ORIGINAL ARTICLE

CONTENTS

Correlation between MRNA IL1B and type, duration of infertility in women with endometriosis on the stage preparing to assisted reproductive technologies using probiotics

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ABSTRACT

Aim: To investigate the correlations between the level of IL1β mRNA gene expression and the type and duration of infertility and to study the levels of IL1β mRNA gene expression in women with endometriosis associated with infertility.

Materials and Methods: 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.

Results: 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.

Conclusions: 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.

KEY WORDS: endometriosis, probiotics, assisted reproductive technologies, infertility, IL1β

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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].

Given the involvement of natural killer (NK) cells in endometriosis due to reduced toxicity, one potential treatment approach is to activate these cells. In animal models, intraperitoneal injections of Lactobacillus gasserii OLL2809, a probiotic that stimulates IL-12 production, resulted in NK cell activation and reduction of ectopic endometrioid lesions. A randomized, double-blind, placebo-controlled trial also showed that this probiotic can alleviate pain associated with endometriosis [5]. Adhesion of endometrial fragments to other tissues is considered to initiate a local inflammatory response that over time develops into chronic inflammation. The inflammatory response in the pelvic cavity largely involves the activation of macrophages, which produce a number of growth-regulating substances. There is evidence that some of the inflammatory factors also stimulate the growth of ectopic endometrial cells in the early stages of endometriosis [6]. 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 [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 [7]. 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 [11].

AIM

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

MATERIALS AND 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.

RESULTS

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

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.

According to the presented table 2, when comparing of IL1β before treatment, statistically significant differences were revealed depending on group ($p < 0.001$) (applied method: Mann-Whitney U-test).

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.

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

Table 2. Analysis of level expression mRNA IL1β before treatment conditioning on group

Level expression mRNA IL1β before treatment					
Variable	Categories	Me	$Q_1 - Q_3$	n	p
Group	Control	1.00	1.00 – 1.00	10	< 0.001*
	Endometriosis	10.35	2.70 – 21.62	20	

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

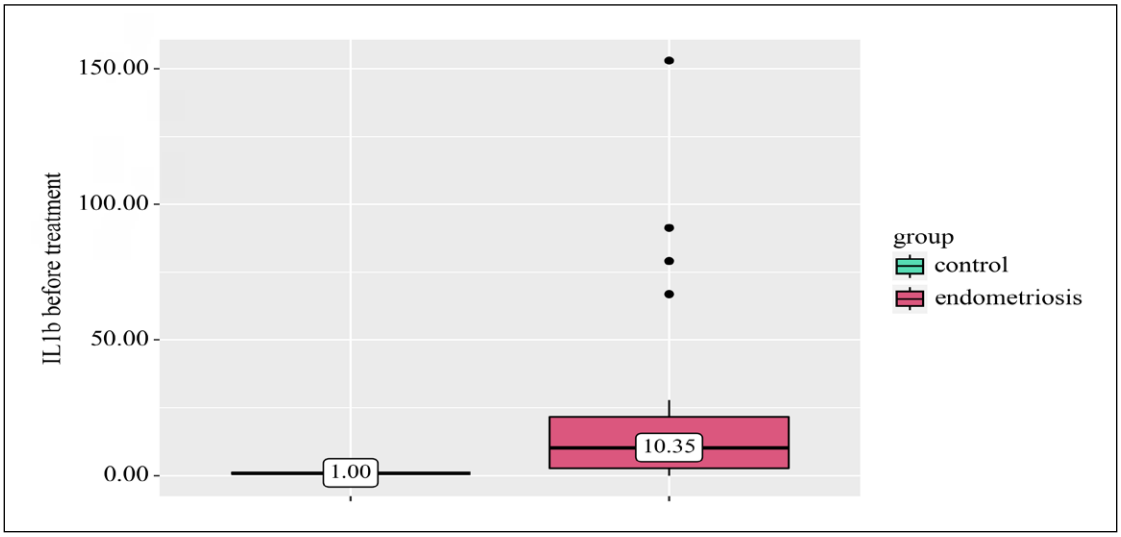

Fig. 1. Analysis of level expression mRNA IL1β before treatment conditioning on group.

