Original Article
Role of MTHFR gene polymorphisms, serum tissue inhibitor of metalloproteinases-1, thymus chemokine-1 and thrombospondin-1 in endometrial cancer

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Abstract: The aim of this study was to evaluate the relationship between the MTHFR gene variants (677 C→T and 1298 A→C), serum tissue metalloproteinases inhibitor (TIMP-1), thrombospondin-1 (TSP-1), thymus chemokine-1 (TCK-1) levels and endometrial cancer. Sixty women were chosen from endometrial cancer patients and fifty-six women without any systemic disease were included as the control group. MTHFR C677T and A1298C MTHFR polymorphisms and their allele frequencies were evaluated with strip assay (Reverse hybridization method). Serum tissue inhibitor of metalloproteinases-1, thymus chemokine-1, and thrombospondin-1 levels were measured with the enzyme-linked ımmunosorbent assay (ELISA). Genotypic distribution and allelic frequencies of MTHFR C677T polymorphism were not associated with endometrial cancer (P>0.05). But, genotypic distribution and allelic frequencies of MTHFR A1298C polymorphism were strongly associated with endometrial cancer (P = 0.047 and P = 0.024). On the other hand, tissue inhibitor of metalloproteinases-1, thrombospondin-1, and thymus chemokine-1 levels were strongly associated with endometrial cancer (P = 0.001, P = 0.02, and P = 0.001 respectively). These results indicate that genotypic distribution and allelic frequencies of MTHFR A1298C polymorphism, tissue inhibitor of metalloproteinases-1, thrombospondin-1 and thymus chemokine-1 may be the prognostic markers in endometrial carcinoma.

Keywords: MTHFR C677T A1298C, gene polymorphisms, TIMP-1, TCK-1, TSP-1, endometrial cancer

Introduction

Methylenetetrahydrofolate reductase enzyme encoded by the MTHFR gene converts 5,10-methylene tetrahydrofolate to 5-methyltetrahydrofolate. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism. Human MTHFR gene is localized on chromosome 1 and encodes MTHFR enzyme consisting of 563 amino acids. Some mutations resulting in inactivation of enzyme in the MTHFR gene lead to hyperhomocysteinemia and homocystinuria which are significant risk factors for cerebrovascular and cardiovascular diseases. According to a large number of literature data, polymorphic variants of MTHFR gene are associated with hyperhomocysteinemia, vascular malformations, neural tube defects, dementia, perinatal mortality, mental disorders, neurodegenerative disorders, migraine and cancer. In the literature, there are studies evaluating the relationship between MTHFR gene polymorphisms and risk of endometrial cancer [1-4]. Endometrial cancer arises from cells in the inner lining of the uterus (endometrium) [5, 6]. There are some specific matrix metalloproteinases (MMPs) and their inhibitors identified and correlated with tumor development [7, 8]. In the regulation of MMP activation, TIMPs (Tissue Inhibitor of Metalloproteinases) play an important role under the in vivo conditions. TIMP-1 binds irreversible

as one to one ratio to the active form of matrix metalloproteinases (MMP) with noncovalent bonds and high affinity. TIMP-1 has an increasing effect on the red blood cell production, also it has directly cellular influence which is associated with binding to cell surface receptors and stimulating effect on replication in various cell types [9]. Another TIMPs was Thymus chemokine 1 (TCK-1) which is a chemoattractant for neutrophils and plays a role in their activation and chemokines altered in expression during specific disease states can serve as useful diagnostic or prognostic biomarkers [10, 11]. The last TIMPs was Thrombospondin 1 (TSP-1) which is an adhesive glycoprotein found in the alpha granules of platelets. It is known that as well as platelets TSP-1 is secreted by many cells. When TSP-1 is released depending on any stimulation, it causes aggregation of platelets. TSP-1 plays a role in the cell adhesion and growth. It helps tumor development by stimulating malignant cells' invasion to the surrounding tissue. Its effect on tumor growth and the endothelium is drawn attention as a significant tumor growth and metastatic factor [12, 13].

