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Original Article

Investigation of resistin 420 and 62 gene polymorphism in patients with endometrial cancer

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ABSTRACT

Objective: We aimed to assess resistin gene polymorphisms, namely 420C > G and 62G > A and their effect on the risk of endometrial cancer (EC).
Materials and methods: Between January 2012 and January 2015, of the total of 183 patients diagnosed with EC, 94 patients were enrolled into the study. Patients with diabetes mellitus, hypertension and history of any other cancer were excluded. To identify the importance of nucleotide polymorphism including 420C > G and 62G > A in the resistin gene, 94 healthy volunteers were included as the control group.
Results: Among the Resistin 420 gene polymorphism profiles, 420 GC (47.9%) was the most common gene polymorphism in the EC group. Also, the polymorphism of 420 CC (57.7%, p: 0.002) lead the list in the control group followed by the 420GC (37.5%) polymorphism. Resistin 62 gene polymorphism analysis demonstrated that the 62GC polymorphism was significantly more common in the EC group ($p < 0.01$), while 62 AG (52.9%) was observed most frequently in the control group bringing about a reduction in the risk of EC ($p < 0.01$, Odds Ratio:0.37). Additionally, the alleles of 420G+ and 62A + were significantly more common in the EC group and the control group, respectively (p:0.02 and p<:0.01). Multivariate regression analysis revealed that the presence of 420G + allele increased the EC risk 1.99 fold while the presence of 62A + allele was shown to decrease the risk of EC (p<:0.01 Odds Ratio:0.038).
Conclusion: Our study for the first time had demonstrated that Resistin 420G > C and 62G > A gene polymorphisms play a role in EC development.

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in developed countries. It was reported that there are 320,000 new cases of EC each year and 42,000 women die from EC or EC related complications. For advanced stage disease, the five years survival rate was shown to decrease from 90% to 15%, thus suggesting that early diagnosis of EC is the critical goal to increase the life expectancy, especially for high risk patients [1]. Obesity, hypertension, diabetes mellitus and unopposed estrogene exposure are well-known risk factors for EC. It is presumed that genetic factors may influence the management strategies for EC patients not harboring the risk factors mentioned above and these genetic factors must be clearly elucidated [2].

Beyond its energy storage function, adipose tissue is accepted as a metabolically active endocrine gland and secretes metabolic active proteins collectively called adipocytokines, which regulate homeostasis, inflammatory response and carcinogenesis [3]. Resistin is one of the relatively new identified adipocytokines. The association of resistin and diabetes mellitus, cardiovascular disease, colon cancer and breast cancer has been demonstrated [4]. Hlavna et al. showed a positive correlation between serum levels of resistin and EC [5]. Furthermore, Ilhan et al. have demonstrated that single nucleotide polymorphism (SNP] in the resistin gene had an impact on EC development [6].

Our objective was to assess the genetic variations of endometrial cancer with respect to resistin and its SNPs. To our knowledge, this

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is the first study searching for two genetic polymorphisms, namely 420C > G and 62G > A and their effect on the risk of EC.

Materials and methods

Following the approval of the local ethics committee, the study was conducted in the Obstetrics and Gynecology Department of Istanbul University Istanbul Medical Faculty. Between January 2012 and January 2015, of the total of 183 patients diagnosed with EC, 94 patients were enrolled into the study. Patients with diabetes mellitus, hypertension and history of any other cancer were excluded. To identify the importance of nucleotide polymorphism including 420C > G and 62G > A in the resistin gene, 94 healthy volunteers were included as the control group. Patients characteristics including, age, body mass index (BMI), the duration of menarcheal estrogen exposure and menopausal status were recorded. Informed consent was obtained from each participant.

Blood samples were obtained from all individuals in EDTA anticoagulant. Total genomic DNA was extracted from peripheral blood (5 mL) samples using a DNA extraction kit (Fermentas, ThermoFisher Scientific, USA), applying the manufacturer's protocol. The polymorphisms were identified using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Two sets of primers (5.0 nmol) were used. For resistin 62G > A region amplification, the forward primer 5-AGA GTC CAC GCT CCT GTG TT-3 and reverse primer 5‘- R-CAT CTC CAG GTT TAT TTC CAG C-3 were used. For 420 C > G region amplification, the forward primer F- 5′-TGT CAT TCT CAC CCA GAG AC-3′ and reverse primer R- 5′-TGG GCT CAG CTA ACC AAA TC-3 were used. The amplification was performed in a volume of 25 μL, containing 0.2 μg genomic DNA, 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 μM of each primer, and 0.5 U Taq DNA polymerase (ThermoFisher Scientific, USA). The PCR initial denaturation was done at 95 °C for 2 min, 35 cycles at 95 °C for 30 s and at 52 °C for 45 s,75 °C for 45 s, and a final elongation step at 72 °C for 7 min. A total of 5 μL of the PCR products was digested with restriction endonucleases for 16 h at 37 °C. The PCR products of digestion were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide.