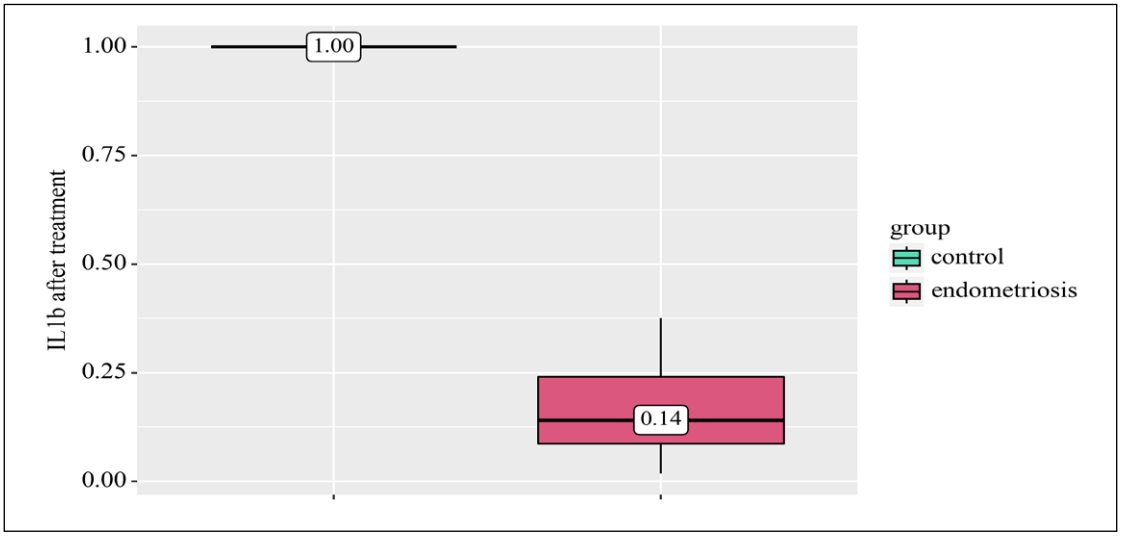

Fig. 2. Analysis of level expression mRNA IL1β after treatment conditioning on group.

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

According to the presented table 3, when comparing of level expression mRNA IL1β after treatment, statistically significant differences were revealed depending on group (p < 0.001) (applied method: Mann-Whitney U-test).

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.

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

In accordance with the presented table 4, when comparing of IL1β level before treatment, statistically significant differences were revealed depending on group (p < 0.001) (applied method: Pearson's chi-square test).

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.

Analysis of level expression mRNA IL1β level after treatment was performed conditioning on group.

In accordance with the presented table 5, when comparing of level expression mRNA IL1β after treatment, statistically significant differences were revealed depending on group ($p < 0.001$) (applied method: Fisher's exact test).

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).

Analysis of level expression mRNA IL1β before treatment was performed conditioning on infertility degree (Fig. 4).

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) (Table 6).

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.

Analysis of level expression mRNA IL1β after treatment was performed conditioning on infertility degree.

According to the presented table 7, when comparing of level expression mRNA IL1β after treatment, statistically significant differences were revealed depending on infertility degree ($p = 0.003$) (applied method: Mann-Whitney U-test).

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.

Analysis of level expression mRNA IL1β before treatment was performed conditioning on infertility degree.

When comparing of level expression mRNA IL1β before treatment depending on infertility degree no statistically significant differences were revealed ($p = 0.075$) (applied method: Pearson's chi-square test) (Table 8).

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 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%.

We performed analysis of level expression IL1β level after treatment conditioning on infertility degree.

According to the presented table 9, when comparing of level expression IL1β after treatment, statistically significant differences were revealed depending on infertility degree ($p = 0.008$) (applied method: Fisher's exact test).

Odds of low were 13.000 times less in 2nd degree group than in 1st degree group, the relative difference in odds was statistically significant (OR = 0.077; 95% CI: 0.012 – 0.482).

Table 3. Analysis of level expression mRNA IL1β after treatment conditioning on group

Level expression mRNA IL1β after treatment					
Variable	Categories	Me	$Q_1 – Q_3$	n	p
Group	Control	1.00	1.00 – 1.00	10	< 0.001*
	Endometriosis	0.14	0.09 – 0.24	20	

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

Table 4. Analysis of level expression mRNA IL1β before treatment conditioning on group

Variable	Categories	Group		p
		Control	endometriosis	
IL1β level before treatment	Normal	10 (100.0)	5 (25.0)	< 0.001*
	High	0 (0.0)	14 (70.0)	
	Low	0 (0.0)	1 (5.0)	

