COX-2 Expression in Gastric Cancer

Handan BEKDEMIR[1], Salim B. TEKIN[2], Mehmet BILICI[2], Kerim CAYIR[2], Cemal GUNDOGDU[3]

[1] Ataturk University, Faculty of Medicine, Department of Internal Medicine
[2] Ataturk University, Faculty of Medicine, Department of Medical Oncology
[3] Ataturk University, Faculty of Medicine, Department of Pathology, Erzurum, TURKEY

ABSTRACT

We evaluated the cyclooxygenase-2 (COX-2) expression in the patients with gastric cancer in our region. Twenty-six patients with gastric cancer were included into the study. Tissue samples were taken from the tumors and adjacent structures. Samples were histopathologically and immunohistochemically examined. COX-2 expressions at the tumors and adjacent tissues were measured. COX-2 was positive in 16 tumor positive tissues (61%) and negative in 6 tumor tissues (23%). There was a significant difference statistically between these two groups (p= 0.005). COX-2 was positive in 14 (63%) of 22 patients who had gastric cancer in intestinal type. COX-2 expression was also positive in 2 (50%) of the remaining 4 cases with gastric cancer in diffuse type. COX-2 was positive in 5 (83%) of 6 well differentiated tumors, in 3 (37%) of 8 moderately differentiated tumors and in 8 (66%) of 12 poorly differentiated tumors. COX-2 was found positive in 5 (83%) of 6 cardia tumors and in 11 (55%) of 20 non-cardia tumors.

In conclusion, COX-2 expression in patients with gastric cancer was higher in tumor tissues than in adjacent tumor-free tissues. There was no statistically significant association between COX2 expression and both histological type and differentiation of the tumors (p > 0.05).

Keywords: Cancer, COX-2 expression, Gastric

ÖZET

Mide Kanserinde COX-2 Ekspresyonu

Bu çalışmada bölgemizdeki mide kanserli olgularda siklooksijenaz-2 (COX- 2) salınımını araştırmak amaçlanmıştır. Çalışmaya endoskopik biyopsi ile mide kanseri tanısı konan 26 hasta (17 erkek, 9 bayan) alındı. Operasyon esnasında tümör ve tümöre komşu normal dokularından yaş doku örnekleri alındı. Örnekler histopatolojik olarak incelendi ve immunohistolojik yöntemle COX-2 antikoru ile boyanan dokular ışık mikroskobunda değerlendirildi. Tümör dokusu ve tümöre komşu normal dokuda COX-2 salınımı araştırıldı. COX-2 çalışmamızdaki 26 olgunun 16 tümör pozitif dokusunda (%61) pozitif iken, sadece 6 tümör negatif dokusunda (%23) pozitif bulundu. Bu iki grup arasında istatistiksel olarak anlamlı bir fark vardı (p= 0.005). İntestinal tip mide kanseri olan 22 tümörün 14'ünde (%63) COX-2 pozitifti. Pozitif COX-2 salınımı geri kalan 4 diffüz tip mide kanserinin 2'sinde de (%50) gösterildi. COX-2 salınımı iyi diferansiye 6 tümörün 5'inde (%83), orta diferansiye 8 tümörün 3'ünde (%37) ve az diferansiye 12 tümörün 8'inde (%66) pozitifti. 6 kardia tümörünün 5'inde (%83) ve 20 kardia dışı tümörün 11'inde (%55) pozitif COX-2 salınımı bulundu.

Sonuç olarak, çalışmada, mide kanserli hastalarda COX-2 salınımının tümöral dokularda, komşu tümöral olmayan normal dokulardakinden daha yüksek olduğunu gösterdik. Tümörün histolojik tipi ve diferensiasyon derecesi ile COX-2 salınımı arasında istatistiksel anlamlı ilişki tespit edilemedi (p > 0.05).