The statistical analyses were performed using the SPSS version 11.0 (SPSS Inc.,Chicago, USA). The differences in genotype distributions and allelic frequencies between two groups were examined using the Fisher exact and the chi-square tests. The odds ratios (OR) and 95% confidence interval (95% CI) ranges were calculated by using logistic regression. Significance was considered at a P value < 0.05.

Results

The results of 94 EC patients and 94 healthy volunteers were compared. The mean age of the EC group and healthy volunteers was 47.8 and 50.2, respectively (p:0.12). The mean BMI was similar for the two groups (p:0.21). 26 patients in the EC group and 28 healthy volunteers in the control group reported the use of hormonal contraception (p:0.30). Preoperative characteristics of patients and healthy volunteers are listed in Table 1.

Endometrioid type was the most common histological EC type (84% of all patients). For all patients, the disease stage at presentation varied from stage 1A to stage 3C. Stage 1B (53 patients, 56.4% of all patients) and stage 1A (16 patients, 17% of all patients) were the most common. Also, 60 patients were diagnosed as grade 1 EC (Table 2).

Among the Resistin 420 gene polymorphism profiles, 420 GC (47.9%) was the most common gene polymorphism in the EC group. Also, the polymorphism of 420 CC (57.7%, p:0.002) lead the list in the control group followed by the 420GC (37.5%) polymorphism. Resistin 62 gene polymorphism analysis demonstrated that the 62GG polymorphism was significantly more common in the EC group (p < 0.01), while 62 AG (52.9%) was observed most frequently in the control group bringing about a reduction in the risk of EC (p < 0.01, Odds Ratio:0.37).

Additionally, the alleles of 420G+ and 62A + were significantly more common in the EC group and the control group, respectively (p:0.02 and p<:0.01). Multivariate regression analysis revealed that the presence of 420G + allele increased the EC risk 1.99 fold while the presence of 62A + allele was shown to decrease the risk of EC (p<:0.01. Odds Ratio:0.038) (Table 3). Associations between histology, stage, grade of EC and 420 and 62 gene polymorphisms are summarized in Table 4.

Discussion

Genetic disorders and gene defects are being accounted for many diseases including rheumatologic diseases, metabolic syndrome or cancer. As most of the various cancers, endometrial cancer has also association with suspected genetic syndromes and genetic mutations [7]. During the last decades, SNP has also emerged as one of the most popular areas of research to investigate the cause of endometrial cancer. SNP is described as a variation of single nucleotide that arises from a specific location in the genome which can cause amino acid changes in the protein and be associated with personal response to pathogens, drugs, vaccines and the development of disease [8]. Recently, the role of SNP is under investigation in diabetes mellitus, hypertension, polycystic ovary syndrome and cancers including colorectal cancer, breast cancer and endometrial cancer.

Resistin is an peptide hormone derived from adipose tissue and encoded by the RETN gene in humans [9]. Previously, the role of resistin in the inflammatory process has been demonstrated. In the presence of infection or irritation, resistin stimulates the expression of pro-inflammatory cytokines including tumor necrosis factor-alfa, interleukin-1, interleukin-6 and interleukin-12 [10,11]. Also, resistin increases the expression of molecules known to cause endothelial dysfunction such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, chemokine ligand 2, vascular endothelial growth factor, endothelin 1 and matrix metalloproteases [12]. Moreover, resistin increases the production of P-selectin and fractalcaline in endothelial cells by MAP kinase pathway. Furthermore proinflammatory factors, themselves upregulate resistin levels. Due to the factors mentioned above neovascularization and inflamation may have a role on tumoral development in endometrial tissue.

Previous reports stated that resistin gene 420 polymorphism is associated with some of the human diseases. Baba et al. lately demonstrated RETN G/G genotype is observed more frequently in patients with polycystic ovary syndrome compared to a control group [13]. In another study, Mahmoudi et al.demonstrated that 420 GG genotype is associated with increased colorectal cancer risk (p = 0.044; OR = 0.53, 95%, Cl = 0.29–0.98) [14]. Similarly, Alharithy et al. showed the potential role of 420 gene polymorphism in colon cancer in a study with 60 patients and 60 healthy volunteers [15]. In our study, we found significant correlation with 420 G > C polymorphism and endometrial cancer development.

Other SNP locations on the RETN gene have been found to be related to other diseases as well. Thammakun et al. reported that resistin gene polymorphism at the position of 62 G > A, predisposes to the development of type 2 diabetes mellitus [16]. Also, Nambiar et al. stated that the polymorphism of resistin 62 gene plays a role in polycystic ovary syndrome [17]. Tan and colleagues demonstrated a statically significant relationship between resistin 62 gene

Table 1
Characteristics of patients with endometrial cancer and healthy volunteers.

