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The Association Between rs11568821 Polymorphism in Programmed Cell Death 1 (PD-1) and the Risk of Endometrial Cancer

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Abstract: *Background:* Endometrial cancer is the second most common gynecological cancer and a leading cause of gynecologic cancer-related deaths worldwide. The aberration in the expression of programmed cell death 1 (PD-1) and its gene polymorphisms have been indicated in several human cancers. In the current study, we aimed to investigate the association between rs11568821 polymorphism in PD-1 and the risk of endometrial cancer. *Methods:* This experiment was a hospital based, case-control study. We enrolled 91 patients with endometrial cancer and 50 healthy individuals as the control in this study. The mean age of patients in the case and control groups were 57.4 ± 9.7 and 55.1 ± 14 years, respectively. Peripheral blood was taken from these individuals, and DNA extraction was carried out. Polymerase chain reaction (PCR) amplified the region containing rs11568821, followed by restriction fragment length polymorphism (RFLP). *Results:* Comparison of disease incidence across rs11568821 genotypes showed significant association in recessive model GG vs. AG+AA (P = 0.028) with GG genotype increasing the risk of endometrial cancer. *Conclusion:* Our results indicated that rs11568821 polymorphism in PD-1 is associated with endometrial cancer. However, further studies in larger cohorts are needed to unravel the exact distribution of the genotypes and alleles of this polymorphism in women with endometrial cancer.

Keywords: Endometrial Cancer, PD-1, rs11568821, PCR-RFLP

1. Introduction

Endometrial cancer is the second most common gynecologic cancer and the second leading cause of gynecologic-related cancer mortality. Based on the national cancer institute's surveillance, epidemiology, and result (SEER) program estimations, about 66,570 new cases were diagnosed with endometrial cancer in the US in 2020,

accounting for 3.5% of all new cancer cases. Endometrial cancer also causes annually about 12,940 people to die of their disease, and this number of deaths is estimated to be 2.1% of all cancer-related mortalities in the US in 2020 [1]. Fortunately, a high proportion of the patients (up to 67%) are diagnosed at the early stages, when a tumor is localized in the uterus. Therefore, these patients can be cured efficiently. According to the estimations, about 95% of these early-detected patients have 5-year overall survival. However, those whose tumors are diagnosed at late stages when cancer is metastasized to a distant organ have significantly lesser survival (only 17.8% of them have 5-year overall survival), reflecting the importance of early diagnosis for endometrial cancer [1, 2]. However, there is an artiste acuity of data regarding the factors predisposing people to endometrial cancer.

Programmed death 1 (PD-1) is a crucial member of the CD28 family of surface molecules with a molecular weight of 55 kDa, expressed on the B cells and T cells surfaces and myeloid dendritic cells [3]. This surface protein plays a crucial role in immunological tolerance. Interaction of PD-1 with its two ligands, including PDL-1 (B7-H1) and PDL-2 (B7-DC), diminishes its activity and proliferation and reduces cytokine secretion, whereas it stimulates programmed cell demise in T cells [4]. The role of PD-1 in the immune tolerance and homeostasis of lymphocytes has been confirmed through investigations using mice with defective PD-1 expression. These experiments have shown that these mice develop various cancers, including endometrial cancers [5].

Blockade of PD-1 or its ligand, PD-L11, with specific antibodies, has shown to re-activate the immune system and is increasingly used as a treatment modality that promises to cure recurrent and metastatic tumors [6]. In endometrial cancer, immune checkpoint inhibitors to PD-L1 and PD-1 are increasingly studied, and they are an attractive option for the treatment [7]. TCGA (The Cancer Genome Atlas) classified endometrial cancer into four distinct molecular subtypes and gave momentum to further research in the targeted therapy [8]. However, it should be noted that various polymorphisms and genetic variants in the DNA sequence of PD-1 may influence the activity of this signaling pathway and the effectiveness of PD-1 targeted drugs. In the present study, we aimed to investigate the association between rs11568821 and the risk of endometrial cancer in Iranian women.

