Medical Journal of Bakırköy

Med J Bakırköy 2021;15:161-166
DOI: 10.4274/BMJ.galenos.2021.12599

Research

The Relationship Between Cyclo-Oxygenase-2 *-1195A/G* Gene Polymorphism and Renal Cell Carcinoma

Siklooksijenaz-2 *-1195 A/G* Gen Polimorfizmi ile Böbrek Hücreli Karsinom Arasındaki İlişki

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ABSTRACT

Objective: This study was aimed to evaluate the association of cyclo-oxygenase 2 (COX-2) -1195A/G polymorphism with initiation and progression of renal cell carcinoma (RCC) and interaction with smoking in RCC patients in a Turkish population.

Methods: The *COX-2 -1195A/G* gene polymorphism was analyzed by method of polymerase chain reaction in DNA samples of 154 healthy controls and 114 patients with RCC.

Results: No significant variation in terms of age, sex, body mass index (BMI) or smoking between RCC patients and controls was observed. There was no statistical significance between *COX-2 -1195A/G* gene polymorphism and onset or progression of RCC in patients and controls ($p>0.05$). In addition, no relationship was identified regarding high stage and poorly differentiated RCC risk after adjusting for age, sex, BMI and smoking status. Furthermore, this polymorphism was not significantly associated with development of RCC when accompanied by smoking status.

Conclusion: Our results showed that the COX-2 -1195A/G polymorphism does not seem to be a major risk factor for both the onset and progression of RCC in a Turkish population.

Keywords: Cyclo-oxygenase-2, single nucleotide polymorphism, renal cell carcinoma

ÖZ

Amaç: Bu çalışmada, Türk toplumundaki böbrek hücreli karsinom (BHK) hastalarında siklooksijenaz 2 *(COX-2) -1195A/G* gen polimorfizminin BHK'nin başlangıcı ve ilerlemesi ile ilişkisi ve sigara kullanımı ile etkileşimi araştırılmıştır.

Gereç ve Yöntem: *COX-2 -1195A/G* gen polimorfizmi, 154 sağlıklı kontrol ve 114 BHK tanısı alan hastaların DNA örneklerinde polimeraz zincir reaksiyonu yöntemi ile incelenmiştir.

Bulgular: BHK'li hastalar ve kontrol grubu arasında yaş, cinsiyet, vücut kitle indeksi ve sigara kullanımı açısından anlamlı bir fark bulunmadı. Hatsa ve kontrol gruplarında *COX-2 -1195A/G* gen polimorfizmi ile BHK'nin oluşumu ve gelişimi arasında istatistiksel olarak anlamlı bir fark saptanmadı ($p>0,05$). Buna ek olarak, yaş, cinsiyet, vücut kitle indeksi ve sigara kullanımına göre düzeltme yapıldıktan sonra da yüksek evre ve yüksek dereceli BHK riski ile ilgili bir farklılık bulunmadı. Ayrıca, *COX-2 -1195A/G* gen polimorfizmi ile sigara kullanımının eşlik ettiği BHK gelişimi arasında bir ilişki de saptanmadı.

Sonuç: Sonuçlarımıza göre *COX-2 -1195A→G* gen polimorfizminin Türk toplumunda BHK'nin başlangıcı ve ilerlemesi bakımından majör bir risk faktörü olmadığı ileri sürülebilir.

Anahtar Kelimeler: Siklooksijenaz-2, tek nükleotid polimorfizmi, böbrek hücreli karsinom

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Cite as: Bingül İ, Küçükgergin C, Erdem S, Tefik T, Şanlı Ö, Seçkin Ş. The Relationship Between Cyclooxygenase-2 *-1195A→G* Gene Polymorphism and Renal Cell Carcinoma in A. Med J Bakırköy 2021;15:161-166

Received: 01.05.2021
Accepted: 22.06.2021

INTRODUCTION

Renal cell carcinoma (RCC) is a common and deadly disease, accounting for about 2% of all cancer diagnoses and 90% of all kidney cancers in adults (1). According to Global Cancer Observatory data, RCC is the seventh most common cancer in the developed world, with more than 400,000 new cases and around 175,000 deaths in 2018 (1,2). Epidemiological researches have suggested that the development of RCC is associated with multiple genetic and environmental factors including age, sex, race, hypertension, obesity, smoking, diet, occupational exposure, and drugs (1,3). In addition, recent studies have reported that genetic variations especially single nucleotide polymorphisms (SNPs) may also be involved in the development of various types of cancer (4,5).

