Introduction

Prostatic carcinoma (PC) is among the most common malignancies [1]. Most studies reflect the etiology of PC is multifactorial. Although, current screening has been realized, the pathogenesis of prostate cancer are still unclear [2]. Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) are the two isoforms of cyclooxygenase, which transform arachidonic acid (AA) into some eicosanoids such as prostaglandin, thromboxane, and prostacyclin which contribute in several normal physiologic processes and inflammation [3]. COX-2 is an inducer of angiogenesis of new blood vessels [4]. COX-2, on the other hand, is an inducible isoform, quickly induced by growth factors, tumor promoters, oncogenes and carcinogens [5]. So, up-regulation of COX-2 is related with carcinogenesis in multiple organ systems such as the large bowel, lung, breast, and prostate [6]. Epidemiological studies have revealed a reduced risk of prostate cancer between men who recurrently take aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) [7]. COX-2 expression in prostate core needle biopsies may display significance in preprostatectomy prognosis [8]. COX-2 is expressed in PC; however, expression is not related to the presence of inflammatory cells infiltration [9]. The aim of this work is to find out the diagnostic value of the mentioned molecule in the core needle biopsies of patients with PC. Other goals are to establish its relation with Gleason score which is known as prognostic factors.

Materials and Methods

A total of 50 PC on core needle biopsy were reviewed for pathology in Sina hospital affiliated Tehran University of Medical Sciences. The average patients’ age was 60 years. All data were collected from pathology reports and patient’s record archives. Cases with incomplete information were excluded. Exclusion criteria were atypical adenomatous hyperplasia or
metastatic involvement. Also, 10 normal prostatic core needle biopsies were selected. All slides were reassessed by an expert pathologist who was unaware of the patients’ history. After diagnosis confirmation based on morphology, appropriate blocks of each sample were selected. The specimens were classified according to the International Society of Urological Pathology (ISUP) consensus conference in 2014 [10].

**Immunohistochemistry staining**

Paraffin blocks were cut with 2 micrometer thickness. Slides were coated with Poly-L-lysine and then deparaffined and rehydrated. Immunohistochemistry (IHC) staining for the detection of COX-2 expression was performed on one representative slide per tumor which exhibited a maximum of tumoral tissue and on normal tissue for comparison. Primary antibody (Polyclonal rabbit anti-human COX-2, Dako) with a dilution of 1/250 was performed according to the manufacturer’s protocol for COX-2 staining. Hematoxylin was used to stain the background. COX-2 immunolabelling was intracytoplasmic and perinuclear considered as positive and base of intensity was graded weak: +1, moderate: +2, and strong: +3 [11].

**Molecular study**

The expression of COX2 was studied by semi-quantitative PCR. The PCR amplification reaction was carried out by AccuPower GreenStar qPCR Premix (Bioneer, Korea) using Stratagene Mx3005PqPCR system (Agilent Technologies, CA, USA). The thermal profile reaction used was 5 minutes at 95°C for 1 cycle, followed by 40 cycles at 94°C for 15 seconds, 55℃ for 30 seconds and 72℃ for 30 seconds. Beta-Actin was amplified as internal control. Arrays were performed and the values were obtained for the threshold cycle (Ct) for each gene and normalized using the housekeeping gene (Beta-Actin) on the same array. The change (ΔΔCt) between the genes of neoplastic and normal tissue and controls was determined. The fold-change was determined using the formula, fold-change = 2(ΔΔCt). The resulting values were reported as fold-change; only genes showing two-fold or greater change were considered. The study endpoint was to first compare gene expressions in PC and normal prostatic tissue. The sequences of the primers and the respective conditions used for COX2 were according to Table 1.

This study is cross-sectional and investigated the association of COX-2 expression by IHC staining and PCR and Gleason score of PC on core needle biopsy specimens in Sina hospital in 2016.

**Statistical Analysis**

For statistical analysis, results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Quantitative variables were also compared with T-test or Mann-Whitney U test. The association between the variables was examined using the Pearson’s or Spearman’s correlation test. For the statistical analysis, the statistical software SPSS version 16 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

**Results**

The database query returned 60 patients. We excluded 10 patients with inadequate specimen. The remaining 50 patients were evaluated who mean age of 60 years.

COX-2 immunolabelling was intracytoplasmic and, in some neoplastic cells, perinuclear; it was demonstrated in neoplastic cells and its intensity was usually mild to moderate. In contrast, all normal tissues showed no staining (Figure 1).

Mean levels of COX-2 mRNA were 2.3-fold higher in PC tissue (n = 50) compared with the paired benign tissue which was similar to other study (1.8-3.4 fold) (Table 2) (12).

The COX-2 gene expression in PC and benign tissue by PCR are shown in Figure 2.

There was no significant difference in the mRNA level of COX-2 gene between the cancer tissues with different Gleason score (P-Value: >0.5). However, the mRNA level of COX-2 gene was considerably higher in the cancer tissue as compared to the normal samples. In other word, the presence of PC induced a 2.3-fold
Evaluation Expression COX-2 in Prostatic Carcinoma by PCR and Immunohistochemistry