2. Materials and Methods

2.1. Patients and Samples

This case-control study was conducted at Friouzgar General Hospital, Iran University of Medical Sciences. In this study, we enrolled the histologically confirmed stages IA to IV endometrial cancer patients referred to this hospital from November 2017 and August 2020. Study subjects were women with the complaint of abnormal uterine bleeding who were admitted for diagnostic curettage. We found 91 patients with abnormal bleeding and uterine cancer with an age of equal to or more than 40 years. The cases with periods less than 40 and those with bleeding due to coagulative disorders or other disorders than uterine cancer were excluded. All of the patients were diagnosed with endometrial cancer by a gynecologist. Their disease was confirmed by evaluating tumor biopsies using PIPELLE or diagnostic curettage in the operating room (if the result showed hyperplasia, D&C would also be performed in the operating room).

Furthermore, we evaluated 50 age- and body mass index (BMI)-matched healthy individuals as the control group. This study was conducted according to the regulations of the Ethics Committee of Iran University of Medical Sciences. Informed written consent was obtained from all the patients and the healthy volunteers. After that, 10 ml of peripheral blood was taken from the patients and the controls and added to EDTA-containing tubes. The samples were stored in a freezer at −20°C until DNA extraction.

2.2. Genetic Analysis

Genomic DNA was extracted from the blood samples using Sinaclon DNA Extraction Kit (CinnaGen, Tehran, Iran). The quality and quantity of the extracted DNA samples were confirmed by running on agarose 1% gel electrophoresis and by reading the absorbances by a NanoDrop-1000 device. The DNA fragment which contained rs11568821 (PD-1.3 G>A) polymorphism was amplified using polymerase chain reaction (PCR) technique with the forward and reverse primers, with the sequence of 5′-CCCCAGGCAGCAACCTCAAT-3′ and 5′-GACCGCAGGCAGGCACATAT-3′, respectively. The total volume of the PCR reaction was 30 µl, each containing 300 ng template DNA, ten mM Tris–HCl, 0.2 mM dNTPs, 2.4 mM $MgCl_2$, 10 pM of forward and reverse primers, and 1-unit Taq DNA polymerase. Thermal cycling conditions for PCR amplification were: 1 cycle of 95°C for 5 min (initiation), and 30 processes of 95°C (40 s), 60°C (50 standard 72°C (40 s), followed by one method of 72°C (10 for the final extension. The amplified fragment was run on a 1.5% agarose gel electrophoresis to confirm the accuracy of the fragment. After that, a restriction fragment length polymorphism (RFLP) technique was carried out to digest the PCR products. For this purpose, 10 µl of each amplified sample was mixed with 1 unit of the restriction enzyme *Pst*I for 5 min at 65°C to allow digestion. After that, the products were run on 2% agarose gel electrophoresis to evaluate the genotypes according to the digestion pattern.

2.3. Statistical Analysis

In this study, we compared the differences in Quantitative variables between two groups using the student t-test. Furthermore, demographic and genetic variables were compared across the groups using the χ^2 test. Quantitative variables were stated as mean ± SD, and P values < 0.05 were considered statistically significant.

3. Results

This case-control study was conducted on 91 women with endometrial cancer referred to the Firouzgar General Hospital, Iran University of Medical Sciences, and 50 healthy women. The mean age of patients in the case and control groups were 57.4±9.7 and 55.1±14 years, respectively. The characteristics of the participants are given in Table 1.

A comparison of disease incidence across the rs11568821 variant did not significantly differ in recessive model GG vs. AG+AA (P=0.028, OR; 2.41, 95%CI; 1.01-5.7). Table 2 shows the distribution of rs11568821 polymorphism genotypes in control and patient groups.

Patients were categorized in terms of type and stage of their cancer, then the frequencies of PD-1.3 alleles and genotypes were evaluated in the arranged groups (Table 3). A similar evaluation was also performed on the grade of cancer (Table 4). Patients with stages 1a and 1b of endometrial adenocarcinoma and patients with grade 1 were the most frequent.

Table 1. *Characteristics of study participants.*

Variables	Groups		P value
	Mean (SD)		
	Case	Control	
Age	57.4 (9.7)	55.1 (14)	0.273
Weight	75.8 (12.7)	73.4 (9.6)	0.188
Height	161 (5.7)	161.3 (5.4)	0.672
BMI	29.3 (5.4)	28.1 (3.7)	0.101

Table 2. *PD-1.3 polymorphism genotypes in cases and controls based on merged AA-plus AG frequency and GG, along with adjusted results by age over 50 in the control group.*

Groups	Genotype number (frequency %)	P value		
	AA+AG			
N (%)	GG			
N (%)	Total			
Case	16 (17.6)	75 (82.4)	91 (100)	0.028*
Control	17 (34)	33 (66)	51 (100)	