Cyclo-oxygenases (COXs), the key enzymes in conversion of arachidonic acid to prostaglandins, are also known as prostaglandin-endoperoxide synthases (6). COXs consist of two isoforms named COX-1 and COX-2. While COX-1 is constitutively expressed in various tissues and maintains homeostasis of various physiological functions, *COX-2* is an inducible form and expressed in response to various factors such as tumorigenic, inflammatory and growth factors (6,7). Therefore, overexpression of COX-2 may contribute to carcinogenesis by increasing cell proliferation, angiogenesis, and inflammation and suppressing apoptosis (7,8,).

The COX-2 gene is located on q25.2-25.3 chromosome 1, including 10 exons and 9 introns with a total length of approximately 8.3 kb (6,7,8). SNP in the *COX-2* gene may affect the activity of that enzyme and consequently alter an individual's susceptibility to different types of cancer. Available data suggested that COX-2 -1195A/G gene polymorphism is associated with the initiation of various cancers including lung cancer (9), epithelial ovarian carcinoma (10), gastrointestinal system cancer (11), and hepatocellular carcinoma (12). However, no association has been found between this polymorphism and development of cancers such as lung (13), oral (14), and RCC (15) in recent studies conducted in different ethnic groups.

Therefore, in the present study, the possible association of the *COX-2 -1195A/G* gene polymorphism on the initiation and progression of RCC in a Turkish population was examined.

METHODS

A total of 114 patients with histopathologically confirmed RCC who experienced radical or partial nephrectomy at the Urology Department of İstanbul Faculty of Medicine were included in this study. All study subjects completed a questionnaire with detailed information. The control group was made up of 154 healthy individuals who were eligible by age, sex, and smoking status, and had no previous or present history of cancer. All individuals were classified as either smokers or non-smokers. The tumor-node-metastasis (TNM) classification system of the American Joint Committee on Cancer (AJCC) and Fuhrman et al. (16) grading system were used to determine of tumor staging and grading, respectively. Based on TNM staging, the patients were assigned into two groups: localized group (Stage I and II) and advanced group (Stage III and IV). They were also divided into two groups using Fuhrman et al. (16) grade: low grade (Grade I and II) and high grade (Grade III and IV). Ethical approval for this study was obtained from the Ethics Committee of İstanbul Faculty of Medicine and informed consent was signed by each participant.

Peripheral blood samples from each RCC patient and each control subject were taken into tubes containing EDTA, and genomic DNA was isolated by using a commercially available kit (Roche Diagnostics, Mannheim, Germany), and stored at -20 0C.

The gene polymorphism of COX-2 -1195A→G (rs rs689466) was genotyped using PCR-RFLP. 5'-CCCTGAGCACTACCCATGAT-3' and 5'-GCCTTCATAGGAGATACTGG -3' were used as forward and reverse primers, respectively, for PCR analysis. The PCR reactions were carried out to amplify the COX-2 gene of the subjects. The amplified PCR products were digested by the PvuII restriction enzyme (Thermo Scientific), and visualized on 3% agarose gel stained with ethidium bromide. Fragment sizes of the COX-2 genotypes were AA (273 bp), AG (273 bp, 220 bp,53 bp) and GG (220 bp, 53 bp).

Statistical Analyses

Data analyses were performed using the Statistical Package for the Social Science (21.0; SPSS Inc., Chicago, IL, USA). For all statistical analyses, $p<0.05$ was considered statistically significant. Mean values were compared between controls and RCC patients by Mann-Whitney U test. Chi-square tests were used to analyze the distributions of genotypes and allele frequencies between patients and controls. Odds ratios (ORs) were determined together with their corresponding 95% confidence intervals (95% CI) by using logistic regression analyses. The chi-squared test was used to test whether the genotype distributions corresponded to the Hardy Weinberg equilibrium (HWE). The power of the study was calculated as 84% with NCSS 2000 statistical

package (NCSS Inc.; Kaysville, UT) to detect an effect size (w) of 0.20 using 2 degrees of freedom (α: 0.05).

RESULTS

The demographic parameters of all subjects and clinicopathological characteristics of the patients are demonstrated in Table 1. There was no statistically significant variation in age, sex, BMI, and smoking status between patients with RCC and controls. The grade distribution of the RCC was low grade (I-II) for 65.8% in patients with RCC and high grade (III-IV) for 34.2% in patients with RCC. In addition, 65.8% of patients with RCC were low stage (I-II), and 34.2% of patients with RCC were high stage (III-IV). Most of the patients with RCC included the low stage and grade.

Genotype distribution of the *COX-2* gene polymorphism was consistent with HWE in both patients (p=0.096) and control groups (p=0.935).

