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Analysis of Involvement of Cytokine Genetic Polymorphisms in Development of Genital Endometriosis.

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ABSTRACT

The article outlines the role of cytokines in development of genital endometriosis. There were detected associations between genetic polymorphisms *IL-6 c.-237 C>G (rs1800795)*, *SDF-1 c.*519 G>A (rs1801157)*, *MIP-1в c.*524 A>T (rs1719153)*, *IL-10 c.-627 A>C (rs180082*), *RANTES c.- 471 G>A (rs 2107538), IL-1в c.-598 T>C (rs16944)* and *I-TAC c.*1539 T>C (rs4512021)* and their combinations and development of genital endometriosis among women in the Central region of Russia.

Keywords: genital endometriosis, cytokines, polymorphism, genetic variations.

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INTRODUCTION

Genital endometriosis is a pathologic process which is characterized by benign growth of tissue beyond the uterine cavity which by its morphological and functional properties is similar to endometrium [1]. In the structure of gynecological incidence genital endometriosis holds the third place after inflammatory diseases and uterine leiomyoma.

The most contemporary theories state that endometrial stroma and glands formation is being influenced by genetic, paracrine and immunologic factors [2]. In this connection a considerable number of works are dedicated to study of genetic principles of endometriosis development [3, 4]. Nevertheless it is worth mentioning that the results obtained by different groups of investigators are contradictory and do not give an unambiguous answer to the question about the role of genetic factors in pathogenesis and clinical features of genital endometriosis. In view of the above this work studies the role of cytokine genetic polymorphisms *IL-6 c.-237 C>G (rs1800795), IL-1в c.-598 T>C (rs16944), IL-1б 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), SDF-1 c.*519 G>A (rs1801157)*, *IL-8 c.-352 A>T (rs4073), MIP-1в c.*524 A>T (rs1719153)*, *RANTES c.- 471 G>A (rs 2107538), MCP-1 c.77-109 G>C (rs2857657)* and *I-TAC c.*1539 T>C (rs4512021)* in development of genital endometriosis.

MATERIALS AND METHODS

There was performed analysis of the observation data for 604 persons: 104 patients with genital endometriosis and 500 females from the reference panel. The patients and reference panels included Russian women, natives of the Central region of Russia and not having family ties among themselves. Clinical laboratory examination of the patients was performed at the gynecology department of the perinatal center of the Bishop Ioasaf Belgorod Regional Clinical Hospital. The patients with genital endometriosis were subject to pelvic organs ultrasonography, hysteroscopy with the subsequent directional biopsy of the lining of the uterus and histologic examination of the scrape, the procedures were performed by means of the common and laboratory methods of examination.

All of the patients with genital endometriosis and individuals from the reference panel were subject to typing of single nucleotide polymorphisms of cytokine genes - *IL-6 c.-237 C>G (rs1800795), IL-1в c.-598 T>C (rs16944), IL-1б 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) SDF-1 c.*519 G>A (rs1801157)*, *IL-8 c.-352 A>T (rs4073), MIP-1в c.*524 A>T (rs1719153)*, *RANTES c.- 471 G>A (rs 2107538), MCP-1 c.77-109 G>C (rs2857657)* and *I-TAC c.*1539 T>C (rs4512021)*.

Venous blood samples with the volume of 8-9 ml drawn from the ulnar vein of the proband were used as a test material. Genomic DNA extraction from peripheral blood was performed by the standard method of phenol-chloroform extraction from frozen venous blood samples. [5]. Analysis of the examined loci was carried out by the method of polymerase chain reaction of DNA synthesis with use of oligonucleotide primers and probes [6-8]. Associations of alleles and genotypes of the studied DNA-markers with development of genital endometriosis were assessed by means of analysis of 2x2 contingency tables which included calculation of χ^2 test with Yates' correction for continuity and odds ratio (OR) with 95% confidence interval. Analysis of the role of combination of cytokine genetic variations in genital endometriosis onset was carried out with the help of APSampler software [9].

RESULTS

The analysis of allelic frequencies of the examined polymorphic markers of genes revealed that for all of the studied loci in the panel with the patients having genital endometriosis and in the sampling population the observable distribution of genotypes complied with theoretical expectations at Hardy–Weinberg equilibrium ($p>0.05$) (Table 1).

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Table 1: Summary information about the studied polymorphisms.

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				χ^2	p
IL-1б c.-949 C>T (rs1800587)	Case	*T*	21.50	0.67	>0.05
IL-1б c.-949 C>T (rs1800587)	Control	*T*	20.40	1.31	>0.05
IL-1в c.-598 T>C (rs16944)	Case	*T*	32.50	0.004	>0.05
IL-1в c.-598 T>C (rs16944)	Control	*T*	34.58	0.25	>0.05
IL-4 c.-589 C>T (rs2243250)	Case	*T*	21.05	0.56	>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*	25.34	0.007	>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*	47.06	1.51	>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*	27.38	3.27	>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*	46.00	2.39	>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*	16.16	0.19	>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*	42.35	0.06	>0.05
*I-TAC c.*1539 T>C (rs4512021)*	Control	*C*	44.15	0.07	>0.05
*MIP-1в c.*524 A>T (rs1719153)*	Case	*T*	27.92	0.32	>0.05
*MIP-1в c.*524 A>T (rs1719153)*	Control	*T*	27.27	1.44	>0.05
MCP-1 c.77-109 G>C (rs2857657)	Case	*G*	18.04	1.60	>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*	23.23	1.74	>0.05
*SDF-1 c.*519 G>A (rs1801157)*	Control	*A*	17.02	0.01	>0.05

Notes: MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium. P values were calculated using the χ^2 test.

