Research Article

A study of COX-2 expression in prostatic adenocarcinoma and its clinical relevance

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Abstract

Objective: The purpose of the present study was to determine the expression of cyclooxygenase-2 (COX-2) in patients with carcinoma of prostate and its clinical significance in relation to clinical pathological parameters. **Materials and Methods:** A total of 100 cases of prostatic tissue specimens consisting of 88 adenocarcinoma prostate and 12 Benign Prostatic Hyperplasia (BPH) were included in the study. Formalin-fixed paraffin-embedded tissue samples were initially stained using Hematoxylin and Eosin stains and graded according to Gleason scoring system. Immunohistochemistry for COX-2 were performed on these blocks. The expression pattern was determined and correlated with clinicopathological parameters. **Results:** A majority of well differentiated prostate carcinoma and BPH cases showed strong COX-2 expression. In contrast poorly differentiated and most of the moderately differentiated cases showed weak positivity. COX-2 expression pattern for different grades were found to be statistically significant (p=0.03). Clinically proven metastatic samples showed negative COX-2 expression. Correlation between serum PSA level with COX-2 expression did not show any significant relationship. **Conclusion:** COX-2 expression was found to be strong in well differentiated carcinoma of prostate irrespective of the PSA levels. Inhibitors of COX-2 may prove useful as a alternative therapeutic adjunct for the treatment of low grade prostatic carcinoma.

Keywords: Cyclooxygenase-2, prostate carcinoma, prostate therapy

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INTRODUCTION

Carcinoma of prostate remains an important public health concern in western countries and an emerging malignancy in developing nation .It is the second most common cancer with 1.1 million men worldwide diagnosed with prostate cancer in 2012, accounting for 15% of the cancers in men[1]. According to National Cancer Institute's (NCI) Surveillance Epidemiology and End Results (SEER) Cancer Statistics Review[(2)] 56.6% of men are diagnosed to have carcinoma between 71 to 90 years .With an increase in life expectancy and growing geriatric population there has been a steady rise in prostate cancer in India also. Intense research in prostate cancer is being carried out in the West, although only few focused studies have been reported in India. Recurrent or chronic inflammation has been implicated in the development of many human cancers, including those of the esophagus, stomach, liver, large intestine and urinary bladder and the elevated levels of pro-inflammatory cytokines influence cell survival growth and differentiation of tumor cells. Cyclooxygenase -2 (COX-2) is an inducible enzyme involved in the synthesis of prostaglandins from arachidonic acid. There are evidences suggesting its role in the development and progression of cancer by facilitating inflammatory response, reducing cell apoptosis, increasing angiogenesis and damaging DNA oxidation[3]. However, controversy still exists regarding

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COX-2 expression in prostate cancer. With this background, we propose to evaluate the expression of COX-2 in prostate cancer and Benign Nodular Hyperplasia (BPH) and correlate it with other clinicopathological factors including, Gleason's score and Serum Prostate-Specific Antigen (PSA) levels.

AIM

The study was undertaken to show the expression pattern of COX-2 in carcinoma prostate. A small number of benign prostatic hyperplasia was also included to note any difference in its staining pattern. An attempt was also made to correlate its expression with tumour grade, serum PSA levels and other clinical parameters.