In the light of all this information, it was aimed to investigate the relationship between the MTHFR gene variants (677 C→T and 1298 A→C), TIMP-1, TSP-1, TCK-1 levels and disease activity in the patients with endometrial cancer in this study.

Materials and methods

This study was approved by the Ethical Committee of Cumhuriyet University with the approval number 2015-07/43. The research was planned between the dates 30.06.2014 and 30.12.2014 in the Obstetrics and Gynecology Department of Cumhuriyet University. Female individuals diagnosed with endometrial cancer and providing research criteria (60 people) were chosen as cancer group and the same number of female without any systemic disease were included in the control group. 4-5 cc of blood was collected into EDTA tubes from patients. DNA was extracted using DNA isolation kit (Invitek Invisorb Spin Blood Kit, Germany). Target genes were amplified in a biotin-labelled amplification reaction. Amplification conditions were organized as follows: pre-PCR, 2 min at 95°C; denaturation, 15 s at 95°C; annealing 30 s at 56°C; extension 30 s at 72°C, and final extension 3 min at 72°C. After PCR amplification, samples were analyzed for MTHFR C677T and MTHFR A1298C gene polymorphisms using reverse hybridization procedure (Vienna Lab, CVD Strip Assay, GMBH, Austria). The amplification products were hybridized to a strip which contains allele-specific oligonucleotide probes. Biotinylated sequences were determined via streptavidin-alkaline phosphatase and color substrates. Hybridization process was performed in an automated incubator (Auto-LIPA, Innogenetics).

MTHFR C677T and A1298C MTHFR polymorphisms and polymorphic alleles were evaluated on the obtained strips. The results were seen in **Tables 1** and **2**. TIMP-1, TCK-1 and TSP-1 levels were measured by using ELISA. The results were seen in **Table 3**. The chi-square (χ^2) and t-test were used for statistical analysis in SPSS 15.0 program (SPSS Inc., Chicago, IL, USA). The χ^2 test is a statistical tool used to examine differences between nominal or categorical variables. Thus, the χ^2 test was used to evaluate the frequencies of alleles and genotypes. The odds ratios were calculated at 95% confidence interval for the association between alleles and risk in the formation of endometrial cancer. Independent-Samples T-test is used to compare the means of the two samples of related data. Therefore, the t-test was used to compare TIMP-1, TCK-1 and TSP-1 concentrations between groups. p value <0.05 was considered statistically significant.

Results

Some possible markers for endometrial cancer were studied in this study. We examined the genotypic distribution and allelic frequencies of MTHFR C677T polymorphism in endometrial cancer patients and healthy individuals. It wasn't seen any statiscall differences (**Table 1**). In contrast to the MTHFR C677T polymorphism, statistically significant differences were seen between the patients and controls in terms of the genotypic distribution and allelic frequencies of MTHFR A1298C (**Table 2**). We also evaluated the levels of TIMP-1, TSP-1 and TCK-1 parameters with regard to the formation of endometrial cancer in this study. It was observed that there was a significant differ-

Table 1. Comparison of Genotypic Distribution and Allelic Frequencies of MTHFR C677T Polymorphism between Endometrial Cancer Patients and Healthy Individuals

Genotypic distribution and Allelic frequencies		Patient		Control		X^2	P
		N	%	N	%		
MTHFR C677T	CC	29	48.3	29	51.8	2.111	0.348
	CT	29	48.3	22	39.3		
	TT	2	3.4	5	8.9		
Allele	C	87	72.5	80	71.4	0.033	0.856
	T	33	27.5	32	28.6		

Odds Ratio: 1.055 (0.594-1.871). Genotypic distribution and Allelic frequencies of MTHFR C677T polymorphism in Endometrial cancer patients and healthy individuals are given in **Table 1** (X^2 = 2.111, P = 0.348, X^2 = 0.033, P = 0.856).