	Endometrial cancer group (n = 94)	Control group (n = 94)	p value
Age ± SD	47.83 ± 9.58	50.20 ± 10.69	0.12
BMI(kg/m^2)	31.3 ± 6.1	30.2 ± 5.5	0.21
Hormonal contraceptive usage	26 (27.7%)	28 (26.9%)	0.30
Age at first menstrual period	13.2 ± 1.5	13.2 ± 1.4	0.86
Age at last menstrual period	51.7 ± 4.5	51.4 ± 4.7	0.63
PCOS[a] history	6 (6.4%)	5 (5.3%)	0.87
Presence of insulin resistance	7 (7.4%)	4 (4.2%)	0.21
HDL level (mg/dl)	50.8 ± 4.1	48.7 ± 6.2	0.44
LDL level (mg/dl)	108.2 ± 15.3	102.7 ± 13.5	0.68
Triglyceride level (mg/dl)	162.4 ± 42 0.1	151.2 ± 43.1	0.15

[a] Polycystic ovary syndrome.

Table 2
Histopathologic results of patients with endometrial cancer.

Histology	
Endometrioid	79 (84%)
Non-Endometrioid	15 (16%)
Stages (FIGO 2009 staging system)	
1A	16 (17%)
1B	53 (56.4%)
2A	4 (4.3%)
2B	6 (6.4%)
3A	4 (4.3%)
3B	0 (0%)
3C	11 (11.6%)
Grade	
1	60 (63.8%)
2	17 (18.1%)
3	17 (18.1%)

polymorphism and diabetes mellitus and hypertension. Tan et al. suggested that resistin 62 gene polymorphism may have an impact on endometrial cancer development beyond diabetes mellitus and hypertension [18]. Our study, for the first time revealed that, 62A + resistin gene allele had a protective role in the development of EC.

Previous studies have investigated the relationship between polycystic ovary syndrome (PCOS) and EC, intensively [19]. Hardiman et al. had demonstrated that PCOS is a predictive factor for EC, however; this finding does not mean the incidence or mortality from EC increased [20]. In a recently published study, authors had stated that most women with PCOS do not face with EC in their lifespan, however, women with PCOS has three times increased risk for EC development when compared with other women without PCOS [21]. In the present study, we did not encounter any difference between patients with and without EC, in terms of PCOS. We

Table 3
Genotype profile of endometrial cancer and control groups.

	Endometrial cancer group (N = 94)	Control group (N = 94)	p value (Univariate regression analysis)	p value (Multivariate regression analysis)	Odds Ratio
Polymorphism					
420 GG	10 (10.6%)	5 (5.3%)	0.12		
420 CC	39 (41.5%)	60 (63.8%)	**0.02**		
420 GC	45 (47.9%)	39 (41.5%)	0.14		
62 GG	39 (41.5%)	15 (15.9%)	**0.00**		
62 AA	34 (36.2%)	34 (36.2%)	0.60		
62 AG	21 (22.3%)	55 (58.5%)	**0.00**	**0.00**	**0.37 (0.18–0.75)**
Alleles					
420G+	55 (58.5%)	44 (46.8%)	**0.02**	**0.02**	**1.99 (1.08–3.65)**
420C+	84 (89.4%)	89 (95.2%)	0.12		
62G+	60 (63.8%)	70 (74.5%)	0.09		
62A+	55 (58.5%)	89 (94.7%)	**0.00**	**0.01**	**0.47 (0.14–0.96)**

Bold values signify statistical difference defined as $p < 0.05$.

Table 4
Genotype profiles according to histology, stage and grade of patients with endometrial cancer.

	420GG	420CC	420GC	p value	62GG	62AA	62AG	p value
Histology				0.86				0.50
Endometroid	9	32	38		34	28	22	
Nonendometroid	1	7	7		5	6	4	
Stage				0.12				0.79
1A	0	4	12		4	7	5	
1B	6	24	23		24	20	9	
2A	1	1	2		2	0	2	
2B	1	2	3		3	3	0	
3A	1	1	2		1	1	2	
3B	0	0	0		0	0	0	
3C	1	7	3		5	3	3	
Grade				0.55				0.76
1	6	26	28		25	24	11	
2	2	9	6		6	5	6	
3	2	4	11		8	5	4	

believed that relatively small study sample may have an effect on these results.

Insulin resistance may have a role on EC development. Hernandez et al. reviewed 25 studies to clarify the impact of insulin resistance on EC and they concluded that insulin resistance is one of predictive factors for EC due to increased activity of insulin-like growth factor-I (IGF-I) and IGF-I receptors and inhibit IGF binding protein-I (IGFBP-I) [22]. In a different study, Trabert et al. have found significant relationship between EC and metabolic syndrome and its components, including higher triglyceride level [23]. Although patients with EC had more common insulin resistance and higher triglyceride levels in our study, the difference was not statically significant ($p = 0.21$ and $p = 0.15$, respectively).

The present study has some limitations. Despite its prospective nature, our study sample is relatively small. Secondly, we only focused on the 420 and 62 polymorphisms in the resistin gene and did not evaluate the resistin level in blood sample. Finally, we did not analyze the potential effect of 420 and 62 resistin gen polymorphisms on endometrial cancer recurrence and patient survival.

Our study had demonstrated that resistin 420 G > C and 62 G > A gene polymorphisms are associated with EC. Our findings should be supported by large sample sized studies with long term outcomes.

Conflicts of interest

There is no financial or non financial conflict of interest in the study.

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