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# Cyclooxygenase-2 (COX-2) Expression in Egyptian Cases of Benign Prostatic Hyperplasia and Prostatic Adenocarcinoma

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**Abstract:** Inflammations, Benign Prostatic Hyperplasia (BPH) and tumors are main pathologic processes that affect the prostate gland. Cyclooxygenase-2 (COX-2) is involved in a variety of important cellular functions, including cell growth and differentiation, cancer cell motility and invasion, angiogenesis. This work aimed to assess the expression of Cox-2 in BPH, prostatic intraepithelial neoplasia(PIN) and prostatic adenocarcinoma to evaluate its role in prostatic cancer. Correlation of its expression with the type of the lesion and tumor grade was also done. COX-2 immunostaining of 42 random cases of prostatic lesions (15 cases of prostatic carcinoma, 10 PIN and 17 BPH) were done. COX-2 immunostaining was positive in 97.6% of cases and negative in only one case (2.4%). The score of its expression in most of BPH cases was weak to moderate while only four (23.5%) show strong expression (score 3). Five (50%) of PIN cases and 13 (86.7%) prostatic cancer cases show strong expression. Prostatic cancer grade 3 cases show COX-2 expression stronger than those of grade 2 with statistical significance. From these results, we can conclude that COX-2 overexpression may play a role in prostatic cancerogenesis and neoplastic progression.

**Key words:** Cox-2 · Prostatic hyperplasia · PIN · Prostatic carcinoma

## INTRODUCTION

The main three pathologic processes that affect the prostatic gland with sufficient frequency are inflammation, benign nodular enlargement and tumors. Among these three conditions, the benign nodular enlargement is by far the most common, followed by prostatic carcinoma [1]. Prostate cancer is one of the most common malignant diseases. It is considered sixth most common cancer in the world and the third in importance in men, so health-care intervention is sought worldwide and in many countries [2].

According to National Cancer Institute, Cairo University, Egypt, Cancer Pathology Registry during years 2003 & 2004 showed an incidence of 49 and 95.74% for prostatic carcinoma and prostatic tumors, respectively among male genital tumors [3].

As regards the diagnostic techniques for prostatic carcinoma, two immunocytochemical markers are used in routinely processed material with polyclonal or monoclonal antisera. They are prostate specific antigen (P.S.A) and prostatic acid phosphatase (P.A.P). Prostatic carcinoma cells are also positive for low molecular weight keratin, leu7, EMA, CEA and cathepsin D [4,5].

The diagnosis of prostate cancer is usually readily made on morphological grounds by use of traditional histological parameters, including architecture, nuclear features and the presence or absence of a basal cell layer. However, in morphologically equivocal cases the histopathologist may have to resort to the use of immunohistochemistry to resolve the differential diagnosis [6].

The identification of an enzyme catalyzing fatty oxidation as a rate limiting step in the progress from normal cell growth through hyperplasia or to neoplasia has opened up a whole new field of cancer research [7]. The cyclooxygenase (COX) isozymes COX-1 and COX-2 catalyze the conversion of arachidonic acid to eicosanoids, namely prostaglandins and thromboxanes via endoperoxides. Both isoforms are expressed in different cell types and tissues [8,9].

COX-2 is an inducible isoform and can be induced in response to mitogenic agents, growth factors, lipopolysaccharides and cytokines. Both isoforms are also responsible for the synthesis of prostaglandin $E_2$ ($PGE_2$) and there is some evidences for a correlation between increased levels of $PGE_2$ and tumorigenesis [10,11].

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Aberrant or increased expression of COX-2 has been implicated in the pathogenesis of many diseases including carcinogenesis [12]. Selective COX-2 inhibition resulted in increased apoptosis and down regulated Bcl-2 expression in androgen sensitive human prostatic cancer cell [13]. Enhanced COX2-induced synthesis of prostaglandins stimulates cancer cell proliferation [14], as well as promotes angiogenesis, inhibits apoptosis and increases metastatic potential [15].

