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RESEARCH ARTICLE

Evaluation of PD-1 Gene Susceptibility and Prognosis in Breast Cancer Patients in Iranian Population

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ABSTRACT

Programmed cell death-1 (PD1) protein has an inhibitory effect that reduces the activity of T cells. In this study, gene expression PD1 in patients with breast cancer compared to healthy subjects discussed. This study was performed on 25 patients with breast cancer as a case group and 25 healthy individuals as a control group. Real-time PCR method was used to evaluate gene expression. For the case group, the mean age was 2.75 ± 12.18 and for the control group 30.6 ± 2.6. The results of our study showed that the expression of PD1 gene in the case group was higher than the control ($p<0.05$). As a result of our study show that expression of gene in the development of breast cancer, So PD1 can be used as prognostic gene in breast cancer study be considered.

Keywords: Breast cancer; Gene expresion; Programmed cell death-1

INTRODUCTION

One of the most common cancers in women, which is the second leading cause of death among women in the United States, is breast cancer[1–3]. Tumor and lymph node resection is a common treatment for breast cancer patients in the form of radiation therapy, hormone therapy or chemotherapy, and often carries many risks for patients with large tumors[4,5]. Most breast cancer patients are treated and survive with these treatments. Gene expression tests can be effective in diagnosing and treating breast cancer[6–8]. Programmed cell death protein is a protein from the family of immunoglobulins that has receptors PDL1 and PDL2 and is known as an inhibitory protein. PD1 is expressed on the surface of T cells and B lymphocytes and normal killer cells[9–11]. Although many studies have shown a link between gene expression and disease pathogenesis, little is known about the expression of PD1 gene and breast cancer[12,13]. Recent studies have shown that PD-1 gene expression is associated with BC pathogenesis, but studies indicate the importance of PD-1 gene in regulating T cell activity[14]. In this study, we investigated the expression of PD1 gene in women with breast cancer in comparison with healthy individuals in Sistan and Baluchestan province, Zahedan.

MATERIALS AND METHODS

Patients and Samples

Blood samples were taken from 25 patients with breast cancer as a case group and 25 healthy individuals as a control group. Patients' clinical information was obtained from their medical records. RNA was collected from all blood samples. The study protocol was also approved by the Medical Research Ethics Committee of Ali Ibn Abitaleb Hospital in Zahedan, Sistan and Baluchestan Province.

PD-1 expression analysis

Expression of PD-1 was analyzed via real time RT-PCR technique. Briefly, EDTA-treated blood sample were collected, and the total RNA was extracted using TRIZOL reagent (Invitrogen) according to manufacturer instruction. The extracted RNA then reversely transcribed into cDNA using Takara cDNA synthesis kit (Takara, Japan). Real time

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Table 1: Primer sets used for PD-1 expression analysis

	Forward primer			Revers primer			Product Length
	Sequence (5'->3')	GC%	length	Sequence (5'->3')	GC%	length	
PD-1	CCGCACGAGGGACAATA	58.8	17	TCTCTCGCCACTGGAAATC	52.6	19	114

PCR was performed using a StepOnePlus real time PCR system (Applied Biosystems) using RealQ Plus 2x Master Mix (Ampliqon) and designed primer listed in Table 1. The β-actin primer was used as the housekeeping control gene.

Statistical Analysis

The SPSS statistical software version 21 was used for all statistical analyses. Differences in characteristics among the genotypes and mRNA expression were compared using t-test for categorical data. Significant level was accepted at $P \leq 0.05$.

RESULTS

A total of 50 patients were recruited in this study, 25 BC patients and 25 healthy controls. The mean age of BC patients and the healthy controls were 2.75 ± 12.18 and 30.6 ± 2.6 respectively. No significant difference was observed in age distribution between the BC patients and control group.

PD-1 mRNA expression analysis

In order to establish the relationship between BC prevalence and PD-1, the PD-1 expression level was determined by Real time PCR. The level analysis of PD-1 showed higher expression in BC patients compared to healthy controls (Table 2). The independent t-test showed that the increased level of PD-1 mRNA was significant ($P < 0.05$).

Table 2: PD-1 expression analysis

Gene	Reaction efficiency	Expression	P-value
PD1	0.8	7.7	0.008

DISCUSSION

After cancers such as lung, stomach and liver cancers, breast cancer is one of the most common cancers in the female population of the world, which can be the most common cause of death in women. programmed cell death is called apoptosis, which is clinically important because failure to control and regulate the mechanism of apoptosis leads to overexpression of certain genes and pathogenesis and plays an important role in the development of many cancers[15]. PD1 protein is a member of the CD28 family of immunoglobulins and has two receptors, PDL1 and PDL2. This protein has an inhibitory role. Inhibitory function and inhibitory signals of PD1 protein through its receptors lead to activation of T cell function, tolerance, and tissue damage due to immunity[16]. There have also been many studies on PD1 gene polymorphisms and their role in breast cancer patients compared to healthy individuals, which have been introduced as prognosticators in the diagnosis and treatment of various cancers, including breast cancer. In addition, gene mutations have played a significant role in the development of breast cancer. Many studies have been conducted in this field. Eight studies (Chang, 2007,55 Cobliegh, 2005,47 Esteva, 2005,48 Gianni, 2005,49 Cronin, 2004,44 Habel, 2006,50 Mina, 2006,51 and Paik, 200428) have examined the expression of different genes in breast cancer compared to healthy individuals using different methods such as real-time PCR or immunohistochemistry[17–24]. Cronin and colleagues showed that there is a significant relationship between PDL1 gene expression and the progression of breast cancer. The group and their colleagues studied the expression of five genes in breast cancer. In a clinical study, Oratz R and colleagues also investigated gene expression variation and their association with a variety of cancers, including breast cancer[25]. They showed that in patients with breast cancer, the expression of PD1 was significantly increased. According to these studies, the expression of PD1 gene can play an effective role in the development and progression of breast cancer. Zhou and colleagues also showed a link between PD1 gene expression and the incidence of breast cancer[26]. They showed that the expression level of PD1 was higher in patients with breast cancer than in the control group. Therefore, gene expression can serve as a prognostic marker in screening and immunotherapy in patients with breast cancer.

CONCLUSION

The results of our study showed that the expression level of PD1 gene in patients with breast cancer was significantly higher compared to healthy individuals and it can be concluded that the expression of PD1 gene can be a prognostic factor in the early diagnosis of breast cancer. Therefore, it is suggested that more studies be performed on the relationship between the expression of more genes and breast cancer. In addition, according to the findings of the study have been obtained only from the people of Sistan-Baluchistan province, fertility studies in different ethnic groups are required.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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