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Associations of Cytokines Genetic Polymorphisms with Development of Endometrial Hyperplasia.

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ABSTRACT

The article covers the results of comparison study of polymorphic variants frequency of cytokine genes in patients with endometrial hyperplasia and women in the control group. There were determined associations of genetic polymorphisms (*+36)A/G TNFR1*, *A/G I-TAC (rs4512021)*, *C/G MCP1 (rs2857657)*, *(+1931)A/T MIP1β*, *(-801)G/A SDF1* and their combinations with endometrial hyperplasia developing in the women of Russia Central Region.

Keywords: endometrial hyperplasia, cytokines, polymorphisms.

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INTRODUCTION

Endometrial hyperplasia is an abnormal diffuse or local proliferation of glandular and stromal constituents of endometrium with predominant affection of glandular structures. According to literature data, frequency of this abnormality is 15-50% among all the gynecological disorders [1].

Hormone-independent proliferation, inflammation, impaired apoptosis, and abnormal neoangiogenesis are of great importance in development of endometrial hyperplasia. Processes of cytokines interaction are of great concern in realization of these mechanisms [2]. In recent times, there are more and more data testifying that signal nucleotide polymorphism, by means of specific genes alleles, has a great importance in underlying risk for a series of disorders. As for endometrial hyperplasia, it was determined some genes which polymorphism is associated with high risk of disorder developing. In this regard, in the recent times, researchers pay careful attention to study of polymorphic markers of cytokine genes at endometrial hyperplasia [3].

MATERIALS AND METHODS

Investigation sampling comprised 502 women; 253 patients had endometrial hyperplasia, and 249 women were from the control group. The case and control group sampling were women of Russian nationality coming from Russia Central Region and not being relatives. Patients with endometrial hyperplasia were made hysteroscopy with the following biopsy of the lining of the uterus and scrape histologic examination.

In the group under study, it was performed typing of nine polymorphic markers of cytokine genes – tumor necrosis factor α ((*-308)G/A TNFα*), lymphotoxin α ((*+252)A/G Ltα*), tumor necrosis factor receptor of the 1st type ((*+36)A/G TNFR1*), tumor necrosis factor receptor of the 2nd type ((*+1663)G/A TNFR2*), factor regulating agitation of normal T-cell expression and secretion ((*-403)A/G RANTES*), interferon of inducible α-chemoattractive agent of T-cells (*A/G I-TAC (rs 4512021)*), macrofage inflammatory protein-1 ((*+1931)A/T MIP1β*), chemoattractive agent of monocyte-1 (*C/G MCP1 (rs 2857657)*), factor-1, produced by stromal cells ((*-801)G/A SDF1*).

As a material, one used black blood in volume of 8-9 ml taken from the median cubital vein of proband. Genomic DNA was extracted from peripheral blood by standard methods [4].

Estimation of all the locuses was performed by method of polymerase chain reaction of DNA synthesis using oligonucleotide primers and probes [5-7]. Genotyping of DNA markers was performed by method of TaqMan probes detection according to data of level value relative to fluorescence of each probe at «IQ5» amplificator with detecting system in real-time mode.

Alleles and genotypes distribution of the studied DNA-markers in the groups of women was valuated with the contingency tables 2x2 analyses, taking into account $\chi 2$ test and Yates correction for regularity and odd ration (OR) with 95% confidence intervals (CI).

Estimation of role of cytokine genetic variants combinations in contraction of endometrial hyperplasia is performed using the standard statistics methods [8].

RESULTS

There were examined 253 women with endometrial hyperplasia (average age 42.05±11.08 years, between 21and 72 years) and 249 women from the control group (average age 41.2±7.5 years, between 120 and 60 years old, $p>0.05$). Main characteristics of the studied groups are given in the Table 1. It was determined, that the control group is completely commeasurable with sampling of patients with endometrial hyperplasia by gender, age, nationality and place of birth, and by height and weight ($p>0.05$).

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Table 1: Characteristics of the subjects from the case and control groups.

Characteristics	Cases	Controls
Total	253	249
Age, yrs	42.05±11.08*	41.2±7.5
Weight, kg	64.8±1.9*	62.4±2.8
Height, cm	164.8±4.1*	167.5±3.7

Note: *p>0.05

Examination of alleles frequency of genes polymorphic markers under study showed (Table 2) that for almost all the examined locuses in the group of patients with endometrial hyperplasia and in population sampling, empiric genotype distribution corresponded to the expected one at Hardy-Weinberg equilibrium (p>0.05).

Table 2: Summary information about the studied polymorphisms.

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				χ^2	p
(-308)G/A TNFα (rs 1800629)	Case	*(-308) A TNFα*	11.66	2.44	>0.05
(-308)G/A TNFα (rs 1800629)	Control	*(-308) A TNFα*	10.08	0.13	>0.05
(+252)A/G Ltα (rs 909253)	Case	*(+252) G Ltα*	26.98	0.72	>0.05
(+252)A/G Ltα (rs 909253)	Control	*(+252) G Ltα*	25.10	0.75	>0.05
(+36)A/G TNFR1 (rs 767455)	Case	*(+36)A TNFR1*	48.35	14.48	<0.001
(+36)A/G TNFR1 (rs 767455)	Control	*(+36) A TNFR1*	49.80	1.04	>0.05
***(+**1663)A/G TNFR2 (rs 1061624)*	Case	***(+**1663)A TNFR2*	41.39	0.10	>0.05
***(+**1663)A/G TNFR2 (rs 1061624)*	Control	***(+**1663) A TNFR2*	37.29	0.20	>0.05
(-403)G/A RANTES (rs 2107538)	Case	*(-403) A RANTES*	19.64	0.04	>0.05
(-403)G/A RANTES (rs 2107538)	Control	*(-403) A RANTES*	16.53	0.02	>0.05
A/G I-TAC (rs 4512021)	Case	*A I-TAC*	40.85	0.08	>0.05
A/G I-TAC (rs 4512021)	Control	*A I-TAC*	44.21	0.007	>0.05
(+1931)A/T MIP1ϐ (rs 1719153)	Case	*(+1931)T MIP1ϐ*	25.51	0.001	>0.05
(+1931)A/T MIP1ϐ (rs 1719153)	Control	*(+1931)T MIP1ϐ*	27.53	0.008	>0.05
C/G MCP1 (rs 2857657)	Case	*G MCP1*	14.48	0.76	>0.05
C/G MCP1 (rs 2857657)	Control	*G MCP1*	17.27	0.49	>0.05
(-810) G/A SDF1 (rs 1801157)	Case	*(-810) A SDF1*	16.70	0.00	>0.05
(-810) G/A SDF1 (rs 1801157)	Control	*(-810) A SDF1*	18.50	1.13	>0.05

