Prognostic Significance of COX-2 Expression in Wilms' Tumor

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Abstract

Objectives: In Wilms Tumor (WT) secondary malignancies caused by the side effects of intensive treatments remain one of the important problems. Therefore, there is a need for new studies to identify low- and high-risk groups for WT and to improve the treatment regimens of children in the low-risk group. Therefore, in our study, we aimed to determine the prognostic significance of the cyclooxygenase-2 (COX-2) biomarker in WT. Materials and Methods: Our study included 24 patients diagnosed with WT between January 2010 and December 2019. The correlation between COX-2 expression and significant prognostic parameters was investigated by studying COX-2 antibody using the immunohistochemical method. Results: COX-2 expression was observed in 22 of the patients, and the expression was more evident especially in the epithelial component. There was no significant correlation between COX-2 positivity and prognostic parameters. Conclusions: In our study, no significant relationship was found between significant prognostic parameters and COX-2 expression. We think that the COX-2 pathway is effective during the development phase of WT, since COX-2 expression was observed in almost all patients, therefore it may be beneficial to add COX-2 inhibitors to the treatment, and that a sufficient number of studies should be conducted in this respect.

Introduction

Wilm's Tumor (WT), also called nephroblastoma, is the most common genitourinary system tumor in children and is caused by mutations or deletions of Wilms Tumor Gene-1 (WT1) located on the short arm of chromosome 11[1]. This tumor with an incidence of 1 in 10,000 children accounts for 6% of all childhood cancers[2]. It usually occurs before 5 years of age in a single kidney and equally in both genders[3]. Although there has been an increase in the survival rate in recent years, the survival rate is still low with 50% in patients with metastasis and recurrence despite intensive treatment regimens[3]. In survived children, late survival and secondary malignancies are observed due to chemo-radiotherapy side effects[4]. Understanding the pathogenesis, progression and metastasis-related factors of this malignancy, which has a high mortality and morbidity rate, is of crucial importance for the development of new treatments. Although it is known that WT1 and kat-catenin mutations are effective in the tumorigenesis of WT, factors effective in the progression are not exactly known[5]. Therefore, new studies are needed to be conducted on this subject.

There are studies suggesting that prostoglandins (PG) have effects on immune system, cell proliferation, apoptosis, and angiogenesis. The cyclooxygenase (COX) enzyme, which plays a role in the synthesis of PGs, has two known isoforms. COX-1 is responsible for the normal physiological effect and found in almost all tissues, while COX-2 can be induced by trauma, inflammatory cytokines, growth factors, and oncogenes. COX-2 increase is responsible for decreased apoptosis, immunosuppression, increased tumor cell proliferation, angiogenesis and metastasis potential in cancerous tissues[6]. Over-expression of COX-2 has been reported in many malignancies, but there are few studies on its role in WT. Therefore, in our study, we aimed to determine the prognostic significance of COX-2 in this tumor and its effects on tumor formation.

Materials and Methods

In our study, the kidney resection materials that were operated between January 2010 and December 2019 with the pre-diagnosis of kidney tumor and diagnosed with WT in our department were examined. Twenty-four patients diagnosed with WT, who did not receive chemo-radiotherapy before surgery, and whose paraffin blocks could be accessed were included in the study. Since the paraffin blocks of 3 patients could not be reached, these patients were not included in the study. The paraffin blocks and glasses of the patients were taken out of the archive and sections were taken again and examined under the light microscope. Four micron-thick sections were taken from the blocks containing the most intensive tumor tissue and placed on the charged glass slides. After keeping at 70°C for 15 minutes, they were placed in the automated immunohistochemistry staining device (Ventana, Roche, USA). After the slides were subjected to deparaffinization and dehydration processes, respectively, they were processed with ULTRA Cell Conditioning Solution, hydrogen peroxidase, and COX-2 antibodies (Nova Castra, Leica, Newcastle, United Kingdom). Immunhistochemical study was performed on the immunohistochemically selected blocks using the COX-2 antibody. Significant stoplasmic and luminal staining of [?]10% in tumoral areas was considered positive [7].

Results were compared with renal pelvis invasion, renal sinus soft tissue invasion, renal sinus lymphovascular invasion, renal vein invasion, renal capsular invasion, perirenal adipose tissue invasion, macroscopic tumor diameter, presence of anaplasia, presence of nephrogenic residue, lymphovascular invasion, TNM stage, and survival, which are significant prognostic parameters. These parameters could not be compared since the lymph node dissection was not performed and the Gerota's fascia was not sent in most of the patients.

Statistical Analysis

The D'Agostino-Pearson test was used to evaluate the normality of data. Normally distributed binary data groups were compared using the independent t-test. Comparison of COX-2 with prognostic factors and the correlation of variables between groups were analyzed by Spearman's rank correlation test and the chi-square test. The test was considered significant when bipolar p-values were <0.05. The statistical analyses were carried out with the Medcalc software (Medcalc ver 16. Ostend, Belgium). Atatruk University School of Medicine local ethics committee approved this retrospective study.

