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RELATIONSHIP BETWEEN mRNA IL1β AND TYPE, OF INFERTILITY IN WOMEN WITH ENDOMETRIOSIS ON THE STAGE PREPARING TO ASSISTED REPRODUCTIVE TECHNOLOGIES

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Анотація. *Ендометріоз часто призводить до безпліддя, що є основною скаргою у жінок репродуктивного віку (35-50%). Незважаючи на значні досягнення, багато проблем залишаються відкритими й сьогодні, і що також дуже важливо, складність виявлення цієї патології на ранніх стадіях, коли лікування може найбільше допомогти. Мета дослідження – дослідити зв'язок між рівнем експресії мРНК гена IL1в та типом безпліддя, а також вивчити рівні експресії мРНК гена IL1в у жінок з ендометріозом, пов'язаним з безпліддям. Для аналізу експресії мРНК гена IL1в та визначення відносної нормалізованої експресії мРНК IL1в використовували метод полімеразної ланцюгової реакції зворотної транскрипції в реальному часі (ЗТ-ПЛР). Досліджувану групу склали 30 жінок з безпліддям, які проходили допоміжні репродуктивні технології. Основну групу склали 20 жінок з діагнозом ендометріозу, які проходили допоміжні репродуктивні технології. Контрольну групу склали 10 здорових жінок. В основній групі рівень експресії мРНК гена IL1в до підготовки становив 26,7877±0,01, що було значно вищим за рівень після підготовки (0,1610±0,01*). Проаналізувавши результати, було виявлено, що середній рівень мРНК IL1в у жінок з ендометріозом, асоційованим безпліддям 1-го ступеня, становить 8,53 у.о., водночас середній рівень мРНК IL1в у жінок з ендометріозом, асоційованим із безпліддям 2-го ступеня, становить 1,0 у.о. Включення пробіотиків до комплексної підготовки до допоміжних репродуктивних технологій призводить до помітного покращення самопочуття пацієнтів.*

Ключові слова: *ендометріоз, пробіотики, допоміжні репродуктивні технології, безпліддя, IL1в.*

Abstract. *Endometriosis often leads to infertility, which is the main complaint in women of reproductive age (35-50%). Despite significant achievements, many problems still remain open today, and what is also very important, the difficulty of detecting this pathology in the early stages, when treatment can help the most. The aim of the study is to investigate the relationship between the level of IL1β mRNA gene expression and the type of infertility and to study the levels of IL1β mRNA gene expression in women with endometriosis associated with infertility. For mRNA gene expression analysis IL1β and determination of relative normalized mRNA expression IL1β used the real-time reverse transcription polymerase chain reaction method (RT-PCR). Examined group consists of 30 infertile women undergoing assisted reproductive technologies. The main group consisted of 20 women diagnosed with endometriosis undergoing assisted reproductive technologies. The control group consisted of 10 healthy women. In the main group, the level of IL1β mRNA gene expression before preparing was 26.7877±0.01, which was significantly higher than the level after preparing (0.1610±0.01). Analized results, it's found out that Mean level of mRNA IL1β in women with endometriosis associated infertility 1-st degree is 8.53 c.u., at the same time Mean level of mRNA IL1β in women with endometriosis associated infertility 2-nd degree is 1.0 c.u. The inclusion of probiotics in a comprehensive regimen of preparation for assisted reproductive technologies leads to a noticeable improvement in the patients well-being.*

Key words: *endometriosis, probiotics, assisted reproductive technologies, infertility, IL1β.*

Connection of the publication with planned research works.

The conducted scientific research is part of the complex research work of the Department of Obstetrics and Gynecology of the Bukovinian State Medical University within the framework of the scientific topic according to the plan of the Ministry of Health of Ukraine: "Preservation and restoration of the reproductive health of women and girls with obstetric and gynecological pathology". State registration number 0121U110020.

Introduction.

Endometriosis is a chronic inflammatory condition characterized by the growth of tissue similar to the lining of the uterus outside the uterus, usually in areas such as the peritoneum, ovaries, and cervix. Clinical symptoms often include progressive dysmenorrhea, chronic pelvic pain, profound dyspareunia, and infertility, which significantly affect the patient's quality of life [1]. It is estimated that approximately 10% of women of reproductive age suffer from endometriosis [2].

