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Plasma microRNA level as a non-invasive predictor in assessing the degree of genital endometriosis lesion.

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Endometriosis has many symptoms that negatively affect reproductive capability and social life of women. This disease is characterized by the proliferation of endometrial tissue outside of the uterine cavity that causes pelvic pain and infertility. The prevalence of endometriosis varies between 5-10% for all women, 20-25% for gynecological patients and reaches 45-50% for women with infertility. The true frequency of endometriosis is not definitely known, and its growth over the past decades is associated with both the increasing frequency of the disease and the improvement of its diagnosis.
Endometriosis occurs irrespective of ethnicity and race, socioeconomic conditions, at any age. Diagnosis of external genