Single nucleotide polymorphism in DNA base excision repair genes *XRCC1* and *hOGG1* and the risk of endometrial carcinoma in the Polish population

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Background: Polymorphisms in the human oxoguanine glycosylase 1 (*hOGG1*) and X-ray repair cross-complementing 1 (*XRCC1*) genes have been extensively studied in the association with various human cancers such as endometrial cancer.
Material and methods: The genotype analysis of *hOGG1* Ser326Cys and *XRCC1* Arg399Gln gene polymorphisms for 150 endometrial cancer patients and 150 controls of cancer-free subjects, in the Polish population, were performed using PCR-based restriction fragment length polymorphism (PCR-RFLP).
Results: Although there were no significant ($p > 0.05$) differences in the frequencies of genotypes or alleles of *hOGG1* genes between patients and controls, the frequency of the *XRCC1* 399Gln allele was significantly greater in endometrial cancer patients compared with controls ($p = 0.033$) with an odds ratio of 1.39 (95% confidence interval 0.99 to 1.95). The distributions of genotypes and alleles of the genes *hOGG1* and *XRCC1* were not significantly associated with different grades of endometrial cancer ($p > 0.05$).
Conclusion: In conclusion, these findings indicated that *XRCC1* Arg399Gln polymorphism may be a genetic determinant for developing endometrial cancer. The *hOGG1* Ser326Cys may not play an important role in susceptibility to endometrial cancer in Polish women.

Key words: endometrial cancer, *hOGG1*, *XRCC1*, polymorphism, PCR-RFLP.

Introduction

Endometrial cancer is one of the most common malignant neoplasms which appear in the uterine body. About 80% of cases are diagnosed after menopause. The highest incidence, estimated at 57-58 years, is moving to the 6th and 7th decade of life at present. Endometrial cancer is the fourth most common female carcinoma [1, 2].

Endometrial cancer oncogenesis is not a fully recognized process regarding many risk factors. Neoplasm genesis is a multi-stage process. Carcinogenic factors influencing our organism mostly do not cause neoplasm development directly, but they induce genesis of endogenous intermediates, for example reactive oxygen intermediates (ROI) or substances oxidized by ROI. These substances may damage the DNA structure and cause point or chromosomal mutations. Some of these mutations lead to cell neoplastic transformation and in consequence to neoplasm development. At each of these stages there are actions of some endogenous or exogenous anticarcinogenic factors (e.g. vitamins A, C, E, glutathione,

enzymes acting as free radical scavengers, DNA self-repairing structures).

For repair of oxidative DNA damage, human cells are supported by five DNA repair systems: direct reversal, mismatch repair, double-strand break repair, nucleotide excision repair (NER) and base excision repair (BER) [3].

The human oxoguanine glycosylase 1 (*hOGG1*) and X-ray repair cross-complementing 1 (*XRCC1*) genes are key genes in the BER pathway [3, 4]. All oxidatively induced DNA lesions and single-strand breaks are repaired via the BER pathway [4]. *XRCC1* and *hOGG1* can be involved in the repair of DNA lesions, which are known to contribute to endometrial cancer.

There have been some reports about the relation between *hOGG1* codon 326 and *XRCC1* codon 399 polymorphisms and risk for several cancers [4-18].

The *hOGG1* G>C transversion at position 1245 of the *hOGG1* gene producing a Ser → Cys substitution at codon 326 (the Ser326Cys polymorphism) was associated with the risk of lung [7, 8], gastric [9] and larynx cancer [10]. On the other hand, *hOGG1* 326Cys allele plays a significant protective effect against breast cancer in European women [11].

Table I. Characteristics of endometrial cancer (n = 150) patients

CHARACTERISTICS	NUMBER OF CASES
Age (years)	
Median	64
Range	52-83
BMI (body mass index) (kg/m^2)	
<24.9	32 (21%)
25-29.9	46 (31%)
>30	72 (48%)
Number of pregnancies	
1	48 (32%)
2-3	102 (68%)
> 4	0
Use of hormone replacement therapy (HRT)	
Yes	96 (64%)
No	54 (36%)
Grading	
G1	67 (45%)
G2	77 (51%)
G3	6 (4%)
Uterine bleeding	
Yes	100 (67%)
No	50 (33%)
Endometrial transvaginal ultrasound (TVU)	
>5 mm	115 (78%)
Diabetes mellitus	
Yes	28 (19%)
No	122 (81%)
Hypertension	
Yes	80 (54%)
No	70 (46%)

The amino acid replacement of *XRCC1*-399Arg to Gln might lead to an increased risk of laryngeal, prostate, pancreatic, lung, breast, and oral carcinoma [12-18]. On the other hand, *XRCC1* codon 399 polymorphism was reported to reduce the risk of bladder cancer [19].