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

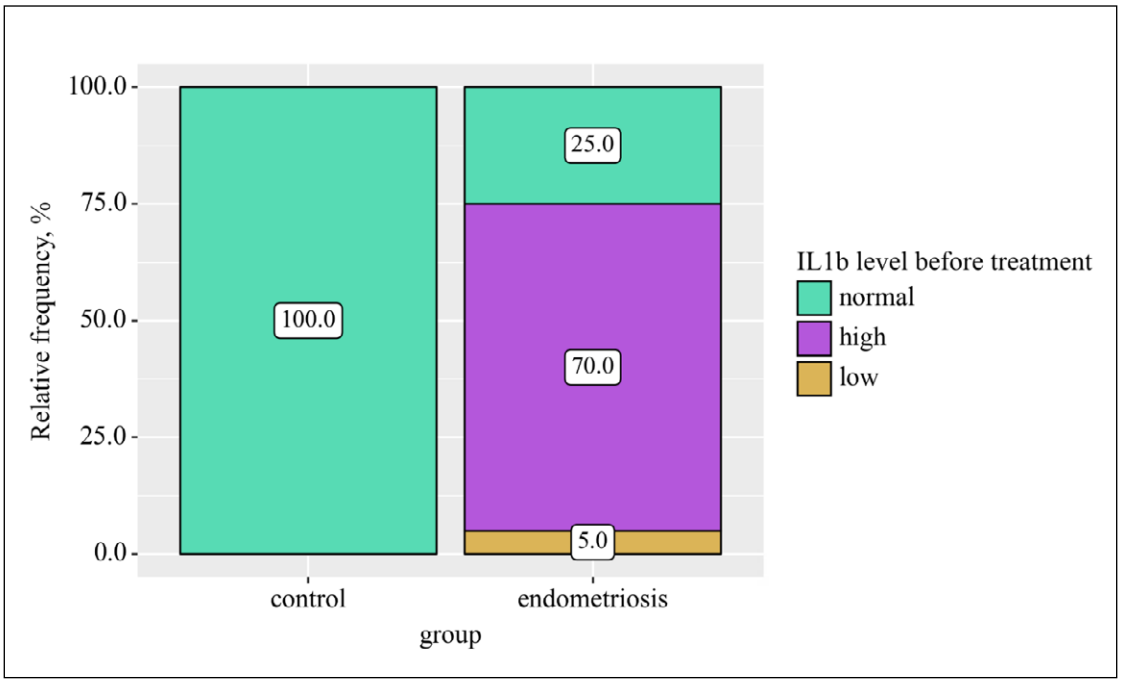

Fig. 3. Analysis of level expression mRNA IL1β level before treatment conditioning on group.

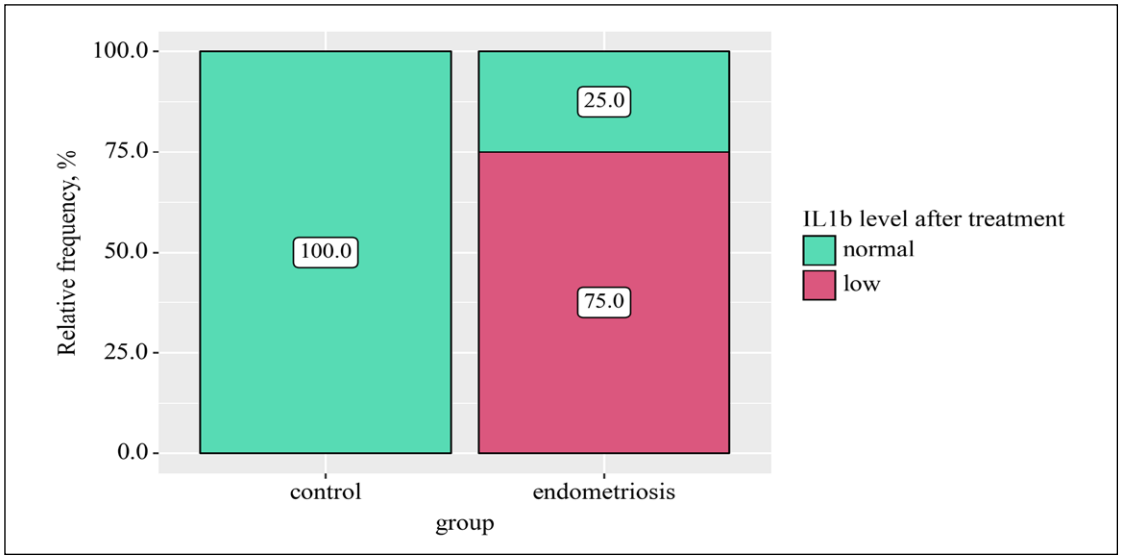

Fig. 4. Analysis of level expression mRNA IL1β after treatment conditioning on group.