Discussion

For several decades, the absence of exact diagnostic and prognostic biomarkers for PC has interested investigators to search for new specific markers to precisely predict recurrent and metastatic PC [13]. Cohesive scientific data from molecular studies supports the hypothesis that abnormal induction of COX-2 and up-regulation of the prostaglandin cascade show an important part in carcinogenesis, and equally, blockade of the process has strong probable for cancer prevention and therapy [14]. Various studies recommend a role for COX-2 in carcinogenesis. However, little is identified about the role sequence variation within the COX-2 gene plays in prostate cancer risk. Panguluri et al. study suggests that variation of the COX-2 promoter may influence the risk and development of prostate cancer [15]. COX-2 is expressed in numerous types of normal epithelial tissues, comprising normal prostate glands in man. Prior research has established that COX-2 expression is maintained during malignant transformation and is expressed in PC [16] which is similar our study and is proved as diagnostic marker. Up-regulated COX-2 may play a role in inducing cell proliferation, differentiation, apoptosis, or angiogenesis [17]. COX-2 overexpression might lead to local persistence of disease and, consequently, more incidence of distant metastasis. Alternatively, COX-2 overexpression might be a risk factor for early metastasis, which might be supported by the conclusion that COX-2 overexpression is accompanying by angiogenesis [18] but in our study is not association between COX-2 expression and tumor grade and Gleason score. So, we couldn’t use it as prognostic factor. Multiple studies have revealed increased expression of COX2 in prostate tumors only, although one study reported overexpression not in tumor tissue but rather in proliferative inflammatory atrophy, a putative precursor lesion of prostate cancer [19] which was according to a number of studies recommend that COX-2 expression is not only an initial happening in the carcinogenesis, but is obligatory during the whole proliferation in all cases with high COX-2 expression in IHC staining of tumor samples. But between COX-2 expression detection by two method and Gleason score of PC was not any relationship (P-Value>0.5).

In comparison between two method including IHC staining and PCR exhibited IHC staining was more sensitive than PCR (P-Value: <0.05). So, for COX-2 expression detection, IHC staining is useful even in small samples. Because PCR cannot detect at least gene expression and expression should be more than detection limit of PCR. Efficacy of PCR in detecting gene proliferation in samples with IHC staining grade of +3 was 100% and in the case of IHC staining grade of + 2 was about 50% (Figure 3). PCR was able to detect gene increase in mRNA expression when compared to normal prostatic tissue.

Table 1. Sequences of the Primers for COX-2 in PCR

| Primers  | Sequence                      | TM  |
|----------|-------------------------------|-----|
| COX-2-F  | 5-ATCATTCACCAGGCAAATTGC-3    | 60.66 |
| COX-2-R  | 5-GGCTTCAGCATAAAGCGTTTG-3    | 60.69 |
| B-ACTIN-F | 5-GCTCCTCTGAGCGCAAGT-3       | 61.6 |
| B-ACTIN-R | 5-TCGTCATACTCTGCTTGAT-3      | 61.96 |

Table 2. Cyclooxygenase-2 (COX-2) Mean Expression Fold Change in Prostatic Carcinoma and Normal Tissue

| Gene   | Prostatic carcinoma Mean±SD | Normal tissue Mean±SD | Fold change | P-value |
|--------|-----------------------------|-----------------------|-------------|---------|
| COX-2  | 5.52 ± 0.42                 | 2.36 ± 0.8            | 2.3         | 0.004   |

P<0.05 have considerate as significant.

Figure 2. The COX-2 Gene Expression in PC and Benign Tissue by PCR. Columns number 1-4 are normal tissue, columns number 6-9 are PC (IHC grade +3), column number 5 is negative control. Non-specific bands show interfering factors.

Figure 3. The COX-2 Gene Expression in PC by PCR. According to IHC Grading. IHC grades are upper picture, N is normal tissue, M is 100-1000bp marker and Co is negative control.
evolutionary process of cancer growth and development [20]. A study by Yoshimura et al. showed the amount and intensity of immunoreactive COX-2 in tumor cells was statistically much greater than those of cells from benign hyperplasia. These outcomes were further confirmed by mRNA analysis, where enhanced expression of COX-2 was detected in prostate cancer tissues. These results lead to the conclusion that human prostate carcinoma cells generated COX-2, and that COX-2 might play an significant role in the proliferation of prostate carcinoma cells which is confirmed by our study [21]. Richardsen et al. study showed high expression of COX-2 was correlated with death from PC [22]. These documents establish that COX-2, are expressed in all stages of human prostate carcinogenesis [23]. Brian L et al. study showed Gleason score, preoperative serum prostatic specific antigen (PSA) level, extraprostatic extension, surgical margin, seminal vesicle invasion, and high COX-2 expression were important predictors of biochemical recurrence [24]. The positive rates of COX-2 were higher in high Gleason score group than those in low Gleason score group. So, the high expressions of COX-2 in PC may be related to tumor pathological grade [25] but we couldn’t establish it in our study. Also, Thibaut et al. study proposed increased levels of this protein in the tumors were all considerably related with higher Gleason scores and pathologic stages, tumor extraprostatic extension and loss of differentiation [26] which in our study only COX-2 expression was seen in neoplastic tissue but was not seen in normal tissue. We couldn’t introduce COX-2 as prognostic factor. Also, some study suggested that overexpression of COX-2 links with T3–T4 stages of PC [27]. Furthermore, patients with high-COX-2 expression revealed lower disease-free and overall survival rates than those with low-COX-2 expression [28]. So, COX2 inhibitors are currently being used in clinical trials for the treatment of PC [29]. This facts suggest that COX-2 is over-expressed in prostate cancer and COX-2 inhibitors may be valuable in combination chemotherapy or chemoprevention for prostate cancer [30]. Therefore, COX-2 might be a potential therapy target for PC and work as a diagnostic and prognostic factor for PC patients.

In conclusion, the expression of COX-2 protein by IHC and PCR in our study offers the valuable diagnostic information but is not well associated with histological grade of prostate carcinoma in biopsies. This may be in part due to the relatively small sample size and various factors such as patient characteristics or procedure specifications.

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