al. [40], Hida et al. [41], Nozoe et al. [42] and Takatori et al. [43] where they reported similar findings in colorectal, lung and esophagus carcinomas.

## LIMITATIONS OF THE STUDY

Our study has some limitations. First, a small sample size was used to identify the value of COX2 expression in colorectal adenocarcinoma because of the short study period. Second, there is no follow-up of the patients because this study is designed and performed recently.

## CONCLUSION

From the results of the present study, it can conclude that the degree of histological differentiation of colorectal adenocarcinoma is inversely correlated

with the intensity of expression of Cox-2. While the area percentage showed direct correlation. COX2 could be used as useful markers to detect invasiveness of colorectal adeno carcinoma. Measuring the intensity of reaction for the used markers is more reliable than measuring the area percentage. This because area percentage showed contradictory findings among the studied cases. A long-term follow-up study will be necessary to identify the clinical value of COX2 expression in colorectal adenocarcinoma. Aspirin can be used for prevention of colorectal adeno carcinoma.

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