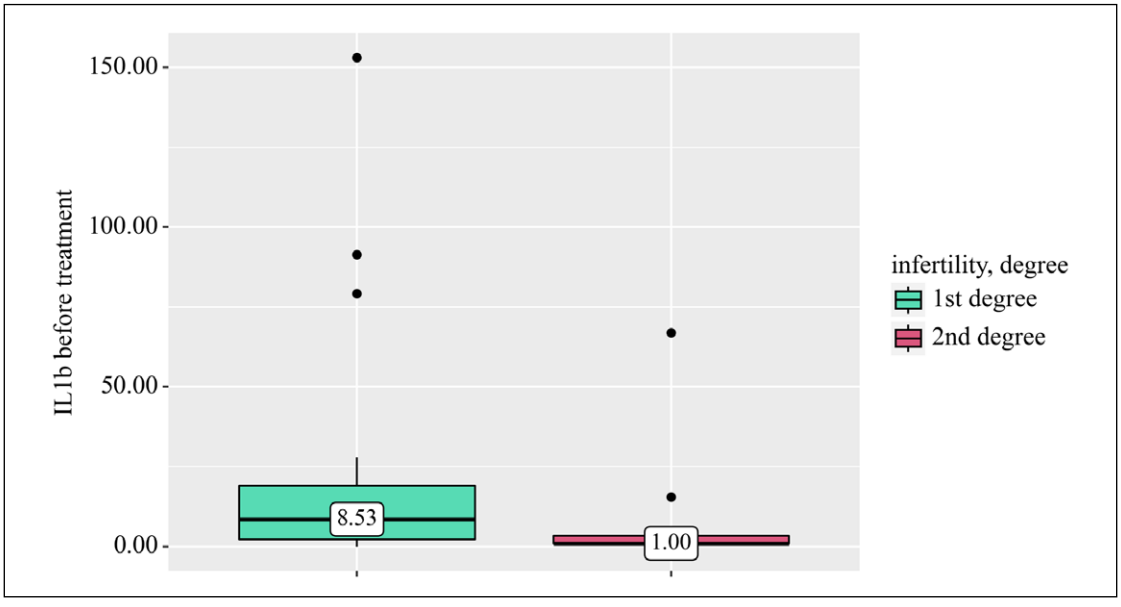

Fig. 5. Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree.

Table 5. Analysis of level expression mRNA IL1β after treatment conditioning on group

Variable	Categories	Group		p
		Control	Endometriosis	
IL1β level expression mRNA after treatment	Normal	10 (100.0)	5 (25.0)	< 0.001*
	Low	0 (0.0)	15 (75.0)	

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

Table 6. 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	

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

Table 7. Analysis of level expression mRNA IL1β after treatment conditioning on infertility degree

Variable	Categories	Level expression mRNA IL1β after treatment		n	p
		Me	$Q_1 – Q_3$	18	
Infertility degree	1st degree	0.14	0.10 – 0.24		0.003*
	2nd degree	1.00	0.30 – 1.00	12	

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

Table 8. Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree

Variable	Categories	Infertility degree		p
		1st degree	2nd degree	
Level expression mRNA IL1β before treatment	Normal	6 (33.3)	9 (75.0)	0.075
	High	11 (61.1)	3 (25.0)	
	Low	1 (5.6)	0 (0.0)	

Table 9. Analysis of level expressionIL1β level after treatment conditioning on infertility degree

Variable	Categories	Infertility degree		P
Level expression mRNA IL1β level after treatment	Normal	1st degree	2nd degree	0.008*
		5 (27.8)	10 (83.3)	
	Low	13 (72.2)	2 (16.7)	

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

During our research we found out that there was no association between level expression mRNA IL1β before treatment and infertility duration.

Observed dependence of level expression mRNA IL1β before treatment from infertility, duration is described by a linear regression equation:

$$Y_{\text{IL1b before treatment}} = 4.283 \times X_{\text{infertility, duration}} - 4.223$$

With an 1 increase of infertility, duration 4.283 change of IL1β before treatment should be expected. According to the coefficient of determination R^2 of the resulting model, 5.9% of the observed variance of IL1β before treatment were explained.

We performed a correlation analysis of the association between infertility, duration and IL1β after treatment. A weak correlation positive association between level expression mRNA IL1β after treatment and infertility duration was estimated (Fig. 8).

