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Research Article

The relationship between single gene polymorphism and response to cisplatin and 5-FU treatment in patients with head and neck cancer

Baş boyun kanserli hastalarda tek gen polimorfizmi ile sisplatin ve 5-FU tedavisine yanıt arasındaki ilişki

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Abstract

Aim: Head and neck cancers are the sixth most common type of cancer worldwide. The treatment process of head and neck cancers is classified as chemotherapy or chemoradiotherapy. In this study, the relationship of ERCC1, XRCC1 and MTHFR genes with treatment response was investigated.

Material and Methods: In the study, 5 ml of blood was collected from the patients to investigate single nucleotide polymorphism, DNA was isolated and investigated by pyrosequencing method.

Results: Patients were evaluated according to RECIST criteria; head and neck computed tomography scans were performed before treatment (4 weeks) and after every three cycles. The overall response rate (RR) was 10 (25%) PD, 7 (17.5%) SD, 9 (22.5%) PR, and 14 (35%) CR. Of the patients who presented with at least one polymorphic variant, four had PD, 3 had SD, 3 had PR and 1 had CR.

Conclusion: In this study, the clinical behaviour of a group of head and neck carcinoma patients was retrospectively evaluated for association with three single nucleotide polymorphisms. These included C8092A in the ERCC1 gene, G28152A in the XRCC1 gene, and C677T and A1298C in the MTHFR gene.

Keywords: Head and neck cancer, SNP, cisplatine, 5-FU

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Öz

Amaç: Baş ve boyun kanserleri dünya çapında en sık görülen altıncı kanser türüdür. Baş ve boyun kanserlerinin tedavi süreci kemoterapi veya kemoradyoterapi olarak sınıflandırılır. Bu çalışmada ERCC1, XRCC1 ve MTHFR genlerinin tedavi ile yanıt ilişkisi araştırıldı.

Gereç ve Yöntemler: Çalışmada tek nükleotid polimorfizmini araştırmak amacıyla hastalardan 5 ml kan alınarak DNA izole edildi ve pirosequencing yöntemiyle araştırıldı.

Bulgular: Hastalar RECIST kriterlerine göre değerlendirildi; tedaviden önce (4 hafta) ve her üç siklustan sonra baş ve boyun bilgisayarlı tomografi taramaları yapıldı. Genel yanıt oranı (RR) 10 (%25) PD, 7 (%17,5) SD, 9 (%22,5) PR ve 14 (%35) CR idi. En az bir polimorfik varyantı olan hastaların dördünde PD, 3'ünde SD, 3'ünde PR ve 1'inde CR vardı.

Sonuçlar: Bu çalışmada bir grup baş boyun karsinomu hastasının klinik davranışı, üç tek nükleotid polimorfizmi ile ilişki açısından retrospektif olarak değerlendirildi. Bunlar arasında ERCC1 geninde C8092A, XRCC1 geninde G28152A ve MTHFR geninde C677T ve A1298C yer alıyordu.

Anahtar Kelimeler: Baş ve boyun kanseri, SNP, cisplatin, 5-FU

Introduction

Head and neck squamous cell carcinoma (HNSCC) generally affect the mouth, lips, nose, sinuses, larynx, and throat. It is the sixth most common cancer worldwide. In men and women, squamous cell carcinoma (SCC) represents 2% and 4%, respectively (1). Chemotherapy, radiotherapy and surgical intervention are used in the treatment of squamous cell carcinoma as well as other cancers. While surgery generally is used to cure SCC (2). Chemotherapy is the most frequently employed to treat patients with advanced or recurring oral SCC (OSCC). The overall 5-year survival rate of patients with advanced HNSCC is still as low as 25% despite improvements in the rate of early detection, multi-drug therapies, and surgical interventions over the past 30 years. The development of drug resistance in patients often leads to treatment failure. The studies indicate that over 90% of cancer patient fatalities and morbidities are attributed to drug resistance (3).