Little is known about *hOGG1* and *XRCC1* polymorphism in endometrial cancer risk. In the available literature not many researchers have investigated the association of *hOGG1* and *XRCC1* polymorphism and endometrial carcinoma [20-22].

Therefore, the aim of this study was to determine the relationship between *XRCC1* (Arg399Gln) and *hOGG1* (Ser326Cys) and endometrial cancer.

Material and methods

Endometrial cancer patients

150 patients with a histologically proven diagnosis of endometrial cancer were included in the study. Table 1 shows characteristics of endometrial cancer subjects. Tumour tissues were obtained from women with endometrial carcinoma treated at the Department of Surgical Gynaecology, Institute of Polish Mother's Memorial Hospital during 2004-2009. The endometrial cancer tissue samples were fixed routinely in formalin and embedded in paraffin. All tumours were graded according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO). DNA from normal endometrial tissue (n = 150) served as a control.

DNA isolation

Archival paraffin-embedded tumour sections on slides were deparaffinized in xylene and rehydrated in ethanol and distilled water. DNA was extracted from material using commercially available QIAmp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to the manufacturer's instructions.

Determination of *hOGG1* genotype

Polymorphism Ser326Cys of the *hOGG1* gene was determined by PCR-RFLP, using primers (5'-GGAAGGTGCTTGGGGAAT-3' and 5'-ACT-GTCACTAGTCTCACCAG-3'). The 25 µl PCR mixture contained about 100 ng of DNA, 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 2 mmol/l of $MgCl_2$ and 1 U of Taq DNA polymerase. PCR products were electrophoresed in a 7% polyacrylamide gel (PAGE) and visualised by ethidium bromide staining. Only one 100-bp fragment was seen in subjects with the Cys/Cys genotype. In subjects with the Ser/Cys genotype, two bands of 100 and 200 bp were seen, whereas in those subjects homozygous for the Ser variant (Ser/Ser), only one 200-bp PCR fragment is seen. All PCR was carried out in a DNA

Thermal Cycler (GeneAmp PCR System 2400; Perkin-Elmer, Norwalk, CT, U.S.A.). After an initial denaturation at 95°C for 5 min, 35 cycles of amplification with denaturation at 95°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 30 sec were performed, followed by a final extension step of 7 min at 72°C. The PCR product was digested overnight with 1 U of *Sat*I at 37°C.

Determination of *XRCC1* genotype

Genotypic analyses of the *XRCC1* gene were carried out by multiplex PCR-RFLP, using primers for codon 399 (5'-TTGTGCTTTCTCTGTGTCCA-3' and 5'-TCCTCCAGCCTTTTCTGATA-3'), which generate a fragment of 615 and 491 bp. Briefly, PCR was performed in 25 µl reaction buffer containing 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 3 mmol/l of $MgCl_2$, about 100 ng of DNA and 1 U of Taq DNA polymerase. The PCR products were digested overnight with 10 U of *Msp*I at 37°C.

For codon 399, the presence of two bands of 375 and 240 bp, respectively, identifies the wild-type Arg allele, while the uncut 615 bp band identifies the mutant Gln allele (indicative of the absence of the *Msp*I cutting site).

Statistical analysis

For each polymorphism, deviation of the genotype frequencies in the controls from those expected under Hardy-Weinberg equilibrium was assessed using the standard χ^2-test. Genotype frequencies in cases and controls were compared by χ^2-tests. The genotypic-specific risks were estimated as odds ratios (ORs) with associated 95% confidence intervals (CIs) by unconditional logistic regression. P-values < 0.05 were considered to be significant.

Results

To verify the association of risk and the genetic change in the base excision repair (BER) pathway in endometrial cancer development, the polymorphisms of *XRCC1* and *hOGG1* in patients and control groups were analysed.