Although the exact cause and development of endometriosis remain unclear, the theory of retrograde menstruation proposed by Sampson in 1921 is widely accepted. Other hypotheses, such as coelomic metaplasia and vascular/lymphatic metastasis [3], have also been proposed, but cannot fully explain all forms of the condition. In addition, factors such as the immune system, hormones, genetics, and the environment are believed to play an important role in the pathogenesis of endometriosis [4].

There is evidence that some of the inflammatory factors also stimulate the growth of ectopic endometrial cells in the early stages of endometriosis [5]. These

compounds can also affect fertility, as well as nociceptors, thus causing infertility and pain. Cytokines are regulatory peptides or glycoproteins that can be produced by virtually every type of nucleated cell in the body and have pleiotropic regulatory effects on many cell types. Unlike hormones, cytokines usually act as paracrine and/or autocrine signals, only occasionally entering the circulation, where they can act as endocrine mediators [5-7]. Macrophages are among the major producers of cytokines, especially interleukins-1 and 6 (IL-1, IL-6) and tumor necrosis factor-α (TNFα); this is probably not the case under normal conditions, but after stimulation by various substances [8]. Interleukins are considered modulators of cell proliferation and as inducers of other cytokines, as a cascade in acute inflammation [9]

Cytokines produced in the uterine environment are involved in the regulation of endometrial growth through steroid-cell and cell-cell interactions [10]. Cytokines may also contribute to the pathophysiology of endometriosis in at least two ways, namely by enhancing the establishment and proliferation of ectopic endometrial implants and by influencing cytokine secretion by macrophages, which can lead to adverse changes. The cytokines IL-1β, IL-6 and TNFα are of great interest because they are partly hormonally regulated and play important roles as mediators of inflammation. IL-1 is involved in the regulation of the immune response and inflammation. There are two different forms of IL-1, α and β, with similar biological activities [11].

IL-1α is present in the endometrium, in both epithelial and stromal cells, at least in the late secretory phase. IL-1β has a similar distribution, usually appearing in lower amounts. IL-1β mRNA is expressed in the endometrium in the late secretory phase and corresponds to serum IL-1β levels, which vary throughout the cycle with maximum values during the secretory phase [12].

The aim of the study.

To study mRNA gene expression level IL1β and estimated correlation between IL1 mRNA β and type, duration of infertility in women with endometriosis on the stage of preparing for assisted reproductive technologies using probiotics

Object and research methods.

For gene expression analysis IL1β mRNA and determination of relative normalized mRNA expression IL1β used the real-time reverse transcription polymerase chain reaction method(RT-PCR).The object for molecular genetic studies using RT-PCR was a fraction of mononuclear cells isolated from whole blood of patients with endometriosis. In this study, we conducted a retrospective analysis of case histories of 30 infertile women undergoing assisted reproductive technologies. The main group consisted of 20 women diagnosed with endometriosis who underwent assisted reproductive technologies. In addition to standard preparation for assisted reproductive technologies, women in the main group received a probiotic containing Lactobacillus 10^{10} manufactured by Unic Biotech Ltd, India. They took one tablet twice a day for one month as part of the general treatment before undergoing assisted reproductive technologies. We determined the level of IL1β expression before and after this stage of preparation. The control group consisted of 10 women who had tubal infertility due to a previous inflammatory disease, but according to the results of a comprehensive clinical and laboratory examination they were equated to healthy women. These women aged 21 to 42 years with a mean age of 29.75 years did not undergo our proposed preparation for ART with the inclusion of a probiotic. This study was conducted at the Bukovinian State Medical University and the "Medical Center of Infertility Treatment" clinic. The value of p (authenticity difference) was determined by Student's table-Fischer. Differences between contrasting averages were considered significant at p<0.05.

Research results and their discussion.

The average age of women in the control group (who did not take the probiotic – 28.78±5.09 years) and the main group (who took the probiotic) 29.54±2.04 (p>0.05). Women in the main and control groups were examined and expression levels were determined IL1β mRNA genes. Expression level IL1β mRNA genes in whole blood in women before preparation for assisted reproductive technologies are given in **table 1**.