In *COX-2 -1195A→G* polymorphism, the AA, AG, and GG genotypes were detected in 86.0%, 12.3%, and 1.8% among patients with RCC, respectively. In the control group, the distributions of the COX-2 genotypes were 77.9% for AA, 20.8% for AG, and 1.3% for GG, respectively (Table 2).

On the other hand, the possible variation in the distributions of genotypes and allele frequencies between smokers and non-smokers was evaluated for RCC susceptibility. No association was observed between *COX-2* gene polymorphism and RCC risk in smokers or non-smokers (Table 3).

In addition, no relationship was found between *COX-2* gene polymorphism and clinicopathological characteristics of RCC (Table 4).

DISCUSSION

The clinical and experimental studies supported the notion that COX-2 has an important role in carcinogenesis by promoting tumor growth, angiogenesis, invasion and metastasis, and inhibiting apoptosis (6,14).

COX-2 is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandin H2, the precursor

Table 1. General characteristics of the controls and patients with renal cell carcinoma (mean ± SD)

Parameters	Controls (n=154)	Patients (n=114)	[a]p
Age (years) (mean ± SD)	56.8±10.7	55.1±9.72	0.070
BMI (kg/m^2) (mean ± SD)	26.7±3.01	27.2±3.50	0.057
Sex (%) (female /male)	41(26.6)/113 (73.4)	43 (37.7)/71(62.3)	0.053
Smoking status (%) (never/current)	65.6/34.4	55.3/44.7	0.086
Grade			
I	-	22 (19.3)	-
II	-	53 (46.5)	-
III	-	27 (23.7)	-
IV	-	12 (10.5)	-
Stage			
I	-	72 (63.2)	-
II	-	3 (2.6)	-
III	-	33 (28.9)	-
IV	-	6 (5.3)	-

[a]p from Pearson's χ^2 test for categorical variables and the Mann-Whitney U or Student's t-tests for continuous variables, SD: Standard deviation, BMI: Body mass index

Table 2. The distribution of genotypes and alleles in controls and patients with renal cell carcinoma

	Controls n (%)	Patients n (%)	p	OR[a] (95% CI)
COX-2-1195A→G				
AA	120 (77.9)	98 (86.0)	-	1.00*
AG	32(20.8)	14 (12.3)	0.077	0.43 (0.17-1.09)
GG	2 (1.3)	2 (1.8)	0.595	1.32 (0.47-3.63)
AG + GG	34 (22.1)	16 (14.0)	0.094	0.57 (0.30-1.10)
Allele				
A	272 (88.3)	210 (92.1)	-	1.00*
G	36 (11.7)	18 (7.9)	0.149	0.64 (0.35-1.17)

[a]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex, BMI, and smoking status

*: Reference genotype, BMI: Body mass index

Table 3. Impact of smoking status on the distribution of genotypes and alleles for patients with renal cell carcinoma

		Controls n (%)	Patients n (%)	p	OR[a] (95% CI)
Non-smokers	**COX-2-1195A→G**				
	AA	79 (77.5)	53 (84.1)	-	1.00*
	AG	22 (21.5)	9 (14.3)	0.231	0.44 (0.12-1.66)
	GG	1 (1.0)	1 (1.6)	0.470	1.71 (0.39-7.31)
	AG + GG	24 (22.5)	10 (15.9)	0.297	0.64 (0.28-1.47)
	Allele				
	A	180 (88.2)	115 (91.3)	-	1.00*
	G	24 (11.8)	11 (8.7)	0.384	0.71 (0.33-1.52)
Smokers	**COX-2-1195A→G**				
	AA	42 (79.2)	45 (88.2)	-	1.00*
	AG	10 (18.9)	5 (9.8)	0.326	0.52 (0.41-1.91)
	GG	1 (1.9)	1(2.0)	0.982	1.01 (0.24-4.16)
	AG + GG	11 (20.8)	6 (11.8)	0.206	2.80 (0.53-14.6)
	Allele				
	A	94 (88.7)	95 (93.1)	-	1.00*
	G	12 (11.3)	7 (6.9)	0.344	2.08 (0.52-8.33)

[a]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex and BMI;

*: Reference genotype, BMI: Body mass index

Table 4. The distribution of genotypes and alleles in patients with renal cell carcinoma according to the grade and stage of the disease