The results of the genotypes of *XRCC1* (Arg399Gln) and *hOGG1* (Ser326Cys) in the endometrial cancer and control groups are shown in Table II. It can be seen from the table that there were significant differences ($p < 0.05$) between the two investigated groups. The women with endometrial cancer showed an incidence of 52 and 48%, respectively, for the Arg and Gln allele of the *XRCC1* gene, whereas the control group showed 65 and 34% for the same alleles. The frequencies of *XRCC1*-399Gln allele in the case group were higher than that of the control group ($p < 0.05$). The variant 399Gln allele was significantly increased in endometrial cancer patients compared with the control group (OR = 1.39, 95% CI: 0.99-1.95; $p = 0.033$) (Table II).

We did not find any significant differences for *hOGG1* genotype frequencies in patients with cancer and controls (Table III). Additionally, there were no differences in the frequencies of alleles between both distributions ($p > 0.05$).

To understand whether the genetic polymorphisms of *XRCC1* (Arg399Gln) and *hOGG1* (Ser326Cys) increased the risk of endometrial cancer development, the different genotypes and the tumour grade evaluated according to FIGO criteria were compared (Table III).

The histological grade was evaluated in all cases (n = 150). 67 cases were grade I, 77 cases were grade II and 6 cases were grade III. Grade II and III were grouped together for the purposes of statistical analysis.

There were no significant differences between distributions of *XRCC1*-Arg399Gln and *hOGG1*-Ser326Cys genotypes in subgroups assigned to histological grades ($p > 0.05$) (Table IV).

No statistically significant differences were observed in the alleles or in the genotype frequencies of the *XRCC1*-Arg399Gln and *hOGG1*-Ser326Cys gene polymorphisms between risk factors of endometrial cancer such as body mass index (BMI), hormone

Table II. Distribution of *XRCC1* genotype frequencies in patients with endometrial cancer and control group

	ENDOMETRIAL CANCER PATIENTS (N = 150)		CONTROLS (N = 150)			
XRCC1-ARG399GLN	NUMBER	%	NUMBER	%	OR (95% PU)[A]	P[B]
Arg/Arg	41	27	64	43	0.64 (0.36-1.12)	0.158
Arg/Gln	73	49	68	45	1.07 (0.67-1.70)	0.8628
Gln/Gln	36	24	18	12	2.0 (0.93-4.29)	0.110
Arg	155	52	196	65	0.79 (0.58-1.07)	0.079
Gln	145	48	104	34	**1.39 (0.99-1.95)**	**0.033**

Data in boldface are statistically significant
[A]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [B]χ^2

Table III. Distribution of *h0GG1* genotype frequencies in patients with endometrial cancer and control group

	ENDOMETRIAL CANCER PATIENTS (N = 150)		CONTROLS (N = 150)			
H0GG1-SER326CYS	NUMBER	%	NUMBER	%	OR (95% PU)[A]	P[B]
Ser/Ser	94	63	105	70	0.89 (0.67-1.19)	0.458
Ser/Cys	46	31	39	26	1.18 (0.87-1.62)	0.271
Cys/Cys	10	6	6	4	1.66 (0.82-3.38)	0.154
Ser	234	78	249	83	0.94 (0.79-1.12)	0.497
Cys	66	22	51	17	1.27 (0.99-1.66)	0.057

[A]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [B]χ^2

Table IV. Dependency of the distribution of genotype frequencies on the tumour grade in patients with endometrial cancer

POLYMORPHISM	GRADE I (%) (N = 67)	GRADE II + III (%) (N = 83)	OR (95% PU)[A]	P[B]
XRCC1-Arg399Gln				
Arg/Arg	17 (25)	32 (38)	0.48 (0.24-0.94)	0.051
Arg/Gln	37 (55)	35 (42)	1.34 (0.77-2.3)	0.292
Gln/Gln	13 (20)	16 (18)	2.04 (0.84-4.90)	0.113
Arg	71 (52)	99 (60)	0.87 (0.67-1.12)	0.296
Gln	63 (48)	67 (40)	1.19 (0.90-1.56)	0.204
h0GG1-Ser326Cys				
Ser/Ser	41 (61)	53 (64)	0.93 (0.62-1.41)	0.751
Ser/Cys	22 (33)	24 (29)	1.12 (0.73-1.72)	0.596
Cys/Cys	4 (6)	6 (7)	1.14 (0.46-2.79)	0.777
Ser	104 (77)	130 (78)	0.96 (0.76-1.23)	0.791
Cys	30 (23)	36 (22)	1.12 (0.79-1.58)	0.497