Table 1 – Expression level IL1β mRNA genes in whole blood in women before preparation for assisted reproductive technologies (M±m)

Group	Expression level IL1β mRNA genes in whole blood		P
	Before preparing (treatment)	After preparing (treatment)	
Main	26,7877±0,01	0,1610±0,01*	<0,001

Examining the data presented in **table 1**, we can distinguish two clear subgroups: the main group, consisting of women with endometriosis who received our proposed training for assisted reproductive technologies, including probiotics, before and after training, respectively. In the main group, the level of IL1β mRNA gene expression before training was 26.7877±0.01, which was significantly higher than the level after training (0.1610±0.01*).

We performed analysis of level expression mRNA IL1β before treatment conditioning on group.

Analized results according **fig. 1**, Mean level of mRNA IL1β in women with endometriosis is 10.35 c.u., at the same time Mean level of mRNA IL1β in women control group is 1.0 c.u..

We also performed analysis of IL1β after treatment conditioning on group.

Analized results according **fig. 2**, Mean level of mRNA IL1β in women with endometriosis is 0.14 c.u., at the same time Mean level of mRNA IL1β in women control group is 1.0 c.u..

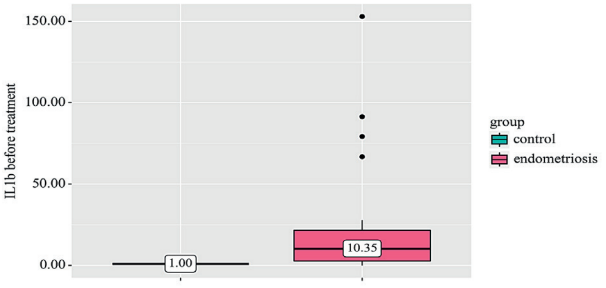

Figure 1 – Analysis of level expression mRNA IL1β before treatment conditioning on group.

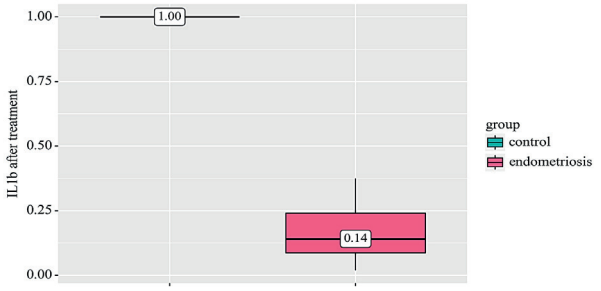

Figure 2 – Analysis of level expression mRNA IL1β after treatment conditioning on group.

Analized results according **fig. 3**, normal level of mRNA IL1β in women with endometriosis is in 25% patients, high level is observed in 70% patients , low level is in only 5% patients., at the same time Mean level of mRNA IL1β in women control group is normal in 100% patients.

Odds of low were 59.182 times greater in women with endometriosis comparing with control group, the relative difference in odds was statistically significant (95% CI: 2.949 – 1187.719) **(fig. 4)**.

Analysis of level expression mRNA IL1β before treatment was performed conditioning on infertility degree **(table 2)**.

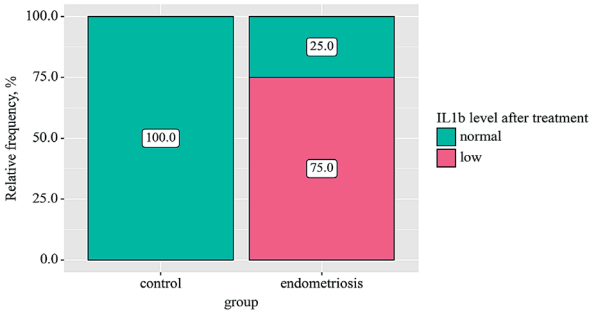

Figure 4 – Analysis of level expression mRNA IL1β after treatment conditioning on group.

According to the data obtained when comparing of level expression mRNA IL1β before treatment statistically significant differences were revealed depending on infertility degree (p=0.050) *(applied method: Mann-Whitney U-test)*.

Analized results according **fig. 5**, Mean level of mRNA IL1β in women with endometriosis associated infertility 1-st degree is 8.53 c.u., at the same time Mean level of mRNA IL1β in women with endometriosis associated infertility 2-nd degree is 1.0 c.u..

