DNA Repair Gene (XPD, XRCC4, and XRCC1) Polymorphisms in Patients with Endometrial Hyperplasia: A Pilot Study

Ebru Öztürk
Sacide Pehlivan
Ozcan Balat
Mete Guroğlu
Huseyin Çağlayan Özcan
Suna Erkılıç

Background:
In this study, we aimed to evaluate the association between endometrial hyperplasia and DNA repair gene (XPD, XRCC4, and XRCC1) polymorphisms.

Material/Methods:
There were 114 cases enrolled in the study in 4 groups: simple endometrial hyperplasia (SH) (Group 1), complex endometrial hyperplasia without atypia (CH) (Group 2), complex atypical endometrial hyperplasia (CAH) (Group 3), and normal endometrium (NE) (Group 4). Of these cases, 37 cases had SH, 36 cases had CH, 16 cases had CAH, and 25 cases had NE. To evaluate an association between atypia and DNA repair genes, we consider a group that included both SH and CH, the endometrial hyperplasia without atypia cases (Group 5). Genomic DNA was isolated from paraffin-embedded endometrial tissue collected from the Pathology Department of Gaziantep University Medical School. Polymerase chain reaction (PCR) and/or restriction fragment length polymorphism (RFLP) method was used for evaluating of XPD (-751), XRCC4 (-1394 and a variable number of tandem repeats in intron 3), and XRCC1 (-399) genes.

Results:
We observed a notable distinction in patients having endometrial hyperplasia without atypia (the SH+CH group) and the CAH group in terms of XPD (-751) gene polymorphisms. A notable contrast was observed in patients with endometrial hyperplasia without atypia (the SH+CH group) and the NE group in terms of XRCC4 (VNTR intron 3) polymorphisms ($P=0.026$, $P=0.018$, respectively).

Conclusions:
It was evident the DNA repair gene XPD and XRCC4 polymorphisms had a role in the pathophysiology of endometrial hyperplasia.

MeSH Keywords: DNA Repair • Endometrial Hyperplasia • Genes, vif

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Background

Uterine endometrioid carcinoma is the most common type of endometrial carcinoma that develops from endometrial hyperplasia, and its characterized histopathological endometrial abnormalities include glandular complexity and cytologic atypia, in the setting of unopposed estrogen exposure [1]. DNA repair defects might affect genome-wide genetic instability, which can induce further cancer progression [2]. X-ray repair cross-complementing 1 and 4 (XRCC1 and XRCC4), and xeroderma pigmentosum complementary group D (XPD) are 3 major DNA base excision repair genes that act interactively in DNA repair processes [3]. Polymorphisms of these genes might alter the rate of gene transcription, the stability of the messenger RNA, or protein functions. It is plausible that variations in these genes affect an individual's capacity to repair damaged DNA, and thus induce cancer development in normal or exposed individuals. In the literature, although there have been many molecular studies on endometrial tumorigenesis including DNA repair gene polymorphisms focusing on invasive lesions, only a limited number of publications have evaluated the genetic alterations that occur in endometrial hyperplasia [4].

The primary objective of this study was to assess the association between endometrial hyperplasia and XPD, XRCC4, and XRCC1 gene polymorphisms. As far as we know, this is the first assessment of the association between these DNA repair gene polymorphisms in patients with endometrial hyperplasia taking into account atypia and the complexity in existing English language literature.

Material and Methods

The Ethics Committed for Clinical Research of Gaziantep University approved this retrospective study. Patients were selected from a pool of female patients receiving treatment at the Obstetrics and Gynecology Department of Gaziantep University between January 2001 and December 2010; all study patients had a successive endometrial aspiration biopsy (Pipelle®) or had a hysterectomy because of abnormal uterine bleeding. Serial specimens of normal endometrium (NE), simple endometrial hyperplasia (SH), complex endometrial hyperplasia without atypia (CH), and complex endometrial hyperplasia with atypia (CAH) were obtained from the Pathology Department of Gaziantep University. In total, 114 cases were enrolled in this study. Of these cases, 37 cases had SH (Group 1), 36 cases had CH (Group 2), 16 cases had CAH (Group 3), and 25 cases had NE (Group 4). To evaluate an association between atypia and DNA repair genes, we also include an SH and CH (Group 5) to represented endometrial hyperplasia without atypia cases. The isolation of genomic DNA was performed using paraffin-embedded endometrial tissues collected from the Pathology Department of Gaziantep University Medical School [5]. The polymorphisms of XPD (-751), XRCC4 (-1394 and variable number of tandem repeat in intron 3), and XRCC1 (-399) genes were evaluated. Genotyping was performed by polymerase chain reaction (PCR) and/or restriction fragment length polymorphism (RFLP) [6]. The computation for Hardy-Weinberg equilibrium was performed utilizing the De-Finetti program. A $\chi^2$ test was used for statistical analysis of genotype and allele frequencies in groups.

