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Associations of Cytokines Genetic Variants with Myomatous Knots Sizes.

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ABSTRACT

The article presents study of cytokines molecular genetic markers' impact on nature of uterus affection with myomatous knots. The authors found associations of genetic polymorphisms - *IL-5 (rs2069812), IL-1α (rs1800587), IL-4 (rs2243250)* with uterus size and relation between polymorphous loci *IL-5 (rs2069812)* and *SDF-1 (rs1801157)* and size of myomatous knots of persons affected by uterine leiomyoma. Also it was found that a candidate gene *MIP-1β (rs1719153)* is involved into buildup of subserous myomatous knots sizes.
Keywords: leiomyoma, myomatous knots, cytokines, polymorphism.

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INTRODUCTION

Leiomyoma is the most widespread tumour among all women's genital zone diseases. It is a benign tumour of monoclonal origin emerging in myometrium. According to present-day ideas, leiomyoma is a monoclonal hormone-sensitive proliferating tissue consisting of phenotypically changed smooth muscle cells [1]. Some researchers believe that the main role in leiomyoma growth belongs to genetic factors [2, 3, 4]. According to data found in the literature leiomyoma growth may be connected with polymorphous variants of specific causalgic genes initiating myomatous knots growth [5].

To that end, the aim of this work was to study associations of cytokines genetic variants with myomatous knots sizes.

MATERIALS AND METHODS

The research group consisted of 394 patients with leiomyoma. The sample of the diseased included women of Russian ethnicity, native of Central region of Russia, who did not related to each other. Patients with leiomyoma passed through an ultrasonic examination of pelvic organs, hysteroscopy with a subsequent target biopsy of lining of uterus and a histologic study of the scrape; we used general clinical and laboratory methods of research.

The group under research passed through the genotyping of nine polymorphous markers of cytokines genes: *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)*.

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]. Molecular genetic 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, 8]. 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 (*IL-4 c.-589 C>T (rs2243250), IL-6 c.-237 C>G (rs1800795))*. 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 (*IL-1α c.-949 C>T (rs1800587), IL-1β c.-598 T>C (rs16944), IL-5 c.-746 T>C (rs2069812), IL-10 c.-627 A>C (rs180082*)).

During studying of relations between molecular genetic markers and myomatous knots nature we used nonparametric statistics methods.

RESULTS

It was found that patients with the genotype *CC IL-5* had the biggest median of uterus volume, which amounted to 235.80 cm^3 (lower quartile – 127.49 cm^3, upper quartile – 363.26 cm^3), whereas patients with the genetic variant *T IL-5* (genotypes *CT* and *TT IL-5*) had the least value – 186.31 cm^3 (interquartile range 128.49 – 353.02 cm^3, p=0.04). Also, at the same time, women with leiomyoma having the genotype *CC IL-5* were found to have the biggest volume of myomatous knots (Me=65.29 cm^3, lower quartile – 14.31 cm^3, upper quartile 142.58 cm^3) in comparison with patients with genotypes *CT* and *TT* (Me=48.09 cm^3, interquartile range 26.49 – 137.17 cm^3, p=0.006).

Genetic variant *GG SDF-1* is also associated with the maximum value of myomatous knots: women with this genotype have myomatous knots with volume Me=56.76 cm^3 (interquartile range 16.78 – 133.27 cm^3), whereas this value in case of patients with genotypes *AG* and *AA SDF-1* equals to Me=55.30 cm^3 (lower quartile 24.86 cm^3, upper quartile 139.22 cm^3, p=0.02).

It was revealed that patients with genotypes *CT* and *TT IL-1α*, and also genotypes *CT* and *TT IL-4* had the biggest volume of uterus – 211.64 cm^3 (interquartile range 151.15 – 392.39 cm^3) and 245.25 cm^3 (interquartile range 170.05 – 365,02 cm^3) correspondingly, and that differs to a statistically significant degree (p=0.04) from similar data of patients with genotype *CC IL-1α* (Me=188.94 cm^3, lower quartile 119.68 cm^3,

upper quartile 328.80 cm^3) and genotype *CC IL-4* (Me=194.63 cm^3, lower quartile 106.29 cm^3, upper quartile 339.94 cm^3) correspondingly.

We found associations of genotypical variant *AA MIP-1β* with the biggest subserous myomatous knots. Patients with this genotype had myomatous knots with volume 60.14 cm^3 (interquartile range 27.08 – 79.76 cm^3, p=0.01), and that significantly exceeds analagous value of patients with genotype *AT* and *TT MIP-1β* (26.69 cm^3, lower quartile 16.45 cm^3, upper quartile 54.49 cm^3, p=0.01).

CONCLUSION

Data obtained during the work speak for a important etiopathogenetic role of genetic polymorphisms *IL-5 (rs2069812), IL-1α (rs1800587), IL-4 (rs2243250), MIP-1β (rs1719153), SDF-1 (rs1801157)* in the character of myomatous knots affection of uterus. As shown in the literature, among interleukins *IL-1α, IL-4, IL-5* play an important role in leiomyoma growth. These interleukins are characterized by many biological effects and function both systemwide and locally. For instance, *IL-1α* is a polyfunctional cytokine and has quite a lot of different functions, targets of which are cells of practically all organs and tissues. It participates in development of a local inflammatory response, an acute phase response during an infectious disease. Involvement of polymorphous variants of *IL-1α* in growth of the biggest myomatous knots may be explained by its participation in processes of proinflammatory cytokines activation (including *IL-4* and *IL-5*). The following biomedical mechanisms may form the basis of the revealed interrelations between *IL-4* and myomatous knots growth. As shown in the literature, interleukin 4 is a a strong growth and differentiating factor for B-lymphocytes, it controls formation of other cytokines by participating in the immune response and inflammatory reactions. At the same time, we know about ability of *IL-4* to take part in mechanisms of cells defending against apoptosis. It is supposed that this can be a direct antiproliferative action caused by cell cycle blocking, or this can be caused by its ability to decrease expression of some cytokines [9]. Association of polymorphous variants of *IL-5* may be caused by this interleukin's involvement in the induction of acute phase proteins synthesis, and hence it can be classified as an inflammation cytokine. As shown in the literature, during leiomyoma an increasing concentration of *MIP-1β* in myomatous knots tissues is registrated. Whereas *SDF1* is a stimulator of angiogenesis in the myometrium, and also contributes to cells survival via apoptosis inactivation [10]. These data speak for an important etiopathogenetic role of genetic polymorphisms *IL-5 (rs2069812), IL-1α (rs1800587), IL-4 (rs2243250), MIP-1β (rs1719153), SDF-1 (rs1801157)* in the character of myomatous knots affection of uterus.

RESUME

Thus, obtained data speak for a significant role of genetic polymorphisms *IL-5 (rs2069812), IL-1α (rs1800587), IL-4 (rs2243250), MIP-1β (rs1719153), SDF-1 (rs1801157)* in the character of myomatous knots affection of uterus. Genetic variants *CC IL-5*, *CT* and *TT IL-1A, CT* and *TT IL-4, AA MIP-1β, GG SDF-1* are associated with the biggest uteri and the biggest myomatous knots of patients with leiomyoma.

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