Anahtar Kelimeler: Kanser, COX-2 ekspresyonu, Mide

INTRODUCTION

Gastric cancer is amongst the most frequent cancer types though with varying distributions worldwide. Despite developments in diagnosis and treatment, gastric cancer is ranked at top amongst cancer deaths today.[1] In Turkey, gastric cancer occupies the fourth rank in all cancers and the first rank in gastrointestinal cancers. According to the recent statistics of the Ministry of Health gastric cancer is the leading cancer type in men in Erzurum. It is the second leading cancer type amongst the cancers suffered by women.[2,3] To date, several laboratory methods were researched in gastric cancers in terms of etiology, early diagnosis, prognosis and follow-up. However, no predictor that could be used in specific diagnosis, follow-up and prognosis of gastric cancer has been found yet.[4] The studies conducted demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) had antineoplastic effects. These drugs were observed to exhibit COX-2-dependent effects in preventing gastrointestinal cancers. Additionally, in colorectal pancreatic and gastric cancers, COX-2 levels were found to be elevated.[5,6] The primary objective of the recent studies is to determine whether COX-2 inhibitors are useful in cancer management or not. In a preclinical study, it was observed that selective COX-2 inhibitors did not result in tumor reduction, but rather decreased tumor growth rate.[7] This suggests that selective COX-2 inhibitors will be much useful when used in combination with standard therapies, and it was demonstrated that selective COX-2 inhibitors increased treatment efficacy when combined with chemotherapy or radiotherapy.[8] This prospective study addresses COX-2 expression in gastric cancer in Erzurum and its vicinity where gastric cancer is most prevalent in Turkey.

MATERIALS AND METHOD

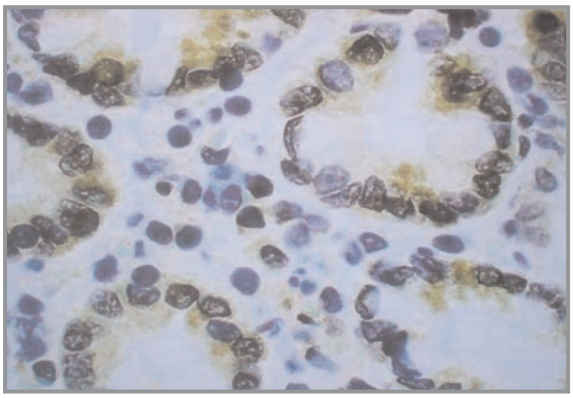
The study included 26 patients (17 Male, 9 Female) who were admit to Ataturk University, Medical Faculty, Internal Medicine and General Surgery Clinic, diagnosed with gastric cancer by endoscopic pathology, were found to have no distant metastasis after staging, and relavent surgery.

From these patients, macroscopically-tumorous wet tissue samples were collected from the resection material, intra-operatively or post-operatively, in sections of 0.5 - 1 cm, and wet tissue samples were collected from adjacent sites thought to be normal. All the patients included in the study were informed about the study, and written consents were obtained with the decision of the Faculty Ethics Committee. Age, gender, blood group, tumor histology and tumor stage of each patient were determined.

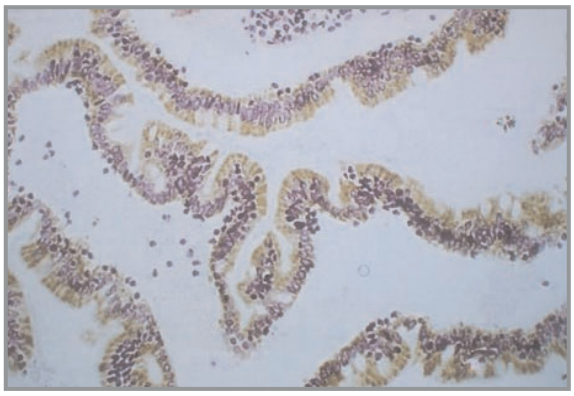
Sections stained with Hematoxylen - Eosine were examined to determine tumorous and non-tumorous regions. From these regions, 4μ-thick sections were taken on Poly-L-Lysine coated glass. Sections collected were allowed to wait in an incubator at a temperature of 45^{0}C. Preparations produced were allowed to remain in 3 different xylol containers for 15 minutes in aggregate so that they would remain for 5 minutes in each container. In the next step, preparations were allowed to remain for 5 minutes in each of the 4 alcohol containers having respectively, 70%, 80%, 90%, and 100% of alcohol concentration, washed in distilled water, and boiled with a 9 pH target-retrieval solution three times at 750 Watts for a total period of 15 minutes so that they would be boiled for 5 minutes at each time. After they were allowed to stand for 20 minutes at room temperature, tissues stained with LESAB-2 method in Dako Auto Stainer Plus system were submerged 3-4 times in hematoxylen which was used as the contrast stain. Preparations were finally washed with distilled water and covered with a coating solution. Tissues immunohistochemically (IHC) stained with COX-2 using the streptavidin-biotine method were analyzed under a light microscope (Picture 1, 2, 3).

