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Study of Cytokines Polymorphous Loci Connections with Rise of Endometrium Proliferative Diseases.

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ABSTRACT

The article presents data on comparative analysis of frequency of cytokines genes polymorphous variants among patients with endometrium hyperplastic processes and the control group women. The authors found associations of genetic polymorphisms *-TAC c.*1539 T>C (rs4512021)*, (*-308)G/A TNFα*, (*+252)A/G Ltα,* (*+36)A/G TNFR1*, *IL-1α c.-949 C>T (rs1800587)* and their combinations with formation of endometrium hyperplastic processes among Russia Central region women.

Keywords: endometrium hyperplastic processes, cytokines genetic variants.

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INTRODUCTION

Endometrium hyperplastic process is a typical representative of endometrium proliferative diseases. This disease is a uterine lining benign pathology characterized by progress of clinicomorphological manifestations from simple and complex hyperplasia to endometrium atypical states [1, 2]. A leading role in endometrium hyperplastic processes pathogenesis is played by relative or absolute hyperestrogenism which conduces to proliferation of glandular and stromal components of uterine lining [3]. At the same time, after many biomolecular analyses on genetic markers determination it has been revealed that endometrium hyperplastic processes pathogenesis involves not only proliferation which depends on hormones but also that one which does not depend on hormones and is stimulated by growth factors and cytokines [4, 5].

In pursuance of the foregoing, in this work we studied the role of cytokines genetic variants (*-308)G/A TNFα*, (*+252)A/G Ltα*, (*+36)A/G TNFR1*, (*+1663)G/A TNFR2*, (*-403)A/G RANTES*, *A/G I-TAC (rs 4512021)*, (*+1931)A/T MIP1β*, *C/G MCP1 (rs 2857657)*, (*-801)G/A SDF1*, *IL-6 c.-237 C>G (rs1800795), IL-1β c.-598 T>C (rs16944), IL-1α c.-949 C>T (rs1800587), IL-4 c.-589 C>T (rs2243250), IL-10 c.-627 A>C (rs180082), IL-5 c. -746 T>C (rs2069812), IL-8 c.-352 A>T (rs4073)* in evolution of endometrium hyperplastic processes among Central Russia women.

MATERIALS AND METHODS

Analysis of cytokines polymorphous loci connections was performed with a sample of 650 women, 150 of whom were patients with endometrium hyperplastic processes and other 500 women were of the control group. The groups of patients and control included women of Russian ethnicity, natives of Central region of Russia, being not related to each other.

The examination of patients was carried out on the basis of the gynecology department of the perinatal centre of Belgorod region clinical hospital of Josaphat the Sanctifier. All patients with endometrium hyperplastic processes passed through hysteroscopy with subsequent biopsy of lining of uterus and histologic study of the scrape.

Among patients with endometrium hyperplastic processes and control group individuals we performed typing of single nucleotide polymorphisms of cytokines genes - (*-308)G/A TNFα*, (*+252)A/G Ltα*, (*+36)A/G TNFR1*, (*+1663)G/A TNFR2*, (*-403)A/G RANTES*, *A/G I-TAC (rs 4512021)*, (*+1931)A/T MIP1β*, *C/G MCP1 (rs 2857657)*, (*-801)G/A SDF1*, *IL-6 c.-237 C>G (rs1800795), IL-1β c.-598 T>C (rs16944), IL-1α c.-949 C>T (rs1800587), IL-4 c.-589 C>T (rs2243250), IL-10 c.-627 A>C (rs180082), IL-5 c. -746 T>C (rs2069812), IL-8 c.-352 A>T (rs4073).*

As the research material we used venous blood – 8-9 ml from a proband's median cubital vein. Extraction of genomic DNA from peripheral blood was performed with the help of standard methods [6].