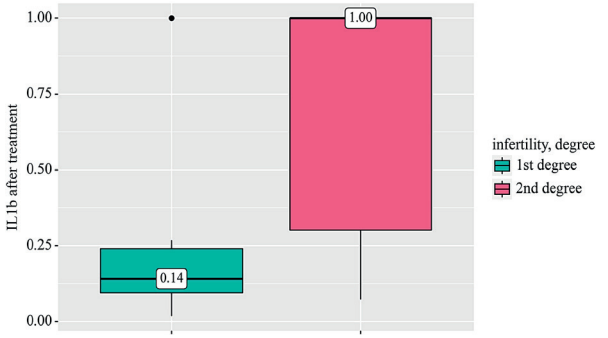

Figure 6 – Analysis of level expression mRNA IL1β after treatment conditioning on infertility degree.

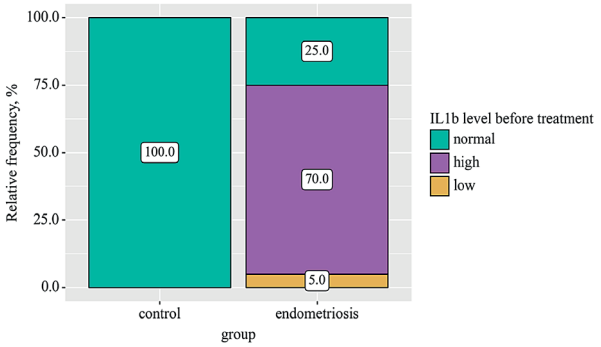

Figure 3 – Analysis of level expression mRNA IL1β level before treatment conditioning on group.

Table 2 – Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree

Variable	Categories	Level expression mRNA IL1β before treatment		n	p
		Me	$Q_1 – Q_3$		
Infertility degree	1st degree	8.53	2.16 – 18.98	18	0.050*
	2nd degree	1.00	1.00 – 3.36	12	

Note: * – differences are statistically significant (p<0.05).

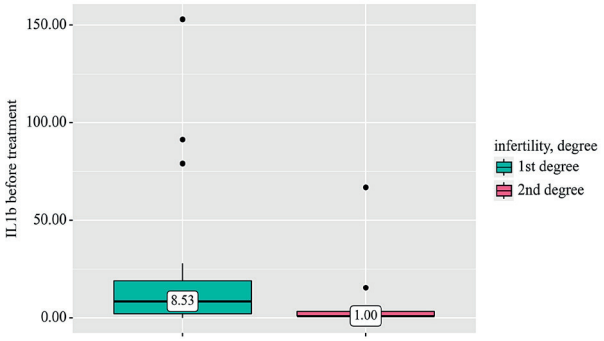

Figure 5 – Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree.

Analized results according **fig. 6**, Mean level of mRNA IL1β after treatment in women with endometriosis associated infertility 1-st degree is 0.14c.u., at the same time Mean level of mRNA IL1β in women with endometriosis associated infertility 2-nd degree is 1.0 c.u..

Analized results according **fig. 7**, normal level of mRNA IL1β before treatment in women with endometriosis associated infertility 1-st degree is in 33,3% , high level – in 61,1%, low level in 5,6%, at the

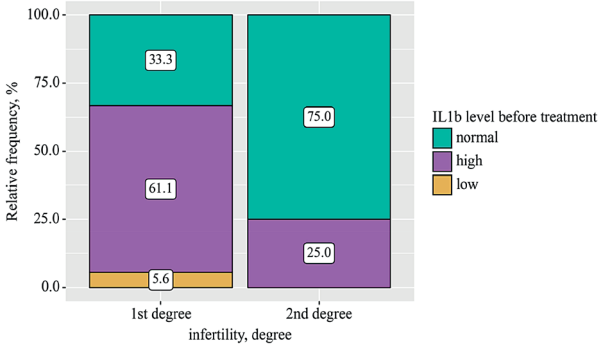

Figure 7 – Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree.

same time normal level of mRNA IL1β in women with endometriosis associated infertility 2-nd degree is in 75% and high level is in 25%.

Conclusions.

The extremely increased expression of IL1β mRNA genes indicates a close relationship between the pathogenesis of endometriosis and inflammation. The inclusion of probiotics in a comprehensive regimen of preparation for assisted reproductive technologies leads to a noticeable improvement in the patient's well-being and a significant decrease in IL1β mRNA gene expression. Therefore, we recommend the proposed preparation for assisted reproductive technologies with the inclusion of a probiotic.

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