Cisplatin, also known as cis-dichlorodiamine platinum, belongs to the platinum-based antineoplastic drug family. Cisplatin has anti-cancer abilities and is a non-specific antineoplastic drug. It interacts with the purine base of DNA, causing DNA damage and thus the death of cancer cells. While 80-90% of patients initially respond positively to cisplatin treatment, some tumour cells become resistant to cisplatin due to tumour heterogeneity. This resistant is 2 times higher in women than in men (4). 5-Fluorouracil (5-FU) is a cytotoxic chemotherapy agent used to treat various cancers including breast, lung, head and neck, stomach and colon cancers. 5-FU primarily inhibits the enzyme thymidylate synthase, preventing the thymidine production needed for DNA synthesis, acting as an antimetabolite to stop cell growth. In addition, 5-FU transform into 5-fluorouridine triphosphate, which penetrates the RNA structure to prevent the production of tumour RNA (5).

X-ray Repair Cross-complementing 1 (XRCC1) is a fundamental gene within the base excision repair (BER) pathway. The substitution of guanine with adenine at position 28152 (G28152A) in exon 10 of the XRCC1 gene results in a substitution of arginine with glutamine at codon 399 (Arg399Gln). This amino acid change causes a decrease in DNA repair capability (6). Glutathione S-transferases play a critical role in the cell's defence system. These phase II detoxification enzymes are responsible for detoxifying several chemotherapeutic drugs, including platinum. The A313G in exon 5 single nucleotide polymorphism, which causes alterations in amino acid is the most prevalent in GSTP1 (6). The C8092A polymorphism in the Excision Repair Cross-Complementing 1(ERCC1) is a single change in DNA nucleotide sequence which replaces cytosine with adenine. Studies indicate that the genotype variant causes decreased enzymatic activity. Furthermore, it is located on the 3' untranslated region of the ERCC1 gene, involved in the translational repression of ERCC1 mRNA and it affects ERCC1 mRNA stability (7). The ERCC1 C8092A polymorphism has supported to predict the overall survival for some cancer patients (8). The T19007C polymorphism, which is synonymous and occurs at codon 118 (converting the common codon usage AAC to an infrequent one, AAT - both coding for asparagine), has been suggested to impair

ERCC1 translation and affects the response to chemotherapy (9). Methylenetetrahydrofolate reductase (MTHFR) exhibits various polymorphisms on chromosome 1p. Among them, the C677T (Ala to Val) and A1298C (Glu to Ala) single nucleotide polymorphisms (SNPs) are the two most frequently associated with altered enzyme activity. The C677T gene polymorphism cause a thermolabile enzyme. TT and CT genotypes experience a reduction in enzyme activity of approximately 70% and 35%, respectively. The A1298C gene polymorphism leads to decreased enzyme activity, although not to the same degree as the C677T gene polymorphism (10-11).

This study uses the basic principles and methods of evidence-based medicine to evaluate the efficacy of platinum and 5-FU-based chemotherapy in head and neck cancer cases of polymorphisms in the XRCC1, ERCC1 and MTHFR gene in the Turkish population. This study is expected to lay the foundation for future investigations into the correlation between platinum-5-FU drug efficacy and gene polymorphisms of XRCC1, ERCC1, and MTHFR.

Material and Methods

Forty volunteer patients with head and neck cancer who were followed up in Pamukkale University Faculty of Medicine, Medical Oncology Outpatient Clinic were included in our study. Approval was obtained from Pamukkale University Non-Interventional Ethics Committee. Informed consent forms were obtained from the participating patients. Patients received cisplatin and 5-FU-based chemotherapy in the first line after diagnosis, and a 3-week treatment regimen was applied. Response was evaluated according to RECIST criteria, with computed tomography scans of the head and neck performed prior to treatment (at 4 weeks), after every three cycles, and after treatment completion (at 4 weeks). 5ml of whole blood was collected from the patients. DNA isolation from the collected whole blood was obtained using qiagen miniBlood kit (Cat no: 51106 Düsseldorf Germany). Single gene variation was analysed from the obtained DNA using pyrosequencing system. Patient data were obtained from patient files. Statistical analysis was performed with SPSS-17 package programme. The results were evaluated at 95% confidence interval. $P<0.05$ was considered statistically significant. Categoric variables with more than two categories were by SPSS for the Cox analysis.