Observed dependence of level expression mRNA IL1β after treatment from infertility duration is described by a linear regression equation:

$$Y_{\text{IL1b after treatment}} = 0.053 \times X_{\text{infertility, duration}} + 0.164$$

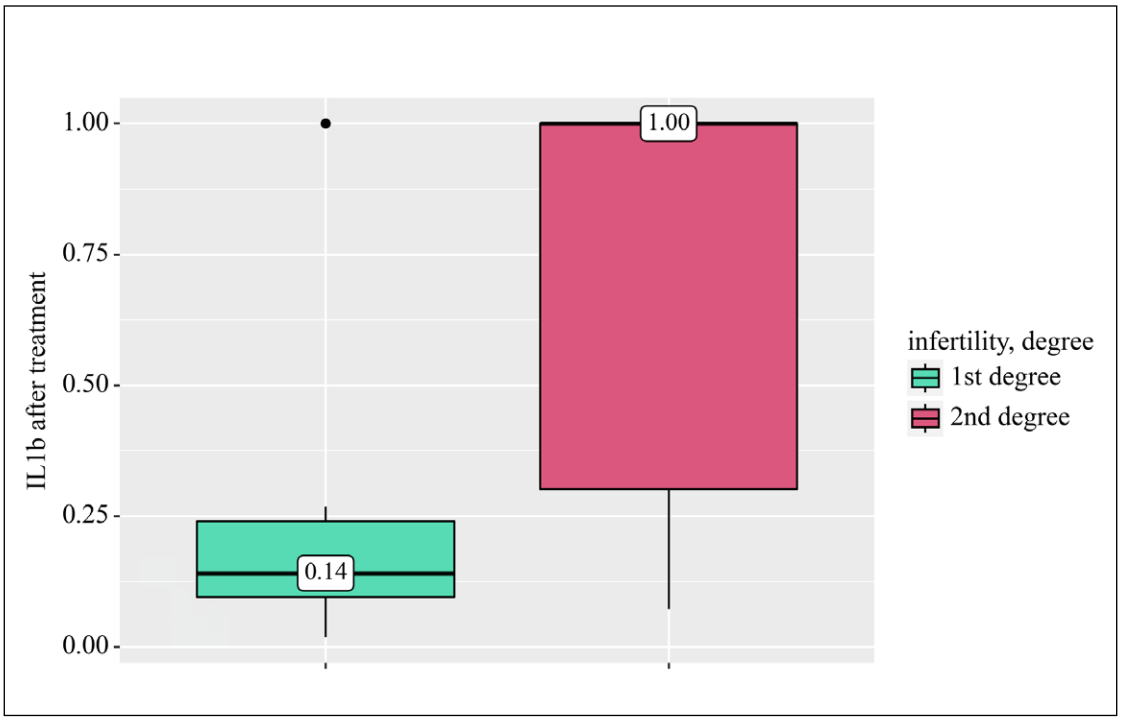

Fig. 6. Analysis of level expression mRNA IL1β after treatment conditioning on infertility degree.

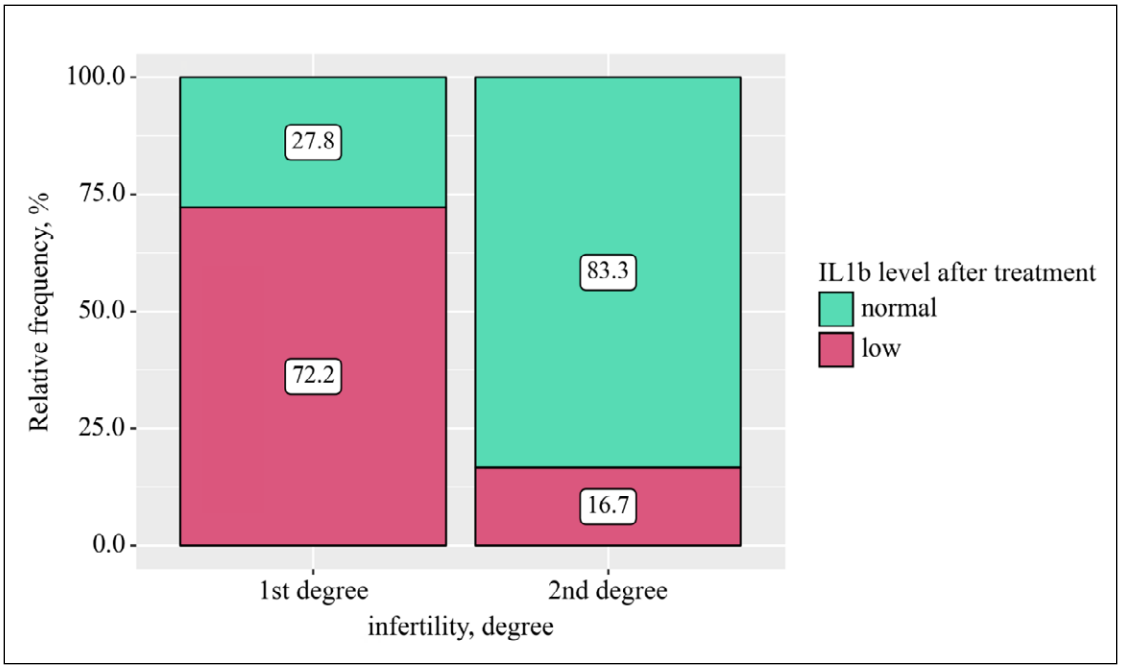

Fig. 7. Analysis of level expression mRNA IL1β before treatment conditioning on infertility degree.