Elevated levels of mRNA and protein of COX2 are known to be associated with various premalignant and malignant lesions of epithelial origin in organs such as esophagus, breast, lung, prostate, bladder, stomach and other cancers, indicating a close involvement of COX2 in tumor progression and other pathological phenotypes in various malignant tumors [16 – 19 ]. Also, it is known to be associated with lymph node metastasis in gastric cancer and to affect the prognosis in primary lung adenocarcinoma [20].

In prostatic adenocarcinoma, COX-2 is highly expressed, whereas it is not detectable in normal epithelial prostate cells [21]. COX-2 over expression has to be found a characteristic of prostatic preneoplastic lesions, prostatic intraepithelial neoplasia, invasion and adhesion of prostatic cancer cells to extracellular matrix (ECM) protein or endothelial cells [22].

The aim of the current study was to examine the expression of COX-2 in series of BPH, PIN and prostatic adenocarcinoma cases using immunohistochemistry, as a trial to define the role of COX-2 in carcinogenesis and its correlation with some clinicopathological features of malignant cases.

## MATERIALS AND METHODS

The materials of this work consisted of 42 cases of prostatic biopsies ( 15 prostatic adenocarcinoma, 10 PIN, 17 BPH) obtained from Pathology Department, Faculty of Medicine, Cairo University during the period from March 2007 to May 2008.

**Histopathological and Immunohistochemical Procedures:** Formalin-fixed and paraffin-embedded blocks were sectioned at 5 μ thickness and examined microscopically using H & E stain to confirm the diagnosis before the immunohistochemistry. In addition, the sections on pretreated (coated) slides (superplus, Menzel-Glaser, Germany) used for immunohistochemistry study were deparaffinized in xylene and rehydrated using ethanol gradients, then pretreated three times in a microwave oven for 5 minutes in citrate solution (Biogenex-Neufahrn, Germany). Endogenous peroxidase activity was blocked by immersion in 3% hydrogen methanol for 20 minutes. Washing three times with cold 0.01M phosphate buffered saline (PBS), then blocking with 10% normal rabbit serum followed by incubation for 30 minutes with COX-2 monoclonal rabbit anti-human antibody (anti-Cox 2, Lab vision, USA, Cat#RB-9072) at a dilution of 1:25. Then, ultravision detection system (HRP/DAB, Lab vision, USA) is added to the slides and incubated for 15 minutes. Finally the diaminobenzidine, (DakoCytomation) was used as a chromogen and hematoxylin as a counterstain. Section untreated with the primary antibody as a negative control were carried out in this study. Positive immunoreactivity to COX-2 gives a brown cytoplasmic staining in tumor cells [23].

All H&E sections were examined for re-evalution of the diagnosis of the prostatic biopsy whether BPH, PIN or carcinoma and Gleason scoring system and grading of prostatic carcinoma.

The entire tissue cox-2 stained sections was scanned to assign the scores cox- 2 expression. For each tissue specimen, the extent and intensity of staining with COX-2 antibodies was graded on a scale from 0 to 4+ on two separate occasions using coded slides and an average score was calculated. Staining was classified into five grades from 0 to 4+ according to the intensity of staining and the number of positive cells. The present study assessed all tissue on the slides to assign the score. A 4+ grade implies that all staining was maximally intense throughout the specimen, whereas a 0 grade implies that staining was absent throughout the specimen. The microanatomic sites of staining also were recorded [24].

**Statistical Analysis:** SPSS version 12.0 (Statistical Package for Social Science) was used for data management. Mean and standard deviation described quantitative data. ANOVA (analysis of variance) was used to compare means of more than 2 groups. Scheffe test made post hoc pair-wise comparisons. Correlation analysis was done with parametric and non-parametric tests. Significant differences and associations were obtained at $P<0.05$.