Results

Patient characteristics and clinical outcomes.

Forty patients were identified between 2008 and 2012 (Table I). The median age was 61.2 years and 75% were male. The oral cavity was the most common primary site (40%). All patients were classified as locally advanced. All patients were treated with cisplatin and 5-FU.

Table 1 Characteristics of the Patients With Head and Neck and of the Treatments Administered (N 40)

Characteristic	No of patients	%
Age		
Median	61,2	
Range	40-78	
Sex		
Female	10	25
Male	30	75
Tumor location		
Oral Cavite	16	40
Oropharynx	12	30
Hypopharynx	4	10
Larynx	4	10
Tongue	2	5
Nasopharynx	2	5
Smoking history		
Never	8	20
Current	32	80

Allele Frequencies

The frequencies of the various gene polymorphisms are shown in Table 2. As a result of the six-gene polymorphism, no patient had common polymorphisms for all genes. Ten patients had two or more polymorphic homozygous variants. Twenty-seven patients had two or more heterozygous genes. No statistically significant association was observed between the presence of simultaneous gene polymorphisms.

Table 2. Allele Frequencies of the Indicated Gene Variants in the Patients with Head and Neck

	Common Homozygotes		Heterozygotes		Polymorhic Homozygotes	
Gene Variant	No	%	No	%	No	%
ERCC1 C8062A	24	60	13	32.5	3	7.5
ERCC1 T19007C	13	32.5	14	35	13	32.5
XRCC1 G28152A	24	60	13	32.5	3	7.5
GSTP1 A313G	13	32.5	25	62.5	2	2.5
MTHFR C677T	20	50	17	42.5	3	7.5
MTHFR A1298C	18	45	13	32.5	9	22.5

Correlation With Clinical Response

After 12 months of follow-up (range, 4 to 12 months; median, 8 months), response/survival data were available for all. At the end of the experiment, 36 patients (90%) were alive, 10 (25%) were alive but progression, and 4 (10%) had died. The overall response rate (RR) was 10 (25%) PD, 7 (17.5%) SD, 9 (22.5%) PR,

and 14 (35%) CR. The results of the analysis of the response rate by genotype are shown in Table 3. Of the patients who presented with at least one polymorphic variant, 4 had PD, 3 had SD, 3 had PR and 1 had CR (P.021).The treatment response analysis indicates that per additional polymorphic variant, the probability of experiencing PD was 2.14 times greater than that of SD (P .048). Furthermore, per additional variant, PD was respectively 2.28 and 1.05 times more likely than PR (P.036) .

Table 3. Response to Treatment of the Advanced Patients with Head and Neck According to Their Genotypes

	Progressive Disease		Stable Disease		Partial Response		Complete Response		p
Gene Variant	No	%	No	%	No	%	No	%	value
ERCC1 8062									0.049
C/C	4	16.6	2	8.4	6	25	12	50	
C/A	4	30.8	4	30.8	3	23	2	15,4	
A/A	2	66.7	1	33.3					
ERCC1 19007									0.052
T/T	2	14.3	3	21.5	4	28.5	5	35.7	
T/C	4	30.8	1	7.7	3	23	5	38.5	
C/C	4	30.8	3	23	2	15.4	4	30.8	
XRCC1									0.042
G/G	5	20.8	3	12.5	4	16.7	12	50	
G/A	3	23	3	23	5	38.5	2	15.5	
A/A	2	66.7	1	33.3					
GSTP1									0.058
A/A	3	23	4	31	3	23	3	23	
A/G	6	24	2	8	6	24	11	44	
G/G	1	50	1	50					
MTHFR 677									0.047
C/C	4	20	2	10	5	25	9	45	
C/T	4	23.5	4	23.5	4	23.5	5	29.5	
T/T	2	66.7	1	33.3					
MTFR 1298									0.040
A/A	4	22.2	2	11.1	4	22.2	8	44.5	
A/C	3	23	3	23	2	15.5	5	38.5	
C/C	3	33.3	2	22.3	3	33.3	1	11.1	