Analysis of all loci was carried out with the help of the method of DNA synthesis polymerase chain reaction using oligonucleotide primers and probes [7]. DNA-markers genotyping was performed with the help of the method of detection of TaqMan probes according to data on value of level of relative fluorescence of each probe, using the amplifier "IQ5" with real-time detection system. And also with the help of the method of analysis of restriction fragment length polymorphism (RFLP) of products of PCR amplification of specific genome sections, using corresponding restriction ferments.

For analysis of the observed genotypes distribution's correspondence to the expected one, premised on Hardy-Weinberg equilibrium, we used the criterion χ^2. Associations of alleles and genotypes of the studied DNA-markers with leiomyoma evolution were estimated with the help of analysis of cross tables 2x2 calculating the criterion $\chi 2$ with Yates correction for continuity and odds ratio (OR) with 95% confidence intervals (CI). Study of the role of interleukins genetic variants combinations in leiomyoma evolution was performed via software APSampler which uses Monte Carlo method in Markovian chains and also Bayesian nonparametric statistics. For multiple comparisons with the purpose of minimization of type I errors we used Bonferroni correction (p_{cor}) and permutational test (p_{perm}) [8].

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RESULTS

As a result of the research it has been found that the group of patients with endometrium hyperplastic processes and the control group are completely equatable regarding sex, age, ethnicity and birth place, and also height and weight (p>0.05).

Analysis of frequencies of the studied genes polymorphous markers alleles has shown that in case of all studied loci in the group of patients with endometrium hyperplastic processes and in the population sampling empirical distribution of genotypes corresponds to the theoretically expected one under Hardy-Weonberg equilibrium (p>0.05) (table 1).

Table 1: Summary information about the studied polymorphisms.

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				χ^2	p
(-308)G/A TNFα (rs 1800629)	Case	*A*	11.48	2.45	>0.05
(-308)G/A TNFα (rs 1800629)	Control	*A*	10.08	0.15	>0.05
(+252)A/G Ltα (rs 909253)	Case	*G*	25.73	0.73	>0.05
(+252)A/G Ltα (rs 909253)	Control	*G*	25.10	0.76	>0.05
(+36)A/G TNFR1 (rs 767455)	Case	*A*	48.45	1.05	>0.05
(+36)A/G TNFR1 (rs 767455)	Control	*A*	49.80	1.03	>0.05
***(+**1663)A/G TNFR2 (rs 1061624)*	Case	*A*	42.40	0.11	>0.05
***(+**1663)A/G TNFR2 (rs 1061624)*	Control	*A*	37.29	0.21	>0.05
IL-1α c.-949 C>T (rs1800587)	Case	*T*	31.85	0.45	>0.05
IL-1α c.-949 C>T (rs1800587)	Control	*T*	20.40	1.31	>0.05
IL-1β c.-598 T>C (rs16944)	Case	*T*	37.88	0.52	>0.05
IL-1β c.-598 T>C (rs16944)	Control	*T*	34.58	0.25	>0.05
IL-4 c.-589 C>T (rs2243250)	Case	*T*	20.77	0.91	>0.05
IL-4 c.-589 C>T (rs2243250)	Control	*T*	18.87	0.19	>0.05
IL-5 c.-746 T>C (rs2069812)	Case	*T*	29.92	4.81	>0.05
IL-5 c.-746 T>C (rs2069812)	Control	*T*	27.59	0,004	>0.05
IL-6 c.-237 C>G (rs1800795)	Case	*C*	46.45	0.78	>0.05
IL-6 c.-237 C>G (rs1800795)	Control	*C*	44.63	0.01	>0.05
IL-10 c.-627 A>C (rs180082)	Case	*A*	23.50	1.61	>0.05
IL-10 c.-627 A>C (rs180082)	Control	*A*	24.44	1.42	>0.05
IL-8 c.-352 A>T (rs4073)	Case	*A*	46.77	0.10	>0.05
IL-8 c.-352 A>T (rs4073)	Control	*A*	48.76	0.94	>0.05
RANTES c.- 471 G>A (rs 2107538)	Case	*A*	15.13	0.83	>0.05
RANTES c.- 471 G>A (rs 2107538)	Control	*A*	17.95	0.17	>0.05
*I-TAC c.*1539 T>C (rs4512021)*	Case	*C*	37.50	0.40	>0.05
*I-TAC c.*1539 T>C (rs4512021)*	Control	*C*	44.15	0.07	>0.05
*MIP-1β c.*524 A>T (rs1719153)*	Case	*T*	27.92	0.07	>0.05
*MIP-1β c.*524 A>T (rs1719153)*	Control	*T*	27.27	1.44	>0.05
MCP-1 c.77-109 G>C (rs2857657)	Case	*G*	12.82	0.004	>0.05
MCP-1 c.77-109 G>C (rs2857657)	Control	*G*	16.87	0.93	>0.05
*SDF-1 c.*519 G>A (rs1801157)*	Case	*A*	18.03	0.02	>0.05
*SDF-1 c.*519 G>A (rs1801157)*	Control	*A*	17.02	0.01	>0.05