It was ascertained that the patients with genital endometriosis demonstrated high frequency of allele *A SDF-1* (23.23%) as compared to the reference group (17.02%, чl=3.86, p=0.05, OR=1.47, 95% CI 1.00 – 2.17).

Bioinformatic approaches allowed to reveal that the following combinations of genetic variations of cytokines are involved in development of genital endometriosis: *C IL-6, A SDF-1, A MIP-1в* and *C IL-10* (OR=5.02); *A SDF-1, G RANTES, A MIP-1в* фтв *C IL-10* (OR=4.00); *A SDF-1, G RANTES, C IL-1в, C IL-10* (OR=3.55); *A SDF-1, A MIP-1в, C IL-10* (OR=3.32), *C IL-6, A SDF1, G RANTES, C IL-10* (OR=3.70), *A SDF-1, A I-TAC, CC IL-1в* (OR=3.76) (Table 2).

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Table 2: Concentration combinations of alleles/genotypes of cytokine genes in patients with genital endometriosis and in the reference panel

SNP 1	SNP 2	SNP 3	SNP 4	Carriage		Fisher's p-value (Bonferroni correction, p_{cor}) Permutation test, p_{perm}	Odds ratio (95% CI)
				Case	Control		
C IL-6	*A SDF1*	*A MIP1в*	*C IL-10*	40.00	11.72	0.0000008 (0.0073) 0.0000003	5.02 (2.52-10.01)
A SDF1	*G RANTES*	*A MIP1в*	*C IL-10*	49.18	19.44	0.000001 (0.01) 0.000008	4.00 (2.15-7.47)
A SDF1	*G RANTES*	*C IL-1в*	*C IL-10*	41.77	16.77	0.000004 (0.03) 0.0001	3.55 (1.92-6.57)
A SDF1	*A MIP1в*	*C IL-10*		48.38	22.00	0.000005 (0.04) 0.0001	3.32 (1.85-5.94)
C IL-6	*A SDF1*	*G RANTES*	*C IL-10*	37.31	13.84	0.000006 (0.05) 0.0004	3.70 (1.95-7.02)
A SDF1	*A I-TAC*	*CC IL-1в*		24.73	8.03	0.000008 (0.05) 0.0005	3.76 (1.95-7.25)

CONCLUSION

The work contains evidences that allele *A SDF-1* is associated with development of genital endometriosis (OR=1.47). It ought to be noted that allele *A SDF-1* is comprised in all combinations of genetic variations of cytokines associated with elevated risk of genital endometriosis development. According to the literature sources *SDF1* functions as a stimulator of angiogenesis due to increased synthesis of *VEGF* (vascular endothelial growth factor), and simultaneously encourages cells survival through apoptosis inactivation by means of apoptosis regulator *BCl-2*, which forms the basis of genital endometriosis pathogenesis. Other chemokines (*RANTES, MIP-1в* and *I-TAC*) according to literature evidences [10] are involved in the inflammatory reactions processes connected with genital endometriosis. In such situation the content of proinflammatory chemokines in peritoneal fluid grows significantly thus counteracting the opportunity to attract immunocompetent and other cells which detect inflammatory reaction to the focus of heterotopias formation and development.

Nosotropic significance of genetic polymorphisms *IL-6 c.-237 C>G (rs1800795)*, *IL-1в c.-598 T>C (rs16944)* and *IL-10 c.-627 A>C (rs180082*) for genital endometriosis formation discovered by our investigation complies with literature evidence stating their medical and biological effects. Thus in case of endometriosis the concentration of *IL-1в* and *IL-6* (which are mainly produced by macrophages) in peritoneal fluid increases. In its turn *IL-1в* and *IL-6* affect the processes of differentiation and growth of cells inclusive of ovarian and endometrial cells. The literature sources state positive association of polymorphism *IL-10 c.-627 A>C (rs180082*) with genital endometriosis [11]. The authors mention that presence of allele C of polymorphism *IL-10 c.-627 A>C (rs180082*) in genotype may account for increase of production of anti-inflammatory IL-10 which is accompanied by suppression of T-lymphocytes proliferation and reduction of level of cytokines of Th1 response. This promotes growth and invasion of new endometrioid heterotopias.

FINDINGS

Therefore the results of work allow making a conclusion that allele *A SDF-1* is associated with genital endometriosis development. Combinations of genetic variations *C IL-6, A SDF-1, A MIP-1в* and *C IL-10* (OR=5.02); *A SDF-1, G RANTES, A MIP-1в* and *C IL-10* (OR=4.00); *A SDF-1, G RANTES, C IL-1в, C IL-10* (OR=3.55); *A SDF-1, A MIP-1в, C IL-10* (OR=3.32), *C IL-6, A SDF1, G RANTES, C IL-10* (OR=3.70), *A SDF-1, A I-TAC, CC IL-1в* (OR=3.76) are risk factors for genital endometriosis development.

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