MATERIALS AND METHODS

A total of 100 cases of prostatic tissue specimens consisting of 88 adenocarcinoma prostate and 12 BPH were retrieved from the surgical pathology files of the Department of Pathology, Sri Ramachandra Medical College and Research Institute, Chennai. The samples included Trans Urethral Resection of the Prostate (TURP) chips and needle core biopsies from the year January 2009 to May 2011.Permission of the institutional ethics committee was obtained prior to commencing the study. Gross findings were recorded and clinical data was obtained from the medical record section and available local area computer network in-service at Sri Ramachandra Medical College and Research Institute, Chennai. Formalin fixed paraffin embedded tissues were stained with Hematoxylin and Eosin. Gleason grading system was used to grade prostate cancer. Among the other histopathological features observed were inflammatory response, adjacent High Grade Prostatic Intraepithelial Neoplasia (HGPIN) and perineural invasion. Immunohistochemistry staining for COX-2 was performed on all the 100 cases. Briefly tissue sections were taken in the 0.1% poly–L-lysine coated slides. After deparaffinization, tissue sections were rehydrated using descending grades of alcohol and water. Antigen retrieval was done by heating in the 0.01 M citrate buffer (pH 6.0). Tris buffer solution (TBS) was used for washing. Hydrogen peroxide (3%) was used to block endogenous peroxidase. This was followed by power block for 10 minutes. Incubation of slides with primary antibody (COX-2) for 1 hour. Followed by rinsing in TBS and then incubated with super enhancer. This was followed by rinse in Tris buffer solution and incubation with secondary antibody for 30 minutes. After cleaning and washing with TBS, excess of buffer was wiped and then slides were incubated with substrate/ chromogens for 5 min. After rinsing in distilled water for a few second, the sections were counter stained with Haematoxylin stain for 3 min, the slides were dehydrated and mounted. Polyclonal antibody directed against COX-2 antigen was observed in the cytoplasm and membrane of the tumor cells and the intensity of COX-2 expression was recorded based on criteria of Krajewska *et al* [(4)] (Immunostaining intensity was rated as follows: 0: none; 1: weak; 2: moderate; and 3: intense. Specimens were considered immunopositive when ≥1% of the tumor cells had clear evidence of immunostaining)

RESULTS

A total of 88 cases of adenocarcinoma prostate and 12 BPH diagnosed between January 2009 to July 2011 at the Department of Pathology, Sri Ramachandra Medical College and Research Institute, Chennai were retrieved and studied. Among the cases studied study 40 were TURP specimen while 60 were needle core biopsies. Patient's age were ranged from 48 years to 92 years. The incidence of prostate cancer was comparatively low below 60 years (7.9 %). The highest incidence was between the age group of 71 to 90 years (67%) (Figure1). The commonest clinical presentation included increased frequency of urine, followed by urgency and urinary retention associated with pain. Hematoxylin and Eosin staining was used to stratify the patients according to Gleason's grades. Among the 88 carcinomas, 20 were well differentiated, 36 were moderately differentiated and 32 were poorly differentiated. (Figure 2) Two cases had adjacent HGPIN, 5 cases had inflammatory response, 22 cases had perineural invasion and 4 cases had radiologically proven metastatic deposit. (Figure 3). Serum PSA was available for 53 cases and was found to be elevated more in the poorly differentiated cases and in bone metastasis as compared to well and moderately differentiated cases (Figure 4). On statistical analysis of the mean PSA values with different grades of the tumor using non parametric test, it was found to be significant ($p = 0.02$). COX-2 expression pattern was seen in the cytoplasm of the cells. The intensity of staining was evaluated in different grades of adenocarcinoma and in BPH. The intensity of staining was found to be inversely proportional to the grades of adenocarcinoma. A majority of well differentiated and BPH cases showed strong COX-2 positivity (Figure 5a and 5b), whereas the poorly differentiated and most of the moderately differentiated cases showed weak positivity (Figure 5c and 5d) (Figure 6). All cases with distant metastasis showed a negative COX2 expression (Figure 5e). There was an inverse correlation between COX-2 expression and different grades of carcinoma and this was found to be statistically significant at $p=0.03$ (Chi square value of 9.09 with Yates continuity correction). There was no significant correlation between Serum PSA levels and COX-2 expression. (Figure 5)