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

It was revealed that frequency of the allele *A I-TAC (rs4512021)* among the patients with endometrium hyperplastic processes (62.50%) was the maximum in comparison with the same parameter of the control group (55.85%) (χ^2=3.83, p=0.05, OR=1.32, 95%CI 1.00 – 1.74).

In the course of studying of the cytokines molecular genetic markers combinations role it has been found that the combination of genetic variants *G TNFα, A TNFR1, A I-TAC, T IL-1α* (41.86%) occurs 2 times more often, *A TNFR1, A I-TAC, T IL-1α* (42.64%) 1.9 times more often, *A TNFα, G Ltα, T IL-1α* (14.81%) 4.5 times more often than in the control group (20.92%, p_{bonf}=0.01, p_{perm}=0.0000004; 22.25%, p_{bonf}=0.02, p_{perm}=0.0000007 and 3.28%, p_{bonf}=0.02, p_{perm}=0.000008, correspondingly). These combinations of cytokines genetic variants increase the risk of endometrium hyperplastic processes progress (OR=2.59 – 5.12).

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CONCLUSION

In the course of analysis of cytokines polymorphous variants role it has been found that allele *A I-TAC (rs4512021)* is associated with endometrium hyperplastic processes formation (OR=1.32). As shown in the literature, *I-TAC* has a positive proliferative effect on endometrium stromal cells, and that is fundamental for endometrium hyperplastic processes formation [9]. With the help of bioinformation approaches we revealed the following "risky" combinations of genetic variants connected with endometrium hyperplastic processes progress: *G TNFα, A TNFR1, A I-TAC, T IL-1α* (OR=2.72); *A TNFR1, A I-TAC, T IL-1α* (OR=2.59); *A TNFα, G Ltα, T IL-1α* (OR= 5.12).

Obtained data speak for an important etiopathogenetic role of these cytokines in endometrium hyperplastic processes progress. For instance, as shown in the literature, *TNFα* and *TNFR1* are main apoptosis mediators, and thus they can take part in endometrium hyperplastic processes pathogenesis [10]. According to the results obtained by us, the allele *T IL-1α* forms a part of all cytokines genetic variants combinations associated with a high risk of endometrium hyperplastic processes progress. As shown in the literature, polymorphism *IL-1α c.-949 C>T (rs1800587)* lies in the gene coding sequence and can influence the protein expression level. Individuals who are homozygous regarding allele *T IL-1A* have plasmis levels *IL-1A* increased in comparison with carriers of other genotypes. It is known that some tumour cells are able to product *IL-1A* and because of that we observe failure of antitumour response realization, and eventually this can lead to a more serious disease state.

RESUME

Thus, it has been found that allele *A I-TAC (rs4512021)* is associated with endometrium hyperplastic processes evolution (OR=1.32). Combinations of alleles *G TNFα, A TNFR1, A I-TAC, T IL-1α* (OR=2.72); *A TNFR1, A I-TAC, T IL-1α* (OR=2.59); *A TNFα, G Ltα, T IL-1α* (OR= 5.12) are factors of risk of endometrium hyperplastic processes progress.

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