Results

The mean age of 24 patients (Male 13, Female 11) included in our study was 38,3 (+-25,7 SD) months. The mean macroscopic tumoral diameter was 9,1 (+-3,8 SD) (Table 1). There was no significant difference between both genders in terms of mean age (p: 0,11). Of the patients, 12 were male and 11 were female, and the male to female ratio was 1.18. Of the patients, 20 were triphasic, 2 were biphasic, and 2 were monophasic. Of the patients, 8 had anaplasia findings, while 2 had nephrogenic residues. When the local tumor spread sizes of the patients were analyzed, of the patiens, 16 had renal pelvis invasion, 15 had renal sinus soft tissue invasion, 8 had renal sinus lymphovascular invasion, 8 had renal vein invasion, 13 had renal capsular invasion, 9 had perirenal adipose tissue invasion. Fifteen of the patients had lymphovascular invasion. When the clinical stages of the patients were analyzed, it was found that of the patients, 3 were Stage 1, 9 were Stage 2, 6 were Stage 3, 4 were Stage 4, and 2 were Stage 5. Of the patients, 4 had distant organ metastasis and 4 had recurrence. Three of the patients died within 3 years.

When COX-2 expression was analyzed, of the patients, 1 showed no expression, while 1 showed weakly positive cytoplasmic staining with 2-5%. These two patients were considered negative. All other patients had COX-2 positivity of [?]10% of the tumoral area. In addition, there was a weak expression of COX-2 in normal tubular structures.

There was no significant correlation between COX-2 positivity and renal pelvis invasion (p: 0.31), renal sinus soft tissue invasion (p: 0.27), renal sinus lymphovascular invasion (p: 0.31), renal vein invasion (p: 0.36), renal capsular invasion (p: 0.9), perirenal adipose tissue invasion (p: 0.71), macroscopic tumor diameter (p: 0.76), presence of anaplasia (p: 0.31), presence of nephrogenic residue (p: 0.67), lymphovascular invasion (p: 0.27), clinical stage (p: 0.49), and survival rate (p: 0.59).

In our study, the expression of COX-2 was observed especially in the epithelial component, and the rates

in the blastemal and epithelial components were 3-5% and 60-70%, respectively (Figure 1-2). No expression was observed in the mesenchymal component.

Discussion

There has been a serious increase in the survival rates of WT in recent years due to the intensive treatment regimens. Unfortunately, late mortality and secondary malignancies caused by the side effects of these intensive treatments remain one of the important problems. For this reason, the aim of recent studies has been to identify low and high-risk groups for WT and to improve the treatment regimens of children in the low-risk group. Determination of risk groups is considered as the first step in the treatment[8]. COX-2 expression is found in most malignancies such as cervix[9], brain[10], breast[11], colon[12] and ovary[13] and is associated with poor prognosis. In our study, we aimed to determine the prognostic significance of the COX-2 biomarker, the role of which has been studied in other malignancies in recent years, but the number of studies on its significance in WT is very few. In recent years, there are studies suggesting that COX-2 has a very important role in tumoral cell cell proliferation, tumor angiogenesis, increased invasion, and metastasis. Its low expression in normal tissues and overexpression in tumoral areas made this marker a therapeutic target[14].

In our study, we detected COX-2 positivity in 22 (92%) of our patients. In their study of 14 patients, Fridman et al. stated that COX-2 expression was observed in all patients, except for one anaplastic WT. They also emphasized that there was expression in the area of lung metastasis in one of the patients. They reported that they observed a weaker expression in the normal kidney tissue compared to the tumoral areas and it was confined to the tubular epithelium in the cortex and medulla[7]. Similarly, in our study, we observed a weak staining with COX-2 in the tubular epithelium. Lee et al. also reported in their study of 26 patients that they observed COX-2 positivity in all patients[15].

According to the International Society of Pediatric Oncology (SIOP) studies, clinical stage, histology, tumor diameter, and response to treatment are among the prognostic factors for WT[16]. Moreover, as in other kidney tumors, renal pelvis invasion, renal sinus soft tissue invasion, renal sinus lymphovascular invasion, renal vein invasion, renal capsular invasion, perirenal adipose tissue invasion, lymphovascular invasion, survival as well as presence of anaplasia and nephrogenic residue are significant prognostic parameters. In our study, there was no significant correlation between COX-2 expression and any of these important parameters.

In the literature, there are studies reporting that COX-2 overexpression is a poor prognostic factor, but few studies have been conducted on COX-2 in the genitourinary system. Oku et al. reported that they observed COX-2 expression in bladder carcinomas, and that increased expression was correlated with high pT stage, decreased cell differentiation (high histological grade), and low survival rate. Based on the fact that COX-2 is correlated with tumoral cell differentiation, they thought that this marker might be effective at every stage of carcinogenesis[17]. In their study, Chen et al. stated that they found a significant correlation between increased COX-2 expression and tendency to invasion in renal cell carcinomas, and that invasion decreased with COX-2 inhibition[18].

There are very few studies investigating the significance of COX-2 expression in WT. With the observation of COX-2 expression in all tumoral areas in WT, Lee et al. emphasized that it was especially more in areas where vascularity was more intense[15]. Giordano et al. observed COX-2 expression in all cases, independent of anaplasia findings, dominant component, presence of heterologous and/or homologous elements, age, gender, stage, and survival. Based on these results, they argued that COX-2 inhibitors could be used to treat all WT cases[19]. Fridman et al. also reported that COX-2 expression was present at all stages of the tumor in WT and not related to the aggressiveness of malignancy[7]. As in our study, studies in the literature have found no significant correlation between significant prognostic parameters and COX-2 expression in WT, and COX-2 positivity has been observed in all or nearly all cases. The observation of COX-2 expression in most cases suggests that the COX-2 pathway is effective in WT development.

There are 3 different components in WT: blastemal, epithelial and stromal. Although some tumors have the predominance of any of these three components, all three components are usually present in most

tumors[2]. In our study, similar to the studies in the literature, all three components were observed in most of our cases. In our study, the expression of COX-2 was observed especially in the epithelial component, and the rates in the blastemal and epithelial components were 3-5% and 60-70%, respectively. COX-2 expression was not observed in the mesenchymal component. When Lee et al examined the prevalence of staining with COX-2, they reported a rate of 69% in the blastemal component and 73% in the epithelial component[15]. Giardino et al. observed COX-2 expression in the blastemal component at a rate of 45%, in the epithelial component at a rate of 7.5%, and in heterologous composite areas at a rate of 25%. Recent SIOP studies are on the prognostic significance of the volume of blastemal component and the determination of biomarkers for resistant blastema. Kinoshita et al. reported that the prognosis of tumors with predominant blastemal component is worse compared with those of other subtypes[20]. Beckwith et al. reported that the diffuse blastemal pattern was more aggressive but the survival rate was higher due to the higher response to chemotherapy[21]. In our study, in addition to the absence of a connection between other prognostic parameters and COX-2 expression, more dominant staining was observed in epithelial component instead of blastamel component, which is a bad prognostic finding.

Based on the effects of COX-2 on tumorigenesis and angiogenesis in recent years, it has been suggested that COX-2 inhibitors can be useful both for preventing and treating cancer[14]. According to the result obtained using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), it has been reported that halting proliferation, angiogenesis and vascular condensation rather than increased apoptosis are effective in halting tumor growth[15]. With the use of the COX2 inhibitor, it has been been observed that changes leading to an increase or decrease in the expression of genes to suppress vascular proliferation and vascular stability in WT are achieved[1]. In their study on rats, Maturu et al. argued that COX-2 inhibitors were effective in tumor progression in WT, and their combined use with other inhibitors may be extremely useful in the treatment. They emphasized that these combinations could be much more advantageous than radiotherapy, conventional cytotoxic therapy and other treatments used for WT, therefore, more studies were needed to be conducted in this regard[22]. In their study on mice, Lee et al. showed that the use of SC-236, a specific COX-2 inhibitor, prevented vascular proliferation in WT, thus reducing tumoral growth[15]. Although the reduction/non-progression of tumoral formation was not investigated in our study with the use of COX-2 inhibitors, the observation of COX-2 expression in most cases suggests that the COX-2 pathway is effective in the development stage of WT. Our study indirectly suggests that the use of COX-2 inhibitors may be effective in the treatment.

Conclusion

The relationship between COX-2 expression and prognosis in some types of cancer has been examined and different results have been reported. There are insufficient studies investigating the relationship between Wilms Tumor and COX-2 expression and prognosis.

In our study, there was no significant correlation between prognostic parameters and COX-2 expression. COX-2 expression was observed in almost all patients, so we think that the COX-2 pathway is effective during the development phase of WT, also therefore it may be beneficial to add COX-2 inhibitors to the treatment. However, it is necessary to support this with larger-scale studies for more objective results.

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Conflict of interest statement

All authors declared that there is no conflict of interest.

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Figure legends:

Figure 1A: Histopathoogical wiev of epithelial component (black arrow) (H&E, x40), **1B:** luminal staining of COX-2 in epithelial component (black arrow) (COX-2, x40).

Figure 2A: Histopathoogical wiev of epithelial (black arrow) and blastemal component (blue arrow) (H&E, x40), **2B:** luminal staining of COX-2 in epithelial component (black arrow) and weak stoplasmic staining in blastamel component (blue arrow) (COX-2, x40).

Table: Demografic datas

Gender (M/F) n (%)	13 (%54) / 11 %46
Age (Mean, ±SD)	38,3 ± 25,7
Tumor size (Mean, ±SD)	9,1 ±3,8
F: *Female,* **M:** *Male*, **SD:** *standart deviation*	**F:** *Female,* **M:** *Male*, **SD:** *standart deviation*

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