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XPD and XRCC1 gene polymorphism in patients with normal and abnormal cervical cytology by pap smear

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Abstract. – **AIM:** The purpose of the present study was to identify the role of abnormalities in DNA repair pathways by measuring the XPD and XRCC1 gene polymorphisms.

MATERIALS AND METHODS: Thirty-five patients with abnormal cervical cytology (study group) and 10 women with normal cytology (control group) were included in the study. The polymorphisms of XRCC1 Arg194Trp, XRCC1 Arg399Gln and XPD Lys751Gln genes were investigated from the blood samples.

RESULTS: There was no statistically significant difference in allele frequencies of XPD gene among the groups ($p = 0.097$), while XRCC1R399Q gene polymorphism was strikingly more frequent in the study group than that of control cases ($p = 0.029$). The prevalence of XRCC1R194W gene polymorphism on the other hand, was similar between the groups ($p = 0.579$).

CONCLUSIONS: Patients with abnormal and normal cervical cytology have similar XPD gene polymorphism. However, the frequency of gene polymorphism in XRCC1 Arg 399 Gln codon was significantly higher in abnormal cervical cytology group.

Key Words:
Cervical intraepithelial neoplasia, Polymorphism, Cervix, DNA repair.

Introduction

Cervical cancer still has been an important public health problem, particularly in developing countries. Globally, it is recognized that nearly half of the patients diagnosed as cervical cancer die due to complications developed secondary to this neoplasia and 85% of the patient population diagnosed as cervical cancer are living in the developing countries[1].

Although the impact of etiologic factors as HPV infection and smoking on cervical dysplasia has been established, the importance of DNA repair genes for cervical cancer and cervical dysplasia is being investigated in recent years[2,3]. Although it is known that many times a day spontaneous oxidative damage occurs in each cell of human beings. DNA fractures are encountered in patients with cervical cancer or cervical smear pathology. In fact as the pathologic degree of abnormal cervical smear increases, the frequency of existent DNA damage increases relative to the patients with normal smear or low-grade cervical pathologies[4-5]. DNA damage is recognized by repair genes and repaired. DNA repair overcomes most of these destructive changes. A variation occurring in alignment of the code in DNA repair genes is called polymorphism[6].

XPD (Xeroderma Pigmentosum complementation group D) gene is a DNA repair gene which is located at 13.2 locus on long arm of the chromosome[3]. Damages and mutations in XPD gene impair DNA repair and also DNA transcription. Polymorphisms on three separate sites such as on 199. (Ile-Met), 312. (Asp-Asn) and 751. (Lys-Gln) codons have been identified[7].

XRCC1 (X-ray crosscomplementing group 1) is another gene involved in DNA repair. Three gene sites with repair functions have been defined. Arg194Tr on exon 6, Arg399Gln on exon 10 and Arg280His on exon 9 are specialized sites for repair function[8]. Although, mutations developed on each three sites induce changes in amino acid chain, Arg399Gln polymorphism site deserves a special importance for DNA repair, in that it couples with binding site of PARP (PolyADP-ribosepolymerase) enzyme which checks audits fractures[9].

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In the present study, polymorphism characteristics of Arg399Gln and Arg194Tr loci which are located on DNA repair genes i.e. XPD (Lys751Gln) and XRCC1 genes were analyzed in serum of the patients with abnormal and normal cervicovaginal cytology.

Materials and Methods

Study Design

Forty-five patients who referred to Outpatient Clinics in the study 2010-2011 years. The patients were prepared in the lithotomy position. A small sample of cells from the surface of the cervix was collected using a spatula or cythobrush. All samples were collected by rotating the device through 360°. The collected material was spread on appropriately labeled slides and immediately sprayed with a fixative. The fixed smears were sent to the Cytology Laboratory and then examined under a microscope. The smears were stained with a Pap-stain and screened by cytologists. The women were grouped for analysis according to the presence or absence of cervical abnormality.

DNA Isolation

From each study participant, 2 ml blood sample was drawn into an EDTA tube. DNA isolation from white blood cells in the peripheral blood sample was performed using a DNA isolation kit (High Pure PCR Template Preparation kit, Roche Diagnostic, Mannheim, Germany) and isolated DNA was kept at –20°C.