With an 1 increase of infertility, duration 0.053 change of level expression IL1β after treatment should be expected. According to the coefficient of determination R^2 of the resulting model, 6.3% of the observed variance of level expression mRNA IL1β after treatment were explained(Fig. 9).

Therefore, our proposed preparation for assisted reproductive technologies with the inclusion of a probiotic is quite effective, as the levels of IL1β mRNA gene expression decreased sharply (Fig. 10).

DISCUSSION

In this study, our main aim was to examine gene expression levelsIL1β mRNAin whole blood in patients with endometriosis associated with infertility and to establish correlations between the type of infertility, its duration at the stage of preparation for assisted reproductive technologies, using a probiotic.

Endometriosis is a common benign gynecological disease characterized by the presence of ectopic endometrium, which causes dysmenorrhea, chronic

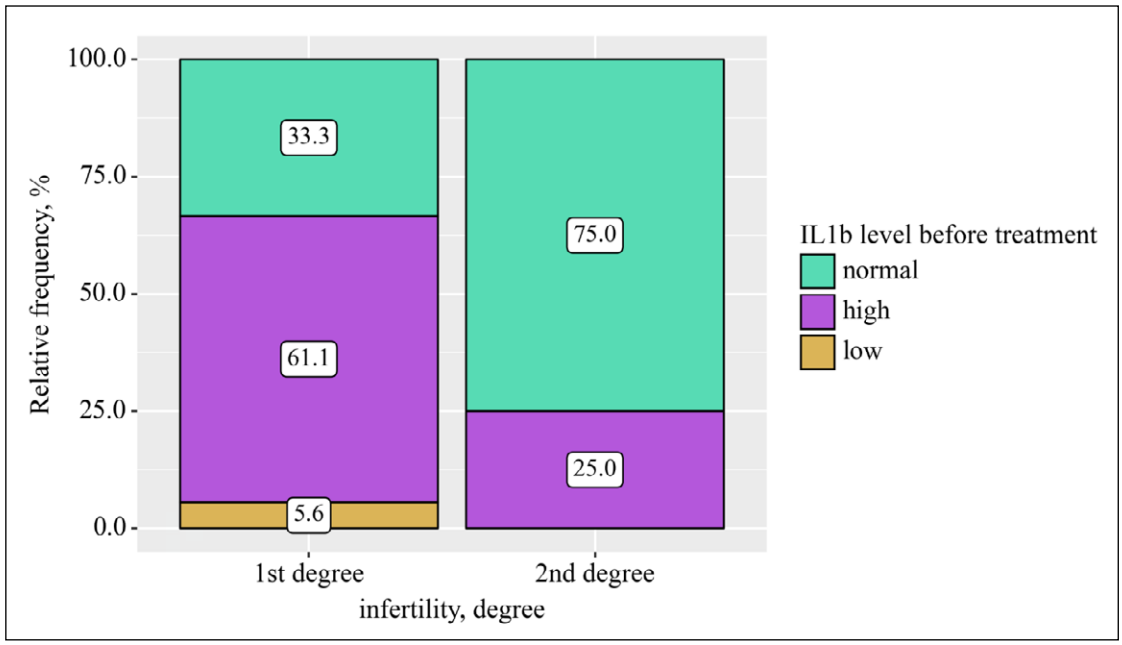

Fig. 8. Analysis of level expression mRNA IL1β after treatment conditioning on infertility degree.