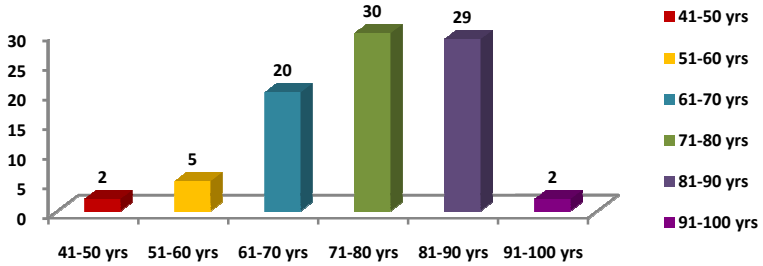

Figure 1: Illustration of incidence of cancer with respect to age

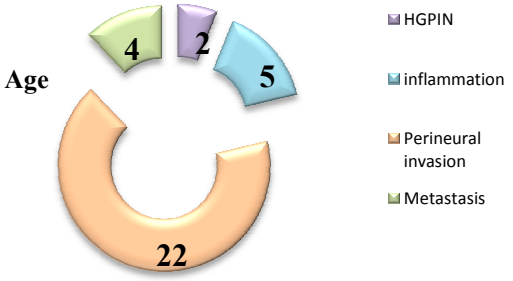

Figure 2: Additional Pathological Findings in Different grades of carcinomas

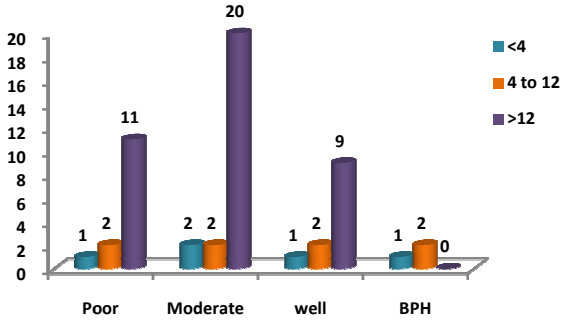

Figure 3: Prostate Specific Antigen (PSA) range associated with the different grades of carcinoma and BPH

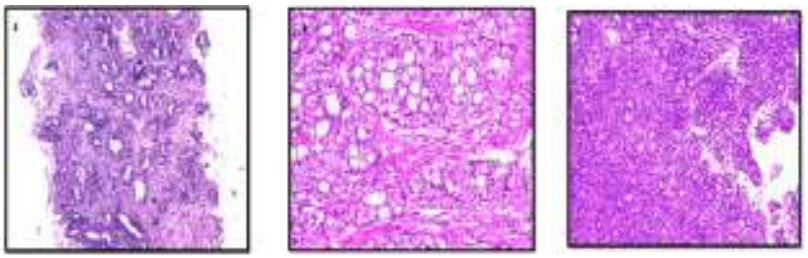

Figure 4: Hematoxylin and Eosin staining procedure for different stages of adenocarcinoma .a- well differentiated carcinoma .b-moderately differentiated .c-poorly differentiated

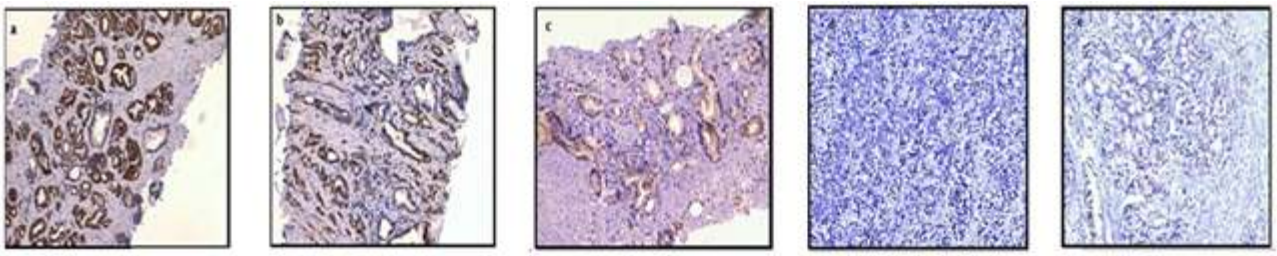

Figure 5: Differentiated carcinomas showing differential immunostaining of COX-2 (100X) .a-Well differentiated carcinoma showing strong positivity (score 3+).b- Moderately differentiated carcinoma showing moderate positivity (score 2+).c- Moderately differentiated carcinoma showing weak positivity (Score 1+).d- Poorly differentiated carcinoma showing negative (score 0) .e- Clinically proven metastasis showing weak to negative (score 0).