## RESULTS

This work studies Cox-2 immunohistochemical staining in forty two randomly collected cases of prostatic lesions, fifteen cases of prostatic carcinoma representing 35.7% of all studied cases (Figs. 5-10), ten PIN cases

Table 1: Gleason scoring in the studied prostatic carcinoma cases

| Gleason score | Number of cases | % |
|---|---|---|
| 5 | 1 | 6.7 |
| 6 | 1 | 6.7 |
| 7 | 6 | 40.0 |
| 8 | 2 | 13.3 |
| 9 | 3 | 20.0 |
| 10 | 2 | 13.3 |
| Total | 15 | 100% |

Table 2: WHO grading of studied prostatic carcinoma cases

| Grade | Number of cases | % |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 8 | 53.3 |
| 3 | 7 | 46.7 |
| Total | 15 | 100% |

Table 3: Cox-2 expression in all studied benign prostatic hyperplasia cases

| COX-2 score | Number of cases | % |
|---|---|---|
| 0 | 1 | 5.9% |
| 1 | 5 | 29.4% |
| 2 | 7 | 41.2% |
| 3 | 4 | 23.5% |
| 4 | 0 | 0% |
| Total | 17 | 100% |

4 out of 17 BPH cases show strong (score 3&4) COX-2 expression (23.5 %).

Table 4: COX-2 expression in all studied PIN cases

| COX-2 score | Number of cases | % |
|---|---|---|
| 0 | 0 | 0% |
| 1 | 2 | 20% |
| 2 | 3 | 30% |
| 3 | 4 | 40% |
| 4 | 1 | 10% |
| Total | 10 | 100% |

5 cases out of 10 PIN cases show strong (score3&4) COX-2 postivity (50%)

Table 5: COX-2 expression in all studied prostatic cancer cases

| COX-2 score | Number of cases | % |
|---|---|---|
| 0 | 0 | 0% |
| 1 | 0 | 0% |
| 2 | 2 | 13.3% |
| 3 | 5 | 33.3% |
| 4 | 8 | 53.4% |
| Total | 15 | 100% |

13 cases out of 15 prostatic carcinomas show strong (score 3&4) COX-2 expression (96.7%)

Table 6: Correlation between the COX-2 expression in BPH, PIN and prostatic carcinoma cases

| Studied cases | Number of cases | Mean | Std. Deviation |
|---|---|---|---|
| BPH | 17 | 1.8 | 0.88 |
| PIN | 10 | 2.4 | 0.97 |
| PC | 15 | 3.4 | 0.74 |

Table 7: Relation between Gleason scoring and COX-2 expression in prostatic carcinoma

| | COX-2 score | | | | | |
|---|---|---|---|---|---|---|
| Gleason score | 0 | 1 | 2 | 3 | 4 | Total |
| 5 | 0 | 0 | 0 | 1 (6.67 %) | 0 | 1 (6.67 %) |
| 6 | 0 | 0 | 1 (6.67 %) | 0 | 0 | 1 (6.67 %) |
| 7 | 0 | 0 | 1 (6.67 %) | 3 (20.00 %) | 2 (13.33 %) | 6 (40.00 %) |
| 8 | 0 | 0 | 0 | 0 | 2 (13.33 %) | 2 (13.33 %) |
| 9 | 0 | 0 | 0 | 1 (6.67 %) | 2 (13.33 %) | 3 (20.00 %) |
| 10 | 0 | 0 | 0 | 0 | 2 (13.33 %) | 2 (13.33 %) |
| Total | 0 | 0 | 2 (13.33 %) | 5 (33.34 %) | 8 (53.33 %) | 15 (100.00 %) |

83% of Gleason score 7 cases showing strong COX-2 expression (score 3&4), increased to 100% among Gleason score 9 cases

Table 8: Relation between WHO grading of prostatic carcinoma and COX-2 expression in prostatic carcinoma

| | COX-2 score | | | | | |
|---|---|---|---|---|---|---|
| Grade | 0 | 1 | 2 | 3 | 4 | Total |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 2 (13.33 %) | 4 (26.67 %) | 2 (13.33 %) | 8 (53.33 %) |
| 3 | 0 | 0 | 0 | 1 (6.67 %) | 6 (40.00 %) | 7 (46.67 %) |
| Total | 0 | 0 | 2 (13.33 %) | 5 (33.34 %) | 8 (53.33 %) | 15 (100.00 %) |