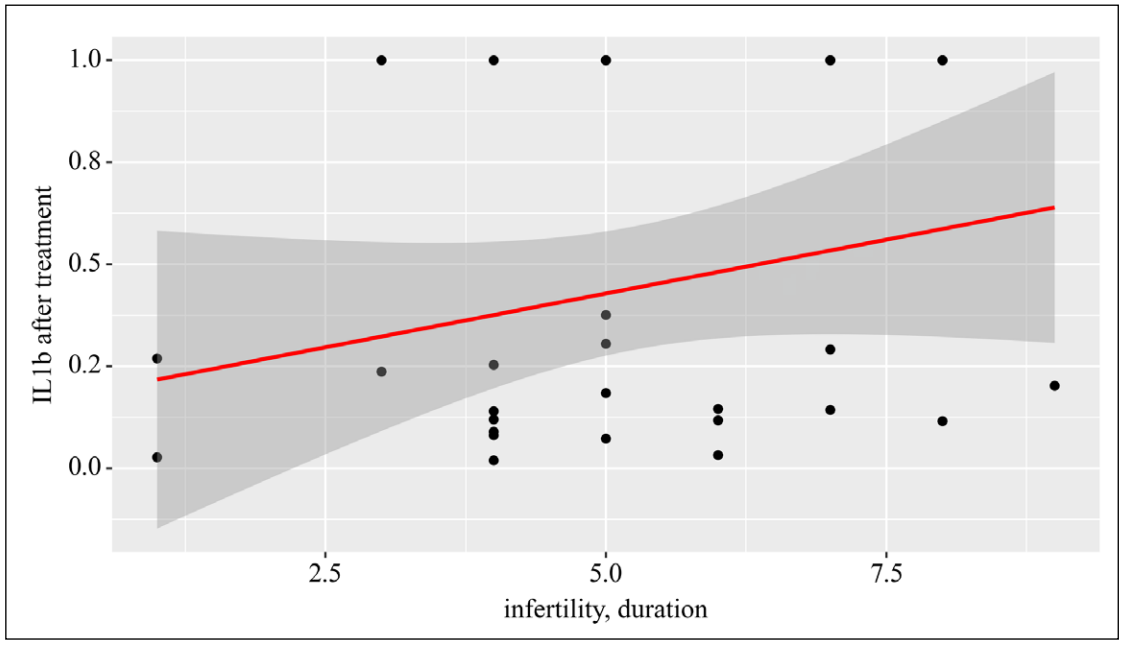

Fig. 9. Regression line characterizing the dependence of level expression mRNA IL1β before treatment from infertility duration.

pelvic pain, and infertility, and is associated with inflammation and immune disorders, as well as changes in ovarian steroid hormone production. The growth and maintenance of the endometrium and endometrioid tissue is regulated by several cytokines and growth factors, such as interleukin (IL) 6, 8, tumor necrosis factor (TNF)α, and vascular endothelial growth factor (VEGF). Retrograde menstruation into the abdominal cavity through the fallopian tubes plays an important role in the pathogenesis of endometriosis. Menstrual fluid is composed of blood cells, endometrial tissue, and waste products that are sources of endometrial cells. However, the profile of bioactive molecules in menstrual blood is unclear [8].

Therefore, our study aimed to investigate this relationship and its potential implications for clinicopathological features. Our results show that IL-1β mRNA gene expression levels have a negative correlation with the duration of infertility, significantly increased IL-1β mRNA gene expression levels in women with primary infertility than with secondary infertility, and may serve as non-invasive markers in women with endometriosis associated with infertility [9].

CONCLUSIONS

The extremely increased expression of IL1β mRNA genes indicates a close relationship between the