[A]Crude odds ratio (OR), 95% CI = confidence interval at 95%, [B]χ^2

replacement therapy (HRT), uterine bleeding, endometrial transvaginal ultrasound, diabetes and hypertension and the women with endometrial cancer.

Discussion

The present study examined whether polymorphism in *XRCC1*-Arg399Gln and *h0GG1*-Ser326Cys gene is related to the development of endometrial cancer. In our present study, the polymorphism of X-ray repair cross complementary 1 (*XRCC1*), a major gene in the BER system, is associated with endometrial cancer, but the polymorphism of *h0GG1* is not associated.

Although there are other SNPs in the *XRCC1* gene, the three *XRCC1* polymorphisms (Arg194Trp, Arg280His and Arg399Gln) have been evaluated as risk factors for cancers in a number of studies.

It was suggested that SNPs in the *XRCC1* gene may alter the ability of XRCC1 to repair damaged DNA, especially SNPs at codon 399.

The *XRCC1*-Arg399Gln gene polymorphism has been studied as a risk factor for various cancers. *XRCC1*-Arg399Gln has been associated with increased risk for lung cancer [23, 24], head and neck cancer [25] and possibly stomach cancer [26].

In contrast, no increased risk was observed for bladder cancer [27], oesophageal cancer [28] and non-melanoma skin cancer [29].

In breast cancer no association between the *XRCC1*-399 Gln/Gln genotype and this carcinoma was found [30]. However, other studies showed an increased risk of breast cancer with this polymorphism [31-33].

In the literature little is known about *XRCC1* Arg399Gln polymorphism in endometrial cancer risk.

Only De Ruyck *et al*. showed that SNPs in *XRCC1* with a combination of different polymorphisms in DNA repair genes (*XRCC3* and *h0GG1*) is associated with an enhanced clinical radiosensitivity in endometrial cancer patients treated with late radiotherapy (RT) [21].

In our work the 399Gln allele was associated with an increased risk for the development of endometrial cancer compared with 399Arg. Our study demonstrated that the 399Gln allele may be a risk factor for this cancer in the Polish population. It is possible

that the presence of the 399Gln allele is in linkage disequilibrium with another, so far unknown, mutation located outside the coding region in the *XRCC1* gene, which may be of importance for the *XRCC1* concentration in plasma.

However, in our study we did not find any association between *hOGG1* gene Ser326Cys polymorphisms and endometrial carcinoma occurrence. This is in line with the reports which indicate that the amino acid replacement of *hOGG1*-326Ser to Cys might lead to lack of association with risk of endometrial carcinoma [21, 22]. Krupa *et al*. suggested that the Ser326Cys polymorphism of the *hOGG1* gene might not be directly involved in the development and/or progression of endometrial cancer in the Polish population; therefore, it may not be useful as an independent marker of this disease [22].

Moreover, we did not find any association of the SNPs in the patient group with cancer progression assessed by BMI, number of pregnancies, HRT, uterine bleeding, endometrial transvaginal ultrasound, diabetes and hypertension and the women with endometrial cancer.

Our results show that the polymorphism of the *XRCC1* gene but not *hOGG1* may be associated with the occurrence of endometrial cancer in Poland.

Therefore, we suggest that different DNA repair systems may play different roles in endometrial carcinoma. These repair systems could be the basis of future surveys. Further studies on the polymorphisms of other genes in NER, BER, or other DNA repair systems are necessary for the detection of a genetic predisposition to endometrial cancer formation and for the investigation of the roles of NER, BER, or other DNA repair genes in cancer formation. In conclusion, *XRCC1* Arg399Glu is correlated with endometrial carcinoma and might become a potential marker for the prediction of endometrial carcinoma susceptibility. It also provides valuable insight into the pathogenesis of endometrial carcinoma.

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