75% of grade 2 cases showed strong COX-2 expression increased to 100% among grade 3 cases

Table 9: COX-2 expression in different PC grades

| Grade of PC | No of cases | Std. Deviation |
|---|---|---|
| 1 (Gleason score 2-4 ) | 0 | 0.00000 |
| 2(Gleason score 5-7 ) | 8 | 0.75593 |
| 3(Gleason score 8-10 ) | 7 | 0.37796 |
| Total | 15 | 0.73679P = 0.04 |

(23.8%) (Figs. 3-4) and seventeen BPH (40.5%) (Figs. 1-2). The Gleason scoring of the studied prostatic carcinoma cases ranging between 5-10 as shown in Table1. Eight (53.3%) of these cases are grade II & seven (46.7%) are grade III according to WHO grading (Table 2).

The scores of expression of COX-2 in the studied cases show negative COX-2 expression is a BPH case which is the only negative case in the all studied cases

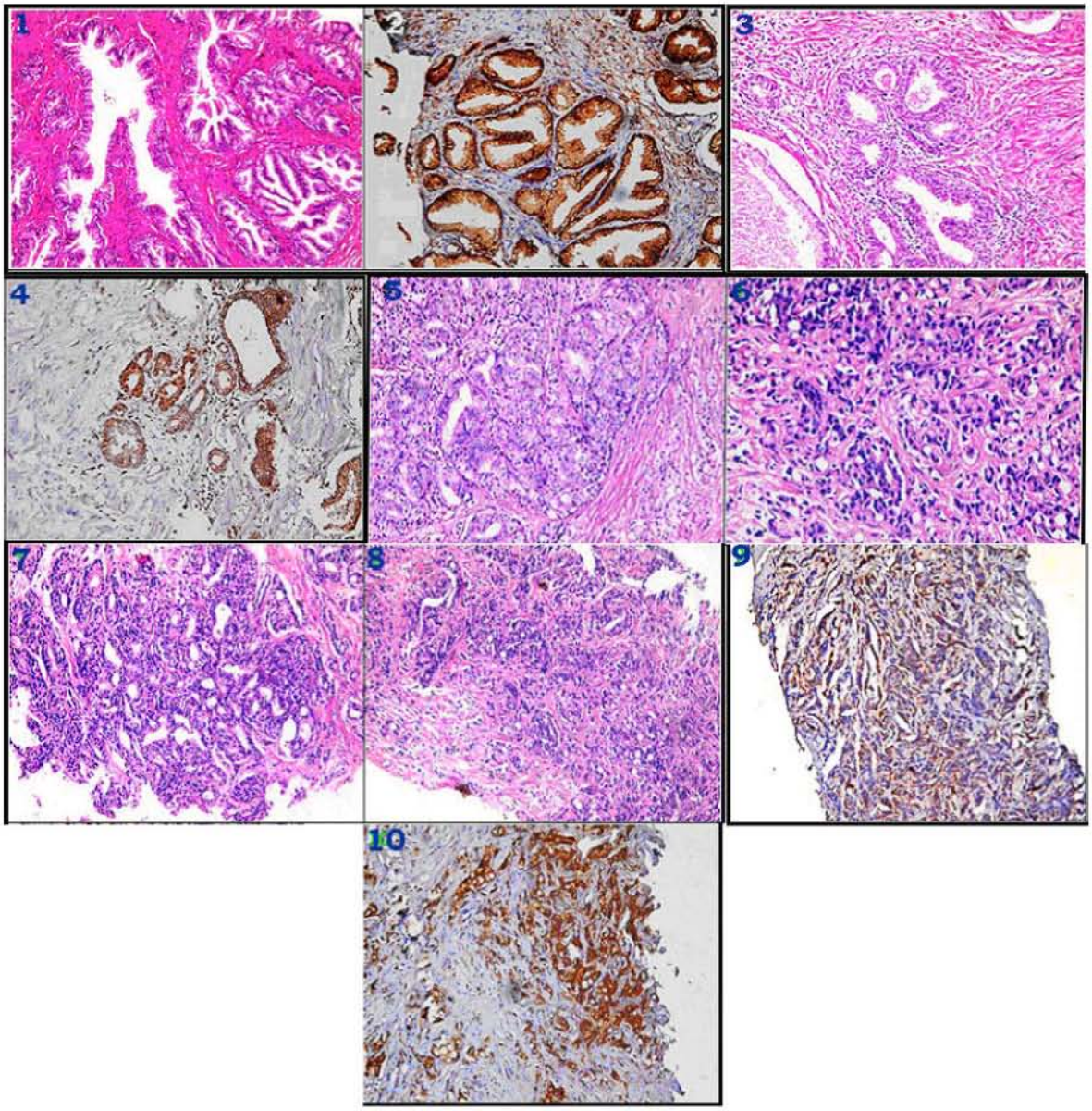


Fig. 1: Benign prostatic hyperplasia. (H&E x 100)
Fig. 2: Diffuse brown cytoplasmic staining denoting strong Cox-2 postivity in a case of benign prostatic hyperplasia. (DAB chromogen and hematoxylin counter stain x 200)
Fig. 3: High grade prostatic intraepithelial neoplasia. (H&E x 200)
Fig. 4: Diffuse brown cytoplasmic staining of Cox-2 in a case of high grade prostatic intraepithelial neoplasia. (DAB chromogen and hematoxylin counter stain x 200)
Fig. 5: Prostatic carcinoma grade II Gleason 7 (3+4) (H&E x 200)
Fig. 6: Prostatic carcinoma grade III Gleason 8 (5+3) (H&E x 400)
Fig. 7: Prostatic carcinoma grade III Gleason 9 (5+4) (H&E x 200)
Fig. 8: Prostatic carcinoma grade III Gleason 10 (5+5) (H&E x 200)
Fig. 9: Prostatic carcinoma grade II Gleason 7 (4+3) showing diffuse brown cytoplasmic staining of Cox-2 (DAB chromogen and hematoxylin counter stain x 200)