* OR; 2.41, 95%CI; 1.01-5.7

Table 3. *Participants' grouping based on the type and stage of cancer and frequency of PD-1.3 polymorphism genotype.*

Type of cancer (stage)	Number	Percentage	AG+AA	GG
Endometrial adenocarcinoma (1a)	44	52.3	8	36
Endometrial adenocarcinoma (1b)	22	26.1	5	17
Endometrial adenocarcinoma (2)	4	4.7	-	4
Endometrial adenocarcinoma (3)	3	3.5	-	3
Endometrial adenocarcinoma (4)	1	1.2	-	1
Clear cell carcinoma	4	4.7	1	3
Papillary serous carcinoma	4	4.7	1	3
Mixed carcinoma	1	1.2	-	1
Sarcoma	1	1.2	1	-
Total	84	100	16	68

Table 4. *Participants' grouping based on the grade of cancer and frequency of PD-1.3 polymorphism type.*

Grade	Number	Percentage	AG+AA	GG
1	57	67.8	11	46
2	10	11.9	2	8
3	17	20.2	3	14
Total	84	100	16	68

4. Discussion

Endometrial cancer is the most common malignancy of the female genital tract and is one of the significant health problems in women and accounts for a substantial cause of women's morbidity and mortality. The estimated incidence of uterus cancer, including the malignancy of endometrium, is more than 3-fold higher than ovarian cancer (66570 vs. 2141. Although its mortality is less than ovarian cancer, the overall mortality rates of these patients are pretty close to ovarian cancer (12940 vs. 13.770) (2020). A plethora of studies have shown that several factors, such as diabetes, insulin resistance, and obesity, are associated with poor prognosis in endometrial cancer; however, the survival prediction of individual patients remains a significant challenge [9], highlighting the need for identifying prognostic factors and unraveling the genetic mechanisms involved in the development of this daunting disease.

It is now well-established that cancer cells take advantage

of several mechanisms to evade immune surveillance and disseminate through the bloodstream [10]. The PD-1/PD-L1 is a major immunosuppressive pathway that facilitates the escape of cancer cells from the immune system. In this regard, the expression of these two proteins and genetic polymorphisms in their sequence is a prognostic marker in various cancers [11-13]. Some studies evaluate the association of polymorphisms in the PD-1/PD-L1 axis and the risk of endometrial cancer. In the present study, we assessed the association between a polymorphism in PD-1, named rs11568821, and the risk of endometrial cancer, in an Iranian population. Our results demonstrated that, although the frequency of GG genotype was significantly higher in the patients than the healthy individuals. The PD-1.3 polymorphism is located in intron 4 of PD-1 and is a guanine (G) to adenine (A) polymorphism. Intron 4 of PD-1 is shown to be involved in the expression of this gene because of acting as an enhancer-like sequence due to the existence of four tandem repeats that contain multiple putative binding sequences of transcription factors [14]. Recent studies have demonstrated that PD-1.3 polymorphism in this region is a regulatory SNP and is associated with vulnerability to some human cancers. PD-1.3 can alter the runt-related transcription factor 1 (RUNX1) binding to PD-1 and affect its transcription rate [15].

Further studies have shown that the A allele of the PD-1.3 polymorphism leads to disturbing the binding site for RUNX1 transcription factors and causes the impaired activity of PD-1 as an inhibitory protein. This effect can result in more significant lymphocyte activity. Therefore, the A allele of the PD1.3 can intensify the antitumor capability of immune responses and diminish the liability of cancers [16]. In line with these observations, our results demonstrated that the presence of both G alleles was associated with endometrial cancer, which was significantly higher than the age-matched controls population. Haghshenas et al. reported no statistically significant difference between the frequency of PD-1.3 genotypes in Iranian women with breast cancer and those without breast cancer field [17].

In conclusion, according to our study, there is a significant association between the PD-1.3 polymorphism and the risk of endometrial cancer. However, it should be noted that in this study, we were able to evaluate a relatively small number of endometrial cancer patients, which may limit the power of the study and influence the results. Further studies, however, are needed to unravel the possible effects of PD-1 polymorphisms, notably PD-1.3 polymorphism, in the predisposition to endometrial cancer.

Statements and Declarations

Conflict of Interest

There was no conflict of interest to report.

Ethical Approval

The ethics committee approved the study at the Tehran University of medical sciences.

Informed Consent

Informed consent was obtained from the study participants.

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