Table 2. Comparison of Genotypic Distribution and Allelic Frequencies of MTHFR A1298C Polymorphism between Endometrial Cancer Patients and Healthy Individuals

Genotypic distribution and Allelic frequencies		Patient		Control		X^2	P
		N	%	N	%		
MTHFR A1298C	AA	20	33.3	28	50	6.096	0.047
	AC	31	51.7	26	46.4		
	CC	9	15	2	3.6		
Allele	A	71	59.2	82	73.2	5.091	0.024
	C	49	40.8	30	26.8		

Odds Ratio: 0.530 (0.304-0.923). Genotypic distribution and Allelic frequencies of MTHFR A1298C polymorphism in Endometrial cancer patients and healthy individuals are given in **Table 2** (X^2 = 6.096, P = 0.047, X^2 = 5.091, P = 0.024).

Table 3. TIMP-1, TSP-1 and TCK-1 parameters in endometrial cancer patients and controls

		N	X	ss	sh_x	t	Sd	P
TIMP-1 (pg/ml)	Patient	60	0.96	0.23	0.03	-18.66	114.89	0.001
	Control	56	0.24	0.20	0.03			
TSP-1 (ng/ml)	Patient	60	0.16	0.10	0.01	2.34	83.41	0.021
	Control	56	0.19	0.05	0.01			
TCK-1 (pg/ml)	Patient	60	1.00	0.75	0.10	-7.79	68.54	0.001
	Control	56	0.23	0.22	0.03			

are in need of appropriate diagnostic facilities. As shown in **Tables 1** and **2**, some possible markers were studied in this study. When the genotypic distribution and allelic frequencies of MTHFR C677T polymorphism in endometrial cancer patients and healthy individuals were examined, it wasn't seen any statistical differences. Likewise, in a study, it was observed no significant association between the MTHFR polymorphisms and ovarian cancer risk [15]. The finding of that study is consistent with our results in relation to the formation of cancer. On the other hand, there were some studies on the association of MTHFR C677T allele with colorectal, endometrial and ovarian cancer [16-18]. Therefore, this study may provide new findings on this topic that genotypic distribution and allelic frequencies of MTHFR C677T polymorphism in endometrial cancer may not biologically relevant marker. The results for genotypic distribution and allelic frequencies of MTHFR A1298C polymorphism in endometrial cancer patients and healthy individuals are shown in **Table 2**. Although MTHFR C677T polymorphism was not statistically significant, there was an important difference between the patients and controls in terms of the genotypic distribution and allelic frequencies of MTHFR A1298C. These findings suggest that MTHFR A1298C polymorphism may play a critical role in relation to endometrial cancer risk. As a matter of fact, some studies have detected the association between the MTHFR polymorphisms and ovarian and cervical cancer risk [19, 20].

Matrix metalloproteinases play an important role in physiological states such as tumor cell invasion and metastasis. There are some fac-

ence between endometrial cancer patients and healthy controls in terms of the levels of TIMP- 1, TSP-1, and TCK-1 (**Table 3**).

Discussion

Endometrial carcinoma is the most common gynecologic malignancy. Understanding of the epidemiology, pathophysiology, and management strategies of this cancer helps the scientists to early diagnose and detect individuals at increased risk [14]. Therefore, the scientists

tors that inhibit MMPs. Among these, specific tissue inhibitor of metalloproteinases (TIMPs) have an essential role in the regulation of the activity of these enzymes. The balance between active metalloproteinase and its inhibitor has been remarked to play an essential role in tumor cell invasiveness [7, 8, 21].

The results gained evaluation of the levels of TIMP-1, TSP-1 and TCK-1 parameters with regard to the formation of endometrial cancer showed that there was significant difference between endometrial cancer patients and healthy controls in terms of the levels of TIMP-1, TSP-1 and TCK-1 (**Table 3**). These results may suggest that TIMP-1, TSP-1 and TCK-1 can be remarked as biomarkers in relation to the formation of endometrial cancer. As a matter of fact, some studies have detected the association between TIMPS and some cancers [22, 23]. Based on the current results, it can be thought that MTHFR A1298C polymorphisms, TIMP-1, TCK-1, TSP-1 can be useful as biomarkers in endometrial cancer. On the other hand, further laboratory trials with larger groups will be required to validate the usefulness of these markers and to get proper information regarding the role of MTHFR A1298C polymorphisms, TSP, TIMP-1 and TCK-1.

Disclosure of conflict of interest

None.

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