Fig. 10: Prostatic carcinoma grade III Gleason 9 (5+4) showing diffuse brown cytoplasmic staining of Cox-2 (DAB chromogen and hematoxylin counter stain x 200)

and most of these BPH cases showed weak to moderate COX-2 immunoreactivity and only four (23.5%) of them show strong COX-2 expression (Score 3) (Table 3). Five (50%) of studied PIN cases and 13 (86.7%) prostatic cancer cases show strong COX-2 expression (score 3&/or 4) Tables4&5. The correlation of COX-2 expression in different studied cases is shown in Table 6.

The most common studied prostatic lesions showing strong COX-2 expression (score 3&4) are prostatic carcinomas. (P = 0.001).

Most of the prostatic cancer cases with high Gleason scoring > 7 show high COX-2 expression score as shown in Table 7.

Poorly differentiated prostatic cancer cases (grade 3) show COX-2 expression stronger than those with moderate differentiation (grade 2). With statististicaly significant difference P= 0.024 This is presented in Tables 8&9.

## DISSCUSION

In this work, BPH, PIN and prostatic carcinoma cases, were investigated as these lesions are of most importance and of sufficient frequency worldwide [1-3]. As COX-2 increased expression has been implicated in carcinogenesis, angiogenesis and increase metastatic potential [12, 15]. Also, it is known to be associated with various premalignant and malignant lesions in organs such as esophagus, breast, lung, bladder, stomach and other cancers, indicating a close involvement of COX-2 in tumor progression [16-19]. So, we investigated Cox-2 expression in these common worldwide lesions to study its potential role in pathogenesis.