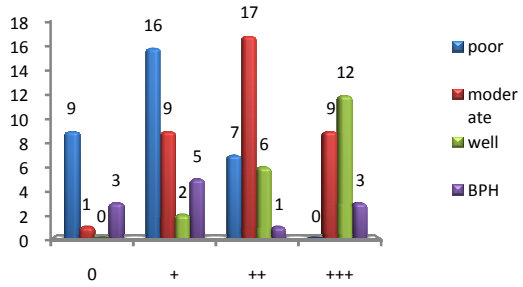

Figure 6: COX2 expression in different grades of prostate cancer

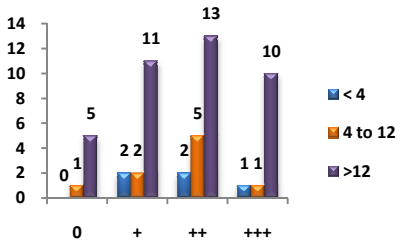

Figure 7: Comparison of Serum PSA level and COX-2 Expression

DISCUSSION

Prostate cancer represents the second leading cause of death in men after lung cancer. Detection at an early stage is very important as this might assist in initiating an early effective therapy. Though, PSA is the best serum indicator for prostatic adenocarcinoma, it has limitations. Currently, there are no markers that differentiate clinically suspicious cases from benign disease and also to determine its clinical behavior. Better indicators of cancer presence and progression are needed to avoid unnecessary treatment, predict disease course and to develop more effective therapy. Availability of such marker may be useful to design appropriate therapy and induct it for clinical use. Cyclooxygenase is one of the key enzymes in the prostaglandin metabolism and is said to have a potential role in the development and progression of tumors. Alteration in the COX-2 expression has been observed more frequently in several types of malignant tumors since COX-2 is present independently in cells during the early stages of cell differentiation or replication.[5] Increased COX-2 expression has been demonstrated in high grade Squamous Cell Carcinoma (SCC) of esophagus,[6] adenomatous and metaplastic lesions of stomach, pre-neoplastic lesions of lung, pre-invasive neoplasias of breast,[7] bladder,[8] and pancreas.[9] The present study was done on 88 cases of histopathological proven prostate cancer and 12 cases of benign prostatic. In this study, the age incidence of ranged from 48 to 92 years and peaked between the age group of 71 to 90 years (67%) indicating that incidence of prostatic cancer steadily increases after the age of 70 years. According to National Cancer Institute's (NCI) Surveillance Epidemiology and End Results (SEER) Cancer Statistics Review, 2010[2] 56.6% of men are diagnosed to have carcinoma between 71 to 90 years and this finding correlates with our data. Clinical symptoms and signs are clues to further investigate in suspicious cases, to rule out malignancy. We evaluated