Discussion

Although many polymorphisms in genes involved in the DNA repair mechanism have been identified, their effects on the biological process have not been clearly explained. Researchers have focused on to explain the function of SNPs in genes implicated in NER/BER in response to chemotherapy based on cisplatin recently. The papers have been reported that ERCC1 C8092A mutation is linked to poor clinical outcomes in patients suffering from stages IIIA-IV lung cancer who have been treated with platinum-based combination therapy (12). Researchers showed that the compound mutation effects of the C8092A and T19007C genotypes act as an independent prognostic factor in T4 breast cancer patients (8). Vaezi et al. reported, that the C8092A polymorphism in the ERCC1 gene is a risk prognostic factor in head and neck cancers (13). Castro et al. (2010) showed that T19007C SNP in the ERCC1 gene has no effect on overall survival, on the contrary to literature including esophageal cancer, colorectal cancer, ovarian cancer and non-small cell lung cancer patients. When we analyzed in terms of treatment response relationship, T19007C polymorphism in ERCC1 gene was not found to be statistically significant and C8092A polymorphism was found to be statistically significant. When we evaluated our results, we found that correlated with the literature.

Genetic polymorphisms in the GSTP1 and XRCC1 genes have been shown to be associated with chemosensitivity and clinical outcomes when evaluated in their single and combined forms (6). In other study, Zhou et al. found statistically significant association of G28152A polymorphism in XRCC1 gene and A313G polymorphism in GSTP1 gene with the treatment response of lung cancer patients. In the meta-analysis of Vaezi et al., it was reported that the G28152A polymorphism in

the XRCC1 gene was associated with response to treatment in the Chinese population but not in the Caucasian race (6). Furthermore, NSCLC patients treated with platinum-based regimens and possessing G28152A polymorphism in the XRCC1 gene exhibit a low objective response rate (14). In our study, G28152A polymorphism in XRCC1 gene was found to be statistically significant in relation to treatment response, and this results are consistent with studies in Chinese and Caucasian populations. Although the A313G polymorphism in GSTP1 gene were significant in the Chinese population, the significant results were not found in our study. So we have thought that it may be due to ethnic origin.

MTHFR serves as a critical enzyme in the 5-FU metabolic process, and its enzyme activity can be diminished due to the MTHFR C677T and A1298C polymorphisms. As a result, there may be a strong link between the MTHFR C677T and A1298C polymorphisms and the effectiveness of 5-FU therapy (15). Some studies have reported a significant association between the C677T genetic variant and increased tumor response rates to 5-FU-based therapy. The A1298C allele has been linked to an increased risk of severe adverse events or poor survival after 5-FU-based chemotherapy (10). In our study, we have found that C677T and A1298C polymorphisms in MTHFR gene was association with increased tumor response rates.

In this study, five single nucleotide polymorphisms, C8092A and T19007C, in ERCC1 gene, G28152A in XRCC1 gene, A313G in GSTP1 gene and C677T and A1298C, in MTHFR gene were retrospectively evaluated for their association with the clinical behavior in a group of head and neck carcinoma patients of West Anatolia, receiving platinum and 5-FU-based chemotherapy. We have demonstrated that this five genotypes have a role as independent prognostic factors for a more favorable clinical outcome in this subset of patients. According to our results, A313G polymorphism in GSTP1 gene and T19007C polymorphism in ERCC1 gene were not found significant. This study that investigate the relationship between single nucleotide polymorphism and response to treatment in head and neck cancers is the first. However, this study is limited in terms of the number of patients.

Conflict of interest

The authors declare that they have no conflict of interest.

Financing

This study did not receive any funding.

Ethic

In this retrospective study, national and international ethical rules were complied with. This study approved Pamukkale University Ethic Committee with number of E-60116787-020-408595.

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