During the analysis, it was examined whether staining occurred or not, and whether the stained areas demonstrated a diffuse or a focal retention of the stain. Staining intensity was expressed as (+1), (+2), (+3), (+4).

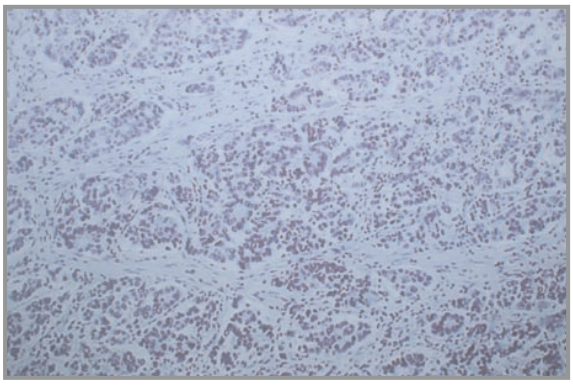
COX-2 expression values and staining scores obtained from this study were evaluated statistically on a computer running on Microsoft Windows XP operating system using the statistic software SPSS 11.5. Chi-square test was employed in comparing tumorous and non-tumorous groups. Fisher's exact test and generalization of Fisher's exact test to rxc table. Values with $p < 0.05$ were considered as significant.



Picture 1. Cancerous tissue showing COX-2 expression (IHC x400)



Picture 2. Paracancerous tissue showing COX-2 expression (IHC x200)



Picture 3. Cancerous tissue not showing COX-2 expression (IHC x100)

RESULTS

There were 17 male (65%) and 9 female (35) patients with mean age being 58.9±9.8 (range: 42-78) years in both genders, 57.6±10 (range: 42-77) years in male subjects and 61.3±9.3 (47-78) years in female subjects. In patients, 11 tumors (42%), 9 tumors (34%), and 6 tumors (23%) were located in the corpus, antrum and cardia, respectively. Histopathologically 4 tumors (13%) were found to be diffuse infiltrative type and 22 tumors (87%) with intestinal type according to Lauren's classification. Two of the cases (8%) had mucinous adenocarcinoma whereas 24 (92%) had adenocarcinoma. Six (24%), 8 (30%) and 12 (46%) of the cases were respectively graded as well-, moderately- and poorly-differentiated. In 24 patients, serosal invasion were identified. Only in 2 cases serosal invasion were not observed. None of the cases had distant metastasis. Pancreatic invasion was identified in one of the cases who underwent surgery, and the surgery was terminated in this patient after getting a pathologic size lymph node for biopsy purposes. Nineteen patients (73%) were applied total gastrectomy and 7 (27%) patients were applied subtotal gastrectomy. Five patients (19%) had first degree relatives with gastric cancer history. Fifteen patients (57%) had a long-term (10-40 years) smoking habit, whereas 11 patients (43%) had never smoked (Table 1). Blood types were found to be A Rh(+), B Rh(+), 0 Rh(+), and AB Rh(+) in 13 (50%), 3 (11%), 7 (29%) and 3 (11%) patients, respectively.

COX-2 was identified as positive in 16 of the tumor-positive tissues of the 26 cases in our study (61%). COX-2 was identified as negative only in 6 of the tumor-negative tissues of the same group of patients (Pictures 2, 3).(%23) COX-2 could not be demonstrated in 20 tumor-negative tissues (Figure 1). The result was significant when rates of staining with COX-2 were statistically compared between tumorous and non-tumorous tissues ($p<0.005$; Table 2). Among the tumor-positive tissues, 4 of the tissues that were positively stained with COX-2 by the immunohistochemical method were stained focally and staining intensity was evaluated as (+1). 3 cases were rated as diffuse (+1), 3 cases as diffuse (+2), 3 cases as diffuse (+3), and 2 cases as diffuse (+4). Among the tumor-negative tissues, 5 of the tissues that were positively stained with COX-2 by the immunohistochemical method were stained focally and staining intensity was evaluated as (+1). Only 1 case was stained at diffuse (+2) intensity.