	Low grade[a] n (%)	High grade[b] n (%)	p	OR[e] (95% CI)
COX-2-1195A→G				
AA	63 (84.0)	35 (89.7)		1.00*
AG	11 (14.7)	3 (7.7)	0.229	0.25 (0.02-2.35)
GG	1 (1.3)	1 (2.6)	0.795	1.21 (0.28-5.12)
AG + GG	12 (16.0)	4 (10.3)	0.402	0.60 (0.18-2.00)
Allele				
A	137 (91.3)	73 (93.6)	-	1.00*
G	13 (8.7)	5 (6.4)	0.548	0.72 (0.24-2.10)
	Low stage[c] n (%)	High stage[d] n (%)	p	OR[e] (95% CI)
COX-2-1195A→G				
AA	64 (85.3)	34 (87.1)	-	1.00*
AG	10 (13.3)	4 (10.3)	0.781	0.78 (0.13-4.47)
GG	1 (1.4)	1 (2.6)	0.843	1.15 (0.27-4.85)
AG + GG	11 (14.7)	5 (12.9)	0.787	0.85 (0.27-2.66)
Allele				
A	138 (92)	72 (92.3)	-	1.00*
G	12 (8)	6 (7.7)	0.934	0.95 (0.34-2.65)

[a]Low grade (I-II), [b]High grade (III-IV), [c]Low stage (I-II), [d]High stage (III-IV), [e]Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, sex, BMI, and smoking status

*: Reference genotype, BMI: Body mass index

of pro-inflammatory mediators such as thromboxane, prostaglandin E2, and prostaglandin I2. Typically, COX-2 expression is often undetectable in normal tissue, however pro-inflammatory stimuli and growth factors induce the expression of COX-2. Therefore, it was proposed that overexpression of COX-2 influenced immune response, cell growth, and proliferation, apoptosis, and promoted tumorigenesis via complex mechanisms (6-8,11).

In a study related to RCC, Cho et al. (17) proposed that the increased expression of COX-2 was associated only with tumor size but not be an effective factor for initiation of RCC. Miyata et al. (18) also demonstrated COX-2 expression was related to tumor status including tumor size and grade. Yoshimura et al. (19) suggested that COX-2 expression was not associated with stage or tumor grade in patients with RCC. In addition, Güçer et al. (20) reported that there was no relationship between COX-2 expression or clinicopathological parameters of RCC. These conflicting results indicate that the underlying mechanism of the regulation of *COX-2* gene expression has yet to be fully explored, and may be affected by genetic variations.

Association of various *COX-2* gene polymorphisms with susceptibility to tumorigenesis has so far been investigated in many published studies. According to these studies, it is generally considered that *COX-2* gene mutations are strongly related to the various types of cancer such as hepatocellular carcinoma, ovarian, lung, and esophagus cancer (9,10,12,21). However, the relationship between this polymorphism and RCC is still unclear.

The *COX-2 -1195A/G* gene polymorphism is a functional SNP resulting from the change of adenine to guanine at position -1195 in the promoter region of this gene. Recent studies have shown that the nucleotide base change of -1195 G to A generates a binding site for c-MYB in the *COX-2* gene promoter region leading to the higher transcriptional activity of this gene. c-MYB, a transcription factor, targets a variety of genes to coordinate the balance between cell division, differentiation, and survival. Therefore, it is suggested that the -1195A→G polymorphism may influence an individual's susceptibility to any type of cancer (11,13,22-24).

This is the only study in the literature that investigated COX-2 -1195A/G polymorphism in RCC performed by Chang et al. (15) in Taiwan, 2014. A total of 92 phenomena with RCC and 580 healthy controls were included in this study. It was reported that the distributions of the genotype of this polymorphism did not differ between the two groups.

In our study we investigated the effect of *COX-2 -1195A/G* gene polymorphism in Turkish patients with RCC and no significant association was identified between this polymorphism or initiation and progression of RCC. In addition, no association was detected between this polymorphism and tumor grade and stage or smoking as well. In the present study, our sample size may be considered as a limiting factor. For this reason, the statistical power of the results may be increased by conducting studies with higher sample numbers.

The variation of ethnicity, control population and sample size may lead to obtaining conflicting results in studies examining the relationship between the gene polymorphism of COX-2 and different types of cancer.

CONCLUSION

In conclusion, this study indicated that *COX-2 -1195A/G* gene polymorphism was not associated with initiation or progression of RCC in the Turkish population. Further functional investigations based on a larger sample size are required in order to clarify the relationship between the COX-2 -1195A→G polymorphism and RCC.

ETHICS

Ethics Committee Approval: This study was approved by the Ethics Committee of İstanbul Faculty of Medicine (date:14.9.2018; number:1254).

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Concept: Ö.Ş., Ş.S. Design: Ö.Ş., Ş.S. Data Collection or Processing: S.E., T.T. Analysis or Interpretation: İ.B., C.K. Literature Search: İ.B., C.K.; Writing: İ.B., C.K., Ş.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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