Results

XPD (-751) A-C genotype and allele frequency of groups are presented in Table 1. A notable distinction was observed in endometrial hyperplasia patients without atypia (SH+CH) (Group 5) and the CAH group (Group 3) for both genotype and allele frequency. A/A homozygotes XPD (-751) gene were significantly more frequent among Group 5 than Group 3 ($P=0.026$) and C/C homozygotes XPD (-751) gene was significantly more frequent among Group 3 than Group 5 ($P=0.026$). While the A allele was significantly more frequent in Group 5 than Group 3, and the C allele was significantly more frequent in Group 3 than Group 5 ($P=0.036, P=0.013$ respectively). The observed genotype counts did not deviate significantly from those expected according to the Hardy-Weinberg equilibrium for XPD gene -751 polymorphisms ($P>0.05$).

XRCC4 gene variable number of tandem repeats in intron 3 (VNTR intron 3) polymorphism I-D genotype and allele frequency of all groups are presented in Table 2. A notable distinction was observed in endometrial hyperplasia cases without atypia (SH+CH) (Group 5) and NE cases (Group 4). While the I allele was significantly more frequent in Group 5 than Group 4, the D allele was significantly more frequent in Group 4 than Group 5 ($P=0.024, P=0.025$ respectively). The observed genotype counts were not significant according to the Hardy-Weinberg equilibrium for XRCC4 gene intron 3 VNTR polymorphisms ($P>0.05$).

We did not observe any noteworthy discrepancy in patients in the SH, CH, CAH, and NE groups in terms of XRCC1 (-399) and XRCC4 (-1394) genotype and allele frequency (data not shown).

Discussion

Endometrial carcinoma is the most common malignant tumor of the female genital organs and classified into 2 histologic subtypes: endometrioid type and non-endometrioid type as exemplified by serous adenocarcinoma [1]. These 2 types of endometrial carcinoma have different molecular genetic changes. In the literature, the mutation of p53 gene has been reported in 90% of non-endometrioid type carcinomas; mutations of
PTEN, KRAS, β-catenin, and p53 genes and microsatellite instability have been reported in 5% to 50% of endometrioid-type carcinomas [7]. However, multiple genetic alterations in endometrial tumorigenesis are not clearly understood [8].
It is known that the most common type of endometrial carcinoma, endometrioid adenocarcinoma, develops from endometrial hyperplasia in the setting of excess estrogen exposure. Epidemiologic studies suggest that the risk of progression to malignancy is low for hyperplasia without atypia, whereas atypia is associated with a significant risk of carcinoma. Despite numerous studies on endometrial tumorigenesis, little is known about the cause of progression from endometrial hyperplasia to carcinoma [9,10].

The XPD protein is a kind of protein that is absolutely necessary for the 5'-3' helicase enzyme to have a role in the DNA repair pathway [11]. Polymorphism -751 in the XPD gene has been associated with a lower DNA repair capacity and a different kind of carcinogenesis [12,13].

A few studies have demonstrated that there is no association between XPD (-751) gene polymorphisms and endometrial cancer, however, in these studies, there was no evaluation of endometrial hyperplasia cases [14,15]. In our study, we observed significant differences in patients with endometrial hyperplasia without atypia (the SH+CH group) and the CAH group. This result suggested to us that the XPD (-751) gene polymorphisms could have a role in the appearance of atypia in endometrial hyperplasia. When AA genotype has a protective role against the appearance of atypia in endometrial hyperplasia, CC genotype could be a predisposing factor for atypia in endometrial hyperplasia.

XRCC4 is generally expressed as a protein (334 amino acids) which plays a role in DNA ligase IV and DNA dependent protein kinase enzyme in the repair of DNA double strand breaks, but its function is unknown [16]. In this study, we evaluated 2 polymorphisms in the XRCC4 gene. This study is the first report on XRCC4 gene polymorphism in endometrial pathology. There have been no studies on the association between endometrial hyperplasia or endometrial carcinoma and XRCC4 gene polymorphism found in the literature.

During our investigation, we noted that patients who had SH or CH and patients in the NE group, in terms of XRCC4 (VNTR intron 3) polymorphisms, showed notable distinctions. These results suggested to us that XRCC4 (VNTR intron 3) gene polymorphisms could have a role on the appearance of endometrial hyperplasia (simple or complex) without atypia. Thus, when DD genotype could have protective role against CH, then II genotype could be a predisposing factor for CH.

The mending of DNA base damage and single-strand DNA breaks is where XRCC1 plays an instrumental part by bringing together a complex of DNA repair proteins including PARP1 and DNA polymerase [17]. Codon 399 is a region that is critical for the role of XRCC1 in single-strand break repair and cell survival [18]. Although there are a few reports about XRCC1 (-399) polymorphisms in endometrial carcinoma, there are no studies on the association between endometrial hyperplasia and XRCC1 gene polymorphism in the English language literature [19,20]. Although Romanowicz-Makowska et al. indicated that XRCC1 A399G polymorphism might be a genetic determinant for developing endometrial cancer [19]. In our study, we observed no association between XRCC1 gene polymorphisms and endometrial hyperplasia including SH, CH, and CAH.