Genotyping Analysis

Determination of polymorphisms (XRCC1 Arg194Trp, XRCC1 Arg399Gln and XPD Lys751Gln) was performed by LightCycler real time PCR (Roche Diagnostics, Mannheim, Germany) using a LightSNiP assay from TIB MOLBIOL (Berlin, Germany). A 20 µl mixed reaction solution which contained 10.4 µl H_2O, 2 µl of LC™ FastStart DNA Master HybProbe kit (Roche Diagnostics), 1.0 µl of LightSNiP reagent mix, 1.6 µl $MgCl_2$ (25 mM) and 5 µl genomic DNA and transferred into capillary tubes. Polymerase chain reaction (PCR) conditions were 10 minutes for initial denaturation at 95°C; 45 cycles at 95°C for 10 seconds for denaturation, 10 seconds at 60°C for annealing and 15 seconds at 72°C for extension. The melting point analysis uses fluorescence resonance energy transfer for detecting a polymorphic site. Melting curve analysis was performed with an initial denaturation at 95°C for 20 s, 20 s at 40°C, slow heating to 85°C with a ramping rate of 0.2°C/s and continuous fluorescence detection. The genotype screening was performed simultaneously for cases and controls.

Viral DNA Isolation

Viral transport media (Digene, AR-MED-Ltd, London, UK) were used for collection of the specimens. All samples were stored at –70°C until the extraction process. Viral DNA was extracted from cervical samples by the automated extraction system (BioRobot EZ1 system, Qiagen, Hilden, Germany).

HPV DNA Detection and Typing

For human papilloma virus (HPV) detection and typing, a chip-based detection system (LCDarray HPV 3.5, Chipron GmbH, Berlin, Germany) was used according to manufacturer's instructions. The specificity of the system was assessed for 32 HPV types (HPV 6, 11, 16, 18, 31, 33, 35, 39, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 70, 72, 73, 81, 82, 83, 84, 90, 91). This method consists of two main stages; hybridization and PCR. In brief, the biotinylated "primer mix HPV MY09/11" was used to generate labeled PCR products (~450 bp length) from the L1 region of the HPV genome. Twelve 1 of prelabeled PCR products were mixed with the hybridization buffer and hybridized to the arrays for 30 minutes at 35°C. Following a short washing step, the arrays were incubated with the secondary label solution for 5 minutes at room temperature and washed again. Hybridization was visualized by the staining solution. Within 1-2 minutes, the substrate formed a dark blue precipitate in those positions where PCR amplicons were bound. Following the staining procedure, high resolution images were taken with a transmission light-scanning device and automated data analysis was done with the "SlideReader" software (LCD-Analysis package, Chipron, Germany).

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Fisher's exact test and chi-square test was used to compare polymorphism characteristics between patients with abnormal smears and the control group. The results for all items were assessed within a 95% reliance and at a level of $p < 0.05$ significance.

Results

This study included 45 participants referred to our Clinics with normal and abnormal cervicovaginal smears. Ten patients (22%) with normal smear test results constituted the control group, while the remaining 35 patients had abnormal cervical cytology. Cervicovaginal smear test results were determined to be ASCUS in eight (17%), ASC-H in eight (17%), LSIL in nine (20%), HSIL in seven (15%) and AGC in three (6.7%) patients. Mean age of the patients with normal smear (37.40±6.72 years), number of pregnancies (2.50±1.71), parities (2.40±1.77), miscarriages (0.1±0.31) and mean number of survived newborns (2.30±1.63) were also determined. Eight of 10 patients with normal cervicovaginal smears were non-smokers and only two patients in this group were current smokers.

Twenty patients in this group were smokers. A statistically significantly difference was found between two groups as for smoking status (p = 0.038). All patients with normal cervicovaginal smear had negative HPV screening test results; while 12 of 35 cases in the patient group HPV positivity was detected. A statistically significant difference was revealed between two groups as for HPV typing (p = 0.031). In a total of six patients HPV type 16, in 3 patients HPV type 53 and in 1 patient HPV type 16 and HPV type 62 and also in 1 case HPV type 54 and 84 combination were detected. These results were summarized in Table I.

In the XPD study group a total of 11 patients had AA, 23 patients had AC and 11 patients had CC alleles. In the XRCC1R399Q group, GG alleles in 20, GA alleles in 19 and AA alleles in eight patients were observed. In the XRCC1R194W study group CC and CT alleles were detected in 37 and 8 patients, respectively. In normal control and patient subgroups, alleles of DNA repair genes were seen in frequencies summarized in Table II. In XPD gene, Lysine (Lys) is coded by allele A, glutamine (Gln) by allele C and in XRCC1R339Q gene, arginine (Arg) by allele G and glutamine (Gln) by allele A, respectively. In XRCC1R194W gene among aminoacids, arginine (Arg) is coded by allele C and tryptophan (Trp) by allele T. When cervicovaginal smear test results were examined as for XPD gene polymorphism, the frequency of gene polymorphism in patients with abnormal smear test results was increased relative to the controls without any statistically significant intergroup difference (p = 0.097).