Table 1. Patients demographics

Patient description	Number	%
Gender		
Male	17	69
Female	9	31
Age	42-78	
Smoking habit		
Yes	14	53
No	12	47
Family History		
Yes	4	19
No	22	81
Histopathology		
Adenocarcinoma	24	92
Well-differentiated	6	25
Moderately-differentiated	8	33
Poorly-differentiated	8	33
Undifferentiated	2	8
Mucinous Adenocarcinoma	2	8
Lauren Classification		
Diffuse type	4	13
Intestinal type	22	87
Tumor location		
Antrum	9	34
Corpus	11	42
Cardia	6	23
Procedure performed		
Total gastrectomy	19	73
Subtotal gastrectomy	7	27

While 5 of 6 cases with well-differentiated adenocarcinoma among the tumor-positive cases (83%) demonstrated COX-2 positivity, 3 of 8 cases with moderately-differentiated adenocarcinoma demonstrated COX-2 positivity (37%). 8 of 12 cases with poorly-differentiated adenocarcinoma demonstrated COX-2 positivity (66%). In 6 of the cases the tumor was located in the cardia and in 5 of them (83%) COX-2 was identified as positive. In 11 of 20 of the cases having a tumor outside the cardia (55%) COX-2 expression was identified as positive. The relationship between gastric cancer histological subtype according to Lauren's classification and COX-2 expression was demonstrated (Figure 2). No significant relationship could be found neither between these subtypes nor between tumor tissue differentiation and COX-2 ($p>0.05$; Tables 3, 4).

DISCUSSION

In this study, we investigated the relationship between gastric cancer and cyclooxygenase-2 (COX-2) which is considered as a potential target in cancer prevention. Studies carried out suggest that COX-2 is important in terms of carcinogenesis in gastrointestinal cancers.[9-19] While selective COX-2 inhibitors were demonstrated to be effective in preventing colorectal adenomas, studies that demonstrate the effects of this group of drugs on patients who are highly susceptible to gastric, esophageal, mouth cavity, dermal and bladder cancer revealed important opinions.[5,9,19-21] COX-2 is effective on several steps that are important in cancer development, which positions it as an important treatment target. Mechanisms in which COX-2 is involved are angiogenesis, apoptosis, immune suppression and inflammation.[20] COX-2 contributes to carcinogenesis development. For example, prostaglandins produced by COX play part in angiogenesis which represents an important mechanism in tumor deve-

Table 2. Comparison between tumorous and healthy tissues for COX-2 expression rate in patients with gastric cancer

	Total Cases	COX-2 expression (+)	COX-2 expression (–)	%	p
Tumor positive tissue	26	16	10	61.5	$p < 0.05$
Tumor negative tissue	26	6	20	23.1	