**Conclusions**

This study demonstrated the correlation of endometrial hyperplasia and variant of XPD, XRCC4 genes. During our investigation, we observed the different steps of endometrial tumorigenesis, including the appearance of endometrial hyperplasia without atypia; once endometrial hyperplasia appeared, progression to atypia was associated with different DNA repair gene polymorphisms. It is evident the DNA repair gene (XPD and XRCC4) polymorphisms have a role in the pathophysiology of endometrial hyperplasia. Nonetheless, a larger study sample, which incorporates endometrial carcinoma studies, will shed more light on this issue.

**References:**

1. Ellenson LH: The molecular biology of endometrial tumorigenesis: Does it have a message? Int J Gynecol Pathol, 2000; 19: 310–13
2. Baak JP, Path FR, Hermens MA et al: Genomics and proteomics in cancer. Eur J Cancer, 2003; 39: 1199–215
3. Jiang J, Zhang X, Yang H, Wang W: Polymorphisms of DNA repair genes: ADPRT, XRCC1, and XPD and cancer risk in genetic epidemiology. Methods Mol Biol, 2009; 471: 305–33
4. Nieminen TT, Gylling A, Abdel-Rahman WM et al: Molecular analysis of endometrial tumorigenesis: Importance of complex hyperplasia regardless of atypia. Clin Cancer Res, 2009; 15: 5772–83
5. Ciftci S, Yilmaz M, Pehlivan M et al: DNA repair gene polymorphisms in multiple myeloma: no association with XRCC1 (Arg399Gln) polymorphism, but the XRCC4 (VNTR in intron 3 and G-1394T) and XPD (Lys751Gln) polymorphisms is associated with the disease in Turkish patients. S Hematol, 2011; 16: 361–67
6. Ünal M, Güven M, Batar B et al: Polymorphisms of DNA repair genes XPD and XRCC1 and risk of cataract development. Exp Eye Res, 2007; 85: 328–34
7. Feng YZ, Shiozawa T, Miyamoto T et al: BRAF mutation in endometrial carcinoma and hyperplasia: Correlation with KRAS and p53 mutations and mismatch repair protein expression. Clin Cancer Res, 2005; 11: 6133–38
8. Morice P, Leary A, Creutzberg C et al: Endometrial cancer. Lancet, 2016; 387: 1094–108
9. Ozler A, Kuscu NK, Temiz P et al: Leptin expression in proliferative, secretory, and hyperplastic endometrial tissues. J Turkish-German Gynecol Assoc, 2011; 12: 157–61
10. Erdem O, Erdem M, Erdem A et al: Expression of vascular endothelial growth factor and assessment of microvascular density with CD 34 and endoglin in proliferative endometrium, endometrial hyperplasia, and endometrial carcinoma. Int J Gynecol Cancer, 2007; 17: 1127–12
11. Jelonek K, Gdowicz-Klosok A, Pietrowska M et al: Association between single-nucleotide polymorphisms of selected genes involved in the response to DNA damage and risk of colon, head and neck, and breast cancers in a Polish population. J Appl Genet, 2010; 51: 343–52
12. Hansen RD, Sørensen M, Tjønneland A et al: XPA A23G, XPC Lys939Gln, XPD Lys751Gln and XPD Asp312Asn polymorphisms, interactions with smoking, alcohol and dietary factors, and risk of colorectal cancer. Mutat Res, 2007; 619: 68–80
13. Wang L, Ma J, Yang B et al: XRCC2 polymorphisms and environmental factors predict high risk of colorectal cancer. Med Sci Monit, 2018; 24: 2858–63
14. Doherty JA, Weiss NS, Fish S et al: Polymorphisms in nucleotide excision repair genes and endometrial cancer risk. Cancer Epidemiol Biomarkers Prev, 2011; 20: 1873–82
15. Weiss JM, Weiss NS, Ulrich CM et al: Nucleotide excision repair genotype and the incidence of endometrial cancer: Effect of other risk factors on the association. Gynecol Oncol, 2006; 103: 891–96
16. Wu PY, Frit P, Meesala S et al: Structural and functional interaction between the human DNA repair proteins DNA ligase IV and XRCC4. Mol Cell Biol, 2009; 29: 3163–72
17. El-Khamisy SF, Masutani M, Suzuki H, Caldecott KW: A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. Nucleic Acids Res, 2003; 31: 5526–33
18. Taylor RM, Thistlethwaite A, Caldecott KW: Central role for the XRCC1 BRCT I domain in mammalian DNA single-strand break repair. Mol Cell Biol, 2002; 22: 2556–63
19. Romanowicz-Makowska H, Smolarz B, Houli A, Szyłło K: Single nucleotide polymorphism in DNA base excision repair genes XRCC1 and hOGG1 and the risk of endometrial carcinoma in the Polish population. Pol J Pathol, 2011; 62: 89–94
20. De Ruyck K, Wilding CS, Van Eijkeren M et al: Microsatellite polymorphisms in DNA repair genes XRCC1, XRCC3 and XRCC5 in patients with gynecological tumors: Association with late clinical radiosensitivity and cancer incidence. Radiat Res, 2005; 164: 237–44