When both groups were screened for XRCC1R399Q gene, gene polymorphism was observed to be statistically significantly higher in the patient group with abnormal cervicovaginal test results (p = 0.029). Even though, the prevalence of XRCC1R194W gene polymorphism in patients with abnormal cervical smear was increased, the difference was not statistically significant (p = 0.579). In XPD group, this polymorphism was detected at a rate of 35% in the patient group with normal Lys allele frequency, 54% in the group with abnormal smear test results, while it was observed in 65% and 46% of the patients with normal Gln allele frequency and abnormal smear test results, respectively. In the patient group, Gln allele frequency was higher than the control group without any statistically significant difference (p = 0.128).

In XRCC1R399Q gene group, Arg allele frequency was 40% in the control and 71% in the patient group, while Gln allele frequency was 60 and 29% in the control and the patient groups, respectively. For this gene group, statistically significant difference was found between patient groups in terms of allele frequencies (p = 0.01). In XRCC1R194W gene group Arg allele frequency was 90% in the control and 91% in the patient group, while Trp allele frequency was 10% in the control and 9% in the patient group. Any statistically significant difference could not

Table I. Demographic characteristics of the patient, and control groups, smoking habits, and cervical smear HPV positivity.

	Patients with normal cervicovaginal smear (n = 10)	Patients with abnormal cervicovaginal smear (n = 35)	*p* value
Age	37.40 ± 6.72	40.89 ± 11.23	0.186
Gravida	2.50 ± 1.71	3.91 ± 2.13	0.290
Parity	2.40 ± 1.77	3.03 ± 1.85	0.903
Abortus	0.1 ± 0.31	0.51 ± 0.887	0.009*
Survived	2.30 ± 1.63	3.03 ± 1.85	0.645
Smoker yes/no	2/8	20/15	0.038*
HPV positivity	–	12	0.0031*

Table II. Number of alleles of DNA repair genes in patients with normal and abnormality cervical smear test results.

Patient groups	XPD			XRCC1R399Q			XRCC1R194W	
	AA	AC	CC	GG	GA	AA	CC	CT
Normal	2	3	5	1	6	3	9	1
ASCUS	2	4	2	3	3	2	6	2
ASC-H	2	5	1	5	3	–	8	–
LSIL	2	5	2	6	3	–	6	3
HSIL	2	5	–	3	4	–	7	–
AGC	1	1	1	2	–	1	2	1

ASCUS: Undetermine significant atypical squamous cell; ASC-H: Atypical squamous cell-high grade; LSIL: Low grade stromal intraepitelial lesion; HSIL: High grade stromal intraepitelial lesion; AGC: atypical glandular cell.

be detected between patient groups as for allele frequencies in this gene group ($p = 0.843$). These results are summarized in Table III.

Discussion

DNA damage is known to occur in cervical dysplasias, cervical smear pathologies, and cervical cancer in association with HPV, smoking, and similar etiologies. Emergent DNA damage causes genomic instability, which triggers oncogenic process. In two separate studies where in situ hybridization and commet assay techniques were used to detect DNA damage in cases with abnormal cervical smear, it was indicated that in the group with abnormal smear test results, the number of DNA fractures were statistically significantly increased relative to the control group. However, within the patient group, in higher grade lesions the frequency of DNA fractures was detected to be increased when compared with lower grade lesions[5,10]. In a similar study performed by Matsuda et al[11] the degree of DNA damage was investigated in patients with normal cervical dysplasia (CIN1-2-3), and in cases diagnosed as cervical cancer. They demonstrated that as the grade of cervical dysplasia approached to that of cervical cancer, the degree of DNA damage increased in direct proportion. Repair genes have a crucial place in the repair DNA damage, which plays a key role in the pathogenesis of cervical pathologies[3]. In the study conducted by Matsuda et al[11] the activity of repair genes were also evaluated, and maximal activity of these genes was observed in cervical cancer, and high grade cervical dysplasias with highest DNA damage. Inadequate repair of DNA damage despite the presence of DNA repair genes has been related to the occurrence of DNA damage exceeding

Table III. Percentages, and number of each of three gene polymorphisms, and allele frequencies in the patient and control groups.

Genotype	Control group (n = 10)	Patient group (n = 35)	*p* value
XPD751			
Lys/Lys	2	9	–
Lys/Gln	3	20	0.097
Gln/Gln	5	6	–
Lys allele frequency	7 (35%)	38 (54%)	0.128
Gln allele frequency	13 (65%)	32 (46%)	–
XRCC1R399Q			
Arg/Arg	1	19	–
Arg/Gln	6	13	0.029*
Gln/Gln	3	3	–
Arg allele frequency	8 (40%)	50 (71%)	0.01*
Gln allele frequency	12 (60%)	20 (29%)	–
XRCC1R194W			
Arg/Arg	9	29	0.579
Arg/Trp	1	6	–
Arg allele frequency	18 (90%)	64 (91%)	0.843
Trp allele frequency	2 (60%)	6 (9%)	–

the repair capacity of the repair genes or functional loss of DNA repair genes[5]. Polymorphisms occurring in association with variations in the sequence of DNA repair genes attenuate or completely abolish the effectiveness of these genes and accelerate tumoral progression