In the current investigation, COX-2 immunostaining in the studied cases were positive in almost all cases (97.6%). The scores of expression of COX-2 in studied cases showed that the only case with negative COX-2 expression is a BPH case and four (23.5%) of BPH show strong COX-2 expression (score 3) while five (50%) of studied PIN cases and 13 (86.7%) prostatic cancer cases show strong COX-2 expression (score 3&/ or 4). There was a significant difference between the studied cases (BPH, PIN and prostatic carcinoma cases as regard the mean of COX-2 expression. Nearly similar results are given by *Yoshimura et al.* [22] Who found that immunoreactive COX-2 was very weak in all BPH samples, PIN samples and normal prostate samples. They found strong expression levels in the low and moderate grades of adenocarcinoma and a very strong expression level in the high grades of adenocarcinomas and suggested that $PG_{E1}$ and $PG_{E2}$ (produced by COX-2) have a role in the proliferation of the malignant cells and metastasis of PC cells. *De Marzo et al.* [25] described the evolution of the proliferative inflammatory atrophy of the prostate to prostate carcinoma and suggested that COX-2, because it has been involved in the induction of inflammation and apoptosis, may play an important role in the evolution of carcinogenesis in the prostate and indicate that the inhibition of COX-2 development may lead not only to the inhibition of proliferation and metastasis of prostatic carcinoma, but also to the inhibition of prostatic carcinogenesis.

*Zha et al.* [26] indicated that COX-2 protein is expressed at very low or undetectable levels in normal prostate and that COX-2 expression is not consistently elevated in PIN or prostate cancer. Furthermore, when staining for COX-2 was observed in prostate cancer, the extent of positive staining did not correlate with established clinical-pathological risk factors (Gleason score or pathological stage). Another study also found low expression of COX-2 in prostate cancer, although they reported more COX-2 expression in higher-grade tumors[27].

*Liu X. et al.* [28] mentioned that discrepancies in published reports include whether or not COX-2 is expressed in normal prostate, which cell types (*e.g.*, epithelial cells, smooth muscle cells, or stroma) express COX-2, whether COX-2 expression is elevated in PIN or prostate carcinoma and whether the expression level of COX-2 in PCa is associated with cellular differentiation or disease progression. Despite these issues, clinical trials of prostate cancer prevention and therapy using selective COX-2 inhibitors have been initiated.

In this work, most of the prostatic carcinoma cases with high Gleason scoring > 7 showed strong COX-2 expression score. The poorly differentiated prostatic cancer cases (grade 3) show COX-2 expression stronger than those with moderate differentiation (grade 2) with statistically significant difference. These results were similar to that given by *Lee et al.* [29] who found that COX-2 was over-expressed in 15 out of 18 (83%) prostatic cancer samples whereas, it was detected only in 22% (4 of 18) BPH and the intensity of immunostaining of COX-2 correlated with the tumor grading. Also, *Madaan et al.* [30] made their study on 30 samples from patients with BPH and 82 with prostatic cancer and showed that COX-2 expression differed markedly between BPH and cancer, also COX-2 expression was significantly higher in poorly differentiated than well differentiated tumors. In spite of, Isbell *et al.* [31] found that no COX-2 expression in benign

prostate and immunostaining in tumors was present only in 17% of cases, they found that COX-2 immunoreactivity is correlated with high tumor grade (Gleason score 8 and 9 versus 5 to 7).

*Hla et al.* [32] mentioned that as it is considered that PGs promote the proliferation and metastasis of cancer cells and secondarily promote the growth of cancer cells by immunosuppression, furthermore, $PG_{E1}$ and $PG_{E2}$, which are derived from COX-2, are known to induce angiogenesis. PG production generally is enhanced in cancer cells, so COX-2 induction has the potential to promote tumor growth and progression.

In conclusion, COX-2 may play a causal role in cell proliferation and carcinogenesis in prostatic carcinoma. The extent of positive staining correlates with established clinical-pathological risk factors (Gleason score and tumor grade)

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