the clinical findings and found that majority (38%) of the patients presented with increased urinary frequency, followed by urgency and urinary retention. However, 20% of the patients were asymptomatic which was also observed in a study by Dan Theodorescu *et al*[11] In our study, Gleason Scoring was used for grading and interpretation of adenocarcinoma of prostate. The strong prognostic value of the Gleason score in prostate cancer is evidenced in a study by Egevad *et al*[12]. However the scoring does not always correlate with clinical behavior. Additionally, the tissue was also examined for presence of HGPIN, inflammation perineural invasion. Among these perineural invasion was most frequently found while inflammation and HGPIN were less rampant. Serum PSA levels were found to be significantly raised in high grade carcinoma as compared to low grade. This is in concordance with the study by Helpap B *et al*[13] where the serum PSA levels correlated directly with the grade of tumor. Serum PSA levels were very high in cases with radiologically proven metastasis and all of these were high grade carcinoma. Similar findings were observed by S. de Sousa *et al*[14] which states that well-differentiated tumors and low PSA levels (PSA <10) are found to have low rate for bone metastasis. These findings emphasize the need for bone scan in patients with high tumor grade and high PSA levels as an important marker for disease staging. The role of COX-2 has been extensively studied in colorectal cancers and it has been found that approximately 50% of adenomas and 80 - 85% of adenocarcinomas show increased expression of COX-2. COX-2 has been implicated in various pre malignant and malignant lesions in organs such as esophagus, breast, stomach and others. However, only a few studies have been undertaken to evaluate the role of COX-2 in carcinomas of prostate. Recent studies on COX-2 expression in prostate indicate variable results and conclusion. Hongtuan Zhang *et al*[15] in a study suggested that there was an association between COX-2 rs2745557 polymorphism and lower prostate cancer risk in Caucasians. In the present study, expression of COX-2 showed significant difference among different grades of prostate cancer and in BPH. This correlates with the study by Yoshimura *et al*[16] which states that there is significant difference in COX-2 expression among BPH and among individual grades of carcinoma. Our study documents that there is increased intensity of COX-2 expression in low grade carcinoma and it is either weak or negative in high grade carcinoma. This is in concordance with the study by Shappell *et al*,[17] that showed low expression of COX-2 in high grade carcinoma. Study by Zha *et al*[18] revealed that COX -2 expression inversely correlates with Gleason score and pathological stage. In contrast, Madan *et al*[19] observed that COX-2 expression markedly differed between BPH and prostate cancer with poorly differentiated tumor showing strong expression. With respect to discrepancies in COX-2 expression in prostate cancer, Liu X *et al*[20] in their study expressed their views on variability for COX-2 positivity. The disparity in results for COX-2 expression could be based on whether or not COX-2 is expressed in normal prostate and other cell in prostate tissue showing positivity (e.g. epithelial cells, smooth muscles or striated cells).. In addition, they also questioned whether the expression of COX-2 is associated with cellular differentiation or disease progression. In our study all cases with distant metastasis and high PSA levels, COX-2 expression was found to be weak or negative. We did not find any significant correlation between PSA and COX-2 expression. Possibly more number of cases with their PSA levels needs to be studied to evaluate the true significance. Thus, COX-2 expression was found to be strongly positive in well differentiated carcinoma of prostate, irrespective of their PSA levels. There are evidences about the apoptotic effect of anti COX-2 antibody (celecoxib) in human prostate carcinoma cells. Ao-Lin Hsu *et al*[21], suggested that celecoxib induces apoptosis, by blocking the activation of the anti-apoptotic kinase Akt (also called protein kinase B). This mechanism is noteworthy because celecoxib displays a significantly higher potency in apoptosis induction than other COX-2 inhibitors. Studies in literature have also found that COX-2 expression to be an independent poor prognostic factor in patients receiving radiation therapy[22]. There was a significant difference in five-year disease-free survival in tumors expressing COX-2 and it may also serve as a prognostic marker. Lymphovascular invasion has been found to be associated with positive COX-2 expression in other tumors like carcinoma of the breast[23]. However in our study metastatic cases showed negative COX- 2 expression.

CONCLUSION

In conclusion our study shows that, COX-2 expression is strongly positive in well differentiated carcinoma of prostate irrespective of their PSA levels. Targeted therapy for COX-2 may be effective on well differentiated prostatic carcinoma since only the low grade prostate cancer express COX-2. Clinical parameters like PSA and Gleason score play a vital role in assessing the tumor grade and progression. High levels of PSA may prove useful in identifying patients with metastatic condition. However, in depth multicentre study with larger sample size may be required to determine the definite role of COX-2 expression in prostate carcinoma and the potential therapeutic role of COX-2 inhibitors.

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