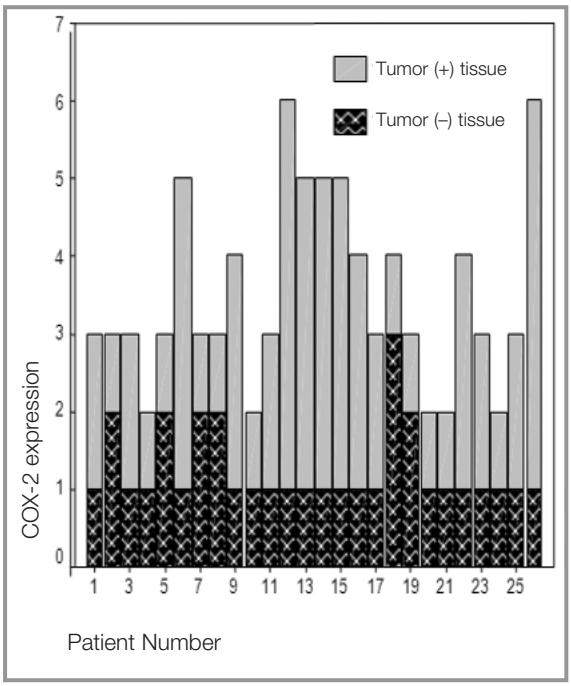


Figure 1. COX-2 expression in tumorous and non-tumorous tissues

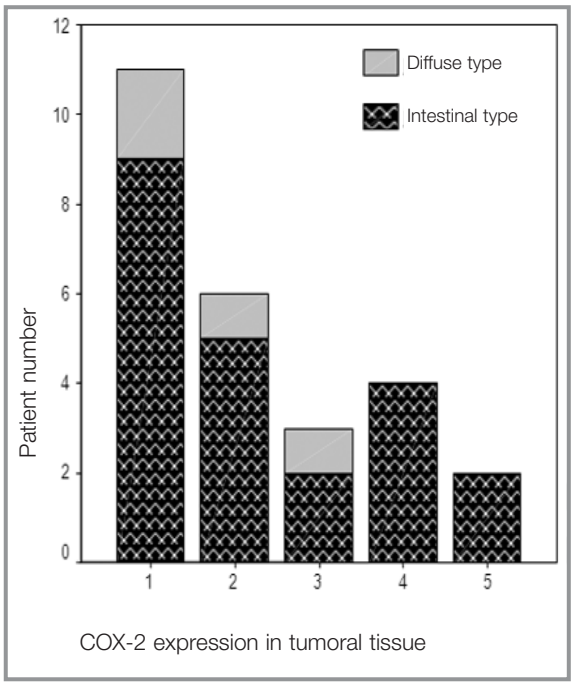


Figure 2. The association between gastric cancer histological subtype according to Lauren and COX-2 expression.

lopment. COX-2 inhibitors have less serious side effects compared with the classic NSAIDs, suggesting that they might be used in the long term for prophylactic purposes in cancers types with moderate risk.[21]

Saukkonen et al. studied COX-2 immunoreactivity in 67 patients with gastric adenocarcinoma and demonstrated COX-2 positivity in 58% of the intestinal-type tumors and 6% of the diffuse-type tumors. These data showed higher expression of COX-2 mRNA, protein, and enzymatic activity in well-differentiated gastric cancer adenocarcinoma cell lines when compared with poorly differentiated cell lines.[11]

Ohno et al collected cancerous and normal tissue samples from 33 patients with adenocarcinoma, and examined both COX-2 positivity immunohistochemically and COX-2 mRNA with reverse transcriptase polymerase chain reaction (RT-PCR). In this study, COX-2 mRNA and protein were found to be elevated in gastric cancer tissues and COX-2 mRNA levels demonstrated correlation with deep invasion. No staining was observed in the stroma and normal tissue surrounding the cancerous tissue. In this study, no relationship was found neither between histological type and differentiation degree of gastric cancer and COX-2 expression nor between distant metastasis and COX-2 level.[13]

Table 3. COX-2 expression rate comparison between intestinal- and diffuse-type stomach adenocarcinomas

	Total Cases	COX-2 expression (+) tissue	COX-2 expression (–) tissue	%	p
Intestinal Type	22	14	8	63	p > 0.05
Diffuse Type	4	2	2	50	

Table 4. Comparison of differentiation degree of gastric cancer against COX-2 expression positivity

Tumor tissue differentiation	Total cases expression (+)	COX-2	%	p
Poorly-differentiated	12	8	66	p= 0.228
Moderately-differentiated	8	3	37	
Well-differentiated	6	5	83	

In our study, consistent with the above mentioned studies, increased COX-2 expression was detected in tumorous tissues. However, no significant relationship was found between histological type of tumor and COX-2 expression. Aside from studies that support our results, there are also studies showing significant COX-2 expression in cases with intestinal-type gastric cancer compared with diffuse-type gastric cancer.[11,13,14] In our study we did not find any significant relationship between tumor differentiation degree and COX-2 expression. However, as we have mentioned above, some studies have shown higher COX-2 expression in well-differentiated tumors.[11]

The difference between the studies may be attributable to the number of patients enrolled, genetic characteristics of the patients and the stage of the disease. Certain factors that are effective in pathogenesis (H. pylori, mutant p53) and the methods used may have influenced the results. However, most of these studies and our study have shown increased COX-2 expression in tumorous tissues. This result supports the opinion that COX-2 is effective in cancer development.

Saukkonen et al. evaluated certain studies showing the relationship between gastric cancer and COX-2, and published the results. In 4 of 5 studies, they observed that aspirin had protective effect against stomach adenocarcinoma. Methods used in these studies for COX-2 expression were also compared. A mean COX-2 expression of 72% was shown in tumor tissue with PT-PCR method. Similarly COX-2 expression in tumor tissue was found to be 73% with immunoblotting method. However, in a few studies where immunohistochemical method was used, this rate varies between 43 and 100%, and is reported to be averagely 62%. These differences were thought to be associated with antibody preparations and their sensitivity. Furthermore, the relationship between tumor localization and COX-2 expression was examined, revealing, however, different results.[10] In our study, 6 tumors were located in the cardia, 9 in the antrum and 11 in the corpus. COX-2 expression was shown in 5 of 6 patients having a tumor located in the cardia (83%) and 11 of 20 patients having a tumor located outside the cardia (55%). In these studies, COX-2 expression was shown in intestinal-type gastric cancers and precursor lesions. COX-2 expression was found to be positive in 58% of intestinal-type tumors and 44% of confirmed dysplastic cases. However, COX-2 expression could only be shown in 1 of 18 diffuse-type tumors that were stained with the immunohistochemical method. While PT-PCR method demonstrated a significantly higher COX-2 expression level in cases with intestinal metaplasia than in those without, the immunohistochemical method revealed a COX-2 expression level close to that of the control group. However, when the dysplastic tissue was compared with the normal tissue, a noteworthy rate of COX-2 expression was detected in the tissue with dysplasia.[14,15]

We employed the immunohistochemical method in our study and found COX-2 positivity in tumor tissue as 61%. But COX-2 expression rates in the tissues could have proved higher if much precise methods mentioned above had been on the same cases. The fact that the study of Saukkonen et al. finds significant COX-2 expression in intestinal-type tumors does not support our findings.[10]

In our study, no significant relationship was identified between histological type and COX-2 expression. The fact that the number of patients were different between that study and our study might and

that only 4 patients had diffuse-type tumors in our study might have influenced the results. There may be different factors in the etiologies of diffuse-type and intestinal-type tumors and etiologic factors may vary from patient to patient. These factors may impact COX-2 expression in patients. On the other hand, in most of the studies and our study, COX-2 expression in tumorous tissues was found to be significantly high. This clearly shows COX-2 existence in tumorous tissue.

In a study, Uefuji et al. examined COX-2 mRNA using RT-PCR method in biopsy specimens collected from 37 cases with stomach adenocarcinoma and 5 healthy volunteers. In the study, COX-2 mRNA was detected in 19 of 37 cases who had a stomach adenocarcinoma (51%). No COX-2 mRNA was shown in stomach biopsy specimens collected from healthy individuals.[17]

In our study, in line with the study of Uefuji et al., COX-2 expression in tumorous tissues was found as 61%. However, COX-2 expression in negative tissue was detected in 6 of 26 patients (23%) in our study, but it was not identified in any of the 5 cases in the above mentioned study. While the control group consisted of 5 healthy volunteers in the study of Uefuji et al., our control group included paracancerous tissues of patients with gastric cancer. Tissues adjacent to the tumor may include inflammation, metaplasic and dysplasic changes. This may be associated with a COX-2 expression of 23% detected in paracancerous tissues. In this study, it was found that in gastric cancers with high tumor diameter COX-2 expression was higher in cases with lymph node metastasis than in those without. In addition, it was detected that COX-2 mRNA expression was greater in cases in Phase III and IV than in cases in Phase I. However, unlike many studies, no relationship was found between histological type and COX-2 mRNA expression.[17] All the patients included in our study had positive lymph node metastasis and none had distant metastasis. For this reason, we did not examine the relationship between tumor stage and COX-2 expression. Yet no significant relationship was found between histological type of tumor and COX-2 expression in our study like the above mentioned study. As a result of the study carried out by Uefuji et al. it was found that COX-2 in cancerous tissue increased tumor invasiveness and metastatic progression through COX-dependent PGE2 biosynthesis, and COX-2 could also suppress local immune response to tumor cells.

It was found that COX-2 could induce the angiogenesis which plays part in tumor development, achieved this by inducing vascular endothelial growth factor, and COX-2 inhibitors did not lead to tumor reduction but reduced tumor growth rate, and demonstrated this action by stimulating treatment factors when combined with standard therapies.[17]

In conclusion, with this study we showed that COX-2 expression was increased in cancerous tissue. However, we could not identify a significant relationship between histological type and differentiation degree of the tumor and COX-2 expression.

REFERENCES

1. Karpeh MS, Kelsen DP, Teper JE. Cancer of the stomach. In: Cancer, Principles and Practice of Oncology. De Vita VT, Hellman S, Rosenburg SA eds. 6th. ed. Philadelphia, Lippincott-Raven, 2001: 1092-1126.
2. Kanserle Savaş Politikası ve Kanser Verileri (1995-1999), T.C.Sağlık Bakanlığı Kanserle Savaş Dairesi Başkanlığı, Bakanlık Yayın No: 618, 2002.
3. Sayek İ. Temel Cerrahi. 1. baskı. Ankara, Güneş Kitabevi, 1991.
4. Sherman CD. Klinik Onkoloji. Türk Kanser Araştırma ve Savaş Kurumu Yayınları, 1990.
5. Reddy BS, Maruyama H, Kelloff G. Dose –related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflamatory drug, during different stages of rat colon tumour development. Cancer Res 47: 5340-5346, 1987.
6. Sawaoka H, Kawano S, Tsuji S, et al. Effects of NSAİDs on proliferation of gastric cancer cells in vitro: posible implication of cyclooxygenase-2 in cancer developmant. J Clin Gastroenterol 27: 47-52, 1998.
7. MasferrerJL, Leahy KM, Koki AT, et al. Antiangiogenic and antitumour activities of cyclooxygenase-2 inhibitors. Cancer Res 60: 1306-1311, 2000.
8. Langman MJ, Jensen DM, Watson DJ, et al. Adverse upper gastrointestinal effects of rofecoxib compared with NSAIDs. JAMA 282: 1929-1933, 1999.
9. Lim JW, Kim H, Kim KH. Expression of ku 70 and ku 80 mediated by NF kappa B and COX-2 is related to proliferation of human gastric cancer cells. J Biol Chem 277: 46093-46100, 2002.
10. Saukkonen K, Rintahaka J, Sıvula A, et al. Cyclooxygenase-2 and gastric carcinogenesis. APMIS 111: 915-925, 2003.

11. Saukkonen K, Nieminen O, Van Ress B, et al. Expression of Cyclooxygenase-2 in Dysplasia of the Stomach and in intestinal-type Gastric Adenocarcinoma. Clin Cancer Res 7: 1923-1931, 2001.
12. Lim H.Y, Joo H.J, Choi J.H, et al. Increased expression of cyclooxygenase-2 protein in human gastric carcinoma. Clin Cancer Res 6: 519-525, 2000.
13. Ohno R, Yoshinaga K, Fujita T, et al. Dept of invasion parellels increased cyclooxygenase-2 levels in patients with gastric carcinoma. Cancer 91: 1876-1881, 2001.
14. Yamagata R, Shimoyama T, Fukuda S, et al. Cyclooxygenase-2 expression is increased in early intestinal type gastric cancer and gastric mucosa with intestinal metaplasia. Eur J Gastroenterol Hepatol 14: 359-363, 2002.
15. Xue YW, Zhang QF, Zhu ZB, et al. Expression of cyclooxygenase-2 and clinocopathologic features in human gastric adenocarcinoma. World J Gastroenterol 9: 250-253, 2003.
16. Uefuji K, Ichikura T, Mochizuki H. Expression of cyclooxygenase-2 in human gastric adenomas and adenocarcinomas. J Surg Oncol 76: 26-30, 2001.
17. Uefuji K, Ichikura T, Mochizuki H. Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. Clin Cancer Res 6: 135-138, 2000.
18. Buskens CJ, Sivula A, Van Rees BP, et al. Comparison of cyclooxygenase-2 expression in adenocarcinomas of the gastric cardia and distal oesophagus. Gut 52: 1678-1683, 2003.
19. Yu LZ, Gao HJ, Bai JF, et al. Expression of cox-2 proteins in gastric mucosal lesions. World J Gastroenterol 10: 292-294, 2004.
20. Dannenberg AJ, Altorki NK, Boyle JO, et al. Cyclooxygenase-2: a pharmacological target for the prevention of cancer. Lancet Oncol 2: 544-552, 2001.
21. Moon RC, Kelloff GJ, Detrisac CJ, et al. Chemoprevention of OHBBN-induced bladder cancer in mice by piroxicam. Carcinogenesis 14: 1487-1489, 1993.

Correspondence
Dr. Mehmet BİLİCİ
Atatürk Üniversitesi Tıp Fakültesi
Yakutiye Araştırma Hastanesi
Medikal Onkoloji Bilim Dalı
25240 Erzurum / TURKEY

Tel: 0.442.2317232
Fax : 0.442.2361301
E-Mail : memetbilici@mynet.com