XDP gene on chromosome 19 is one of these repair genes. Three different regions are defined on this gene and in our study its 751. (Lys-Gln) codon was analyzed. Some literature studies investigated the correlation between codon 751 of XPD gene and various malignancies. Sobti et al[12] analyzed the association between the prostate cancer and XPD gene polymorphism, in their study which included 150 prostate cancer patients and equal numbers of benign prostatic hyperplasia patients and healthy individuals. Among these three groups, a statistically significant difference could not be found as for XPD gene polymorphism. In their study, Ozdemir et al[13] compared a total of 90 patients with diagnoses of Acute Lymphoblastic Leukemia (ALL) and Burkitt's Lymphoma (BL) with 99 patients with normal XPD gene codon 751 polymorphisms. At the end of the study they couldn't see any statistically significant intergroup difference. Huang et al[14] investigated XPD gene codon 751 polymorphism in the etiopathogenesis of squamous esophageal cell in 213 patients with esophageal tumor and compared their results with those of 358 patients in the control group. Again, they could not detect a statistically significant difference between 2 groups as for XPD gene polymorphism. He et al[3] compared patients with cervical intraepithelial dysplasia or cervical cancer patients with the control group in terms of XPD gene polymorphism. They included 200 patients diagnosed as cervical intraepithelial neoplasia (CIN), 134 cases with squamous cell cancer of cervix and 200 control subjects in their study. However, they could not reveal any statistically significant difference among patients with CIN, cervical cancer and controls as for XPD gene polymorphism. Even though our study is the first investigation of its kind in the medical literature, it yields similar results with abovementioned studies. In our research, any significant difference was not detected between the group with cervical dysplasia and the control group as for XPD gene polymorphism

XRCC1 gene can accomplish its DNA repair function on its three different regions. In the literature miscellaneous reports demonstrated association between XRCC1R399Q gene polymorphism with various malignancies. Kumar et al[15] compared 278 patients diagnosed as squamous cell cancers of head and neck region with completely normal 278 individuals and observed that the prevalence of XRCC1 Arg 399 Gln gene polymorphism was statistically significantly higher relative to normal subjects. Studies investigating XRCC1 Arg 399 Gln gene polymorphism in cervical cancer have indicated that frequency of polymorphism in patients with cervical cancer was statistically significantly higher when compared with the control group[16-17]. Only one literature study has so far investigated polymorphism in this gene group and in patients diagnosed as cervical dysplasia. In this investigation, a significant difference could not be detected between the patients diagnosed as cervical dysplasia and the control group in terms of XRCC1 Arg 399 Gln gene polymorphism[18]. Also in our study the frequency of gene polymorphism in XRCC1 Arg 399 Gln codon was significantly higher in the cervical dysplasia group when compared with the control group.

In our research codon 194 of XRRCC1 gene was also analyzed. In the development of other organ tumors (breast, esophagus, head-neck) various results have been observed for this gene polymorphism[19]. However, in a literature study performed with patients diagnosed as cervical cancer, any significant difference could not be found between the patient and the control groups as for XRCC1 Arg194Trp gene polymorphisms[20]. Also in patients diagnosed as cervical intraepithelial neoplasia, the frequency of polymorphism in this gene had not significantly differed from the control group[18].

We have also investigated the effects of smoking and HPV[21] which are held responsible in the etiology of cervical lesions, among other causative factors. Cigarette usage increases the incidence of cervical dysplasias. Plummer et al[22] demonstrated that the prevalence of invasive cervical cancer and cervical dysplasia in smoking quitters or current smokers was significantly higher when compared with non-smokers. Nowadays, only recognized causative agent of cervical cancer and dysplasias is HPV infection. In more than 95% of the patients diagnosed as cervical cancer, HPV DNA has been detected. Besides, incidence of HPV infection increases in cervical dysplasias[23]. Also in our study, the frequency of HPV, and smoking were increased in cases with abnormal cervicovaginal smear when compared with the control group.

Conclusions

DNA repair genes are regions of the gene which repair the emergent damage rapidly and effectively. Polymorphism occurring in only specific regions of these genes is effective not only in gynecologic cancers, also in the development of many organ tumors. In many literature reports, polymorphism in XRCC1 Arg399Gln gene region appears to be more influential in gynecologic and non-gynecologic tumorigenesis. Herein, in our study, this gene polymorphism was found to be statistically significantly at a higher rate in patients diagnosed as cervical dysplasia when compared with the control group.

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