Notes: MAF, minor allele frequency; Hardy-Weinberg equilibrium. P values were calculated using the χ2 test.

It was determined that the risk factor of endometrial hyperplasia was genotype *(+36)AG TNFR1* (χ2=11.06, p=0.002, p_{cor}=0.006, OR=1.87, 95%Cl 1.28-2.73), and protective factor of endometrial hyperplasia is a genetic variant *(+36)AA TNFR1* (χ2=5.45, p=0.017, p_{cor} =0.051, OR=0.58, 95% Cl 0.37-0.92).

While using bio-informational approaches, there were determined combinations of cytokine genetic variants associated with high risk of endometrial hyperplasia: *(+36)AG TNFR1*, *A I-TAC* и *C MCP1* (OR=1.98); *(+36)AG TNFR1* and *C MCP1* (OR=1.94); *(+36)AG TNFR1* and *(+1931) A MIP1ϐ* (OR= 1.91); *+36AG TNFR1*, *A I-TAC* and *-801G SDF1* (OR= 1.91) (Table 3).

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Table 3: Concentration combinations of alleles/genotypes of cytokine genes in patients with endometrial hyperplasia and in the control group

SNP 1	SNP 2	SNP 3	Carriage		Fisher's p-value (Bonferroni correction, p_{cor})	Odds ratio (95% CI)
			Case	Control		
(+36) AG TNFR1	*A I-TAC*	*C MCP1*	51.06	34.45	0.0002 (0,002)	1.98 (1.37-2.87)
(+36) AG TNFR1	*C MCP1*		60.91	44.49	0.0002 (0,001)	1.94 (1.36-2.79)
(+36) AG TNFR1	*(+1931) A MIP1ϐ*		57.98	41.98	0.0003 (0,002)	1.91 (1.33-2.74)
(+36) AG TNFR1	*A I-TAC*	*(-801) G SDF1*	51.90	34.45	0.0003 (0,004)	1.91 (1.32-2.75)

Afterwards

As the result of comparative analyses of alleles and genotypes frequencies of polymorphic markers of cytokine genes among the endometrial hyperplasia subjects and the control group, it was determined that the risk factor of this disorder was a genotype (*+36)AG TNFR1* (OR=1.87), and protective factor of endometrial hyperplasia is a genetic variant (*+36)AA TNFR1* (OR=0.58). According to literature data, *TNFR1* can participate both in the apoptosis process and in inflammation reactions. These abnormal mechanisms are the basis for endometrial hyperplasia formation [9].

According to our obtained data, there were determined "risk" combinations of genetic variants connected with endometrial hyperplasia developing: (*+36)AG TNFR1*, *A I-TAC* and *C MCP1* (OR=1.98); (*+36)AG TNFR1* and *C MCP1* (OR=1.94); (*+36)AG TNFR1* and (*+1931) A MIP1ϐ* (OR= 1.91); (*+36)AG TNFR1, A I-TAC* and (*-801)G SDF1* (OR= 1.91).

Abnormal importance of these cytokines genes while endometrial hyperplasia formation, detected while our research, match the literature data by their medical and biological effects in the organism. Thus, *MCP-1* performs regulation of apoptosis through *Fas*/*FasL* system. *I-TAC*, being an important chemo attractive agent for T-lymphocytes and monocytes in the inflammatory tissue, assures production of anti-inflammatory cytokines. In a series of works dedicated to *SDF1*medical and biological effects, it was confirmed that is was angiogenesis stimulating factor in endometrium due to increase of *VEGF* synthesis (growth factor of vascular endothelium), and it also favors to cells surviving by apoptosis inactivation through *BCl-2* (apoptosis regulator *BCl-2*) [10]. Existence of connection of genetic polymorphic cytokines *(+36) A/G TNFR1, A/G I-TAC (rs4512021)*, (*+1931)A/T MIP1ϐ*, *C/G MCP1 (rs 2857657)*, (*-801)G/A SDF1* with endometrial hyperplasia developing may testify about their possible involvement to this abnormality pathogenesis.

CONCLUSIONS

Consequently, this work results let draw conclusion that molecular and genetic marker of high risk for endometrial hyperplasia is a genetic variant (*+36) AG TNFR1* (OR=1.87), and protective factor of endometrial hyperplasia is a genetic variant (*+36) AA TNFR1* (OR=0.58). Combinations of genetic variants (*+36) AG TNFR1* with alleles *A I-TAC* and *C MCP1* (OR=1.98), *C MCP1* (OR=1.94), (*+1931)A MIP1ϐ* (OR=1.91), *A I-TAC* and *(-801)G SDF1* (OR=1,91) are associated with endometrial hyperplasia developing.

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