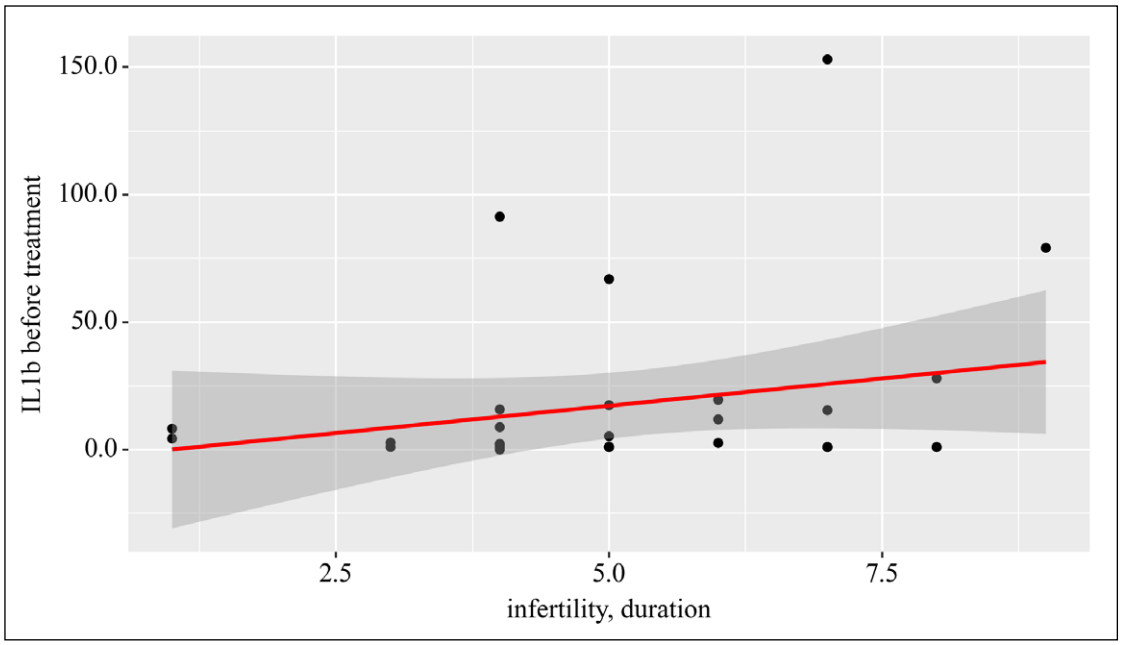

Fig. 10. Regression line characterizing the dependence of level expression mRNA IL1β after treatment from infertility duration.

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. IL-1β mRNA gene expression levels have a negative correlation with the duration of infertility, significantly increased IL-1β mRNA gene expression levels in women with primary infertility than with secondary infertility .Therefore, we recommend the proposed preparation for assisted reproductive technologies with the inclusion of a probiotic.

REFERENCES

1. Gao Y, Shen M, Ma X et al. Seven Hormonal Biomarkers for Diagnosing Endometriosis: Meta-Analysis and Adjusted Indirect Comparison of Diagnostic Test Accuracy. J Minim Invasive Gynecol. 2019;26:1026-35. doi: 10.1016/j.jmig.2019.04.004. DOI
2. Kolanska K, Alijotas-Reig J, Cohen J et al. Endometriosis with infertility: A comprehensive review on the role of immune deregulation and immunomodulation therapy. Am J Reprod Immunol. 2021;85(3):e13384. doi: 10.1111/aji.13384. DOI
3. Pant A, Moar K, K Arora T, Maurya PK. Biomarkers of endometriosis. Clin Chim Acta. 2023;549:117563. doi: 10.1016/j.cca.2023.117563. DOI
4. Lamceva J, Uljanovs R, Strumfa I. The Main Theories on the Pathogenesis of Endometriosis. Int J Mol Sci. 2023;24(5):1-13. doi: 10.3390/ijms24054254. DOI
5. Bulun SE, Yildiz S, Adli M et al. Endometriosis and adenomyosis: shared pathophysiology. Fertil Steril. 2023;119(5):746-50. doi: 10.1016/j.fertnstert.2023.03.006. DOI
6. Anastasiu CV, Moga MA, Elena Neculau A et al. Biomarkers for the Noninvasive Diagnosis of Endometriosis: State of the Art and Future Perspectives. Int J Mol Sci. 2020;21(5):1-24. doi: 10.3390/ijms21051750.191. DOI
7. Werdel R, Mabie A, Evans TL et al. Serum Levels of Interleukins in Endometriosis Patients: A Systematic Review and Meta-analysis. J Minim Invasive Gynecol. 2024;31(5):387-96. doi: 10.1016/j.jmig.2024.02.011. DOI
8. Nabhan A, Salama M, Elsayed M et al. Indicators of infertility and fertility care: a systematic scoping review. Hum Reprod Open. 2022;2022(4):hoac047. doi: 10.1093/hropen/hoac047. DOI
9. Khodaverdi S, Mohammadbeigi R, Khaledi M et al. Beneficial Effects of Oral Lactobacillus on Pain Severity in Women Suffering from Endometriosis: A Pilot Placebo-Controlled Randomized Clinical Trial. Int J Fertil Steril. 2019;13(3):178-83. doi: 10.22074/ijfs.2019.5584. DOI
10. Jia-Juan Wu, Qin-Yu Zhou, Dong-Mei Liu et al. Evaluation of the safety and probiotic properties of Lactobacillus gasseri LGZ1029 based on whole genome analysis. Food science & Technology. 2023;187:1-7. doi: 10.1016/j.lwt.2023.114759. DOI
11. Taylor RN, Berga SL, Zou E et al. Interleukin-1β induces and accelerates human endometrial stromal cell senescence and impairs decidualization via the c-Jun N-terminal kinase pathway. Cell Death Discov. 2024;10:288. doi: 10.1038/s41420-024-02048-6. DOI

Ethical approval for this study was obtained from the Medical Ethics Committee of the Bukovinian State Medical University, Chernivtsi, Ukraine (approval ID: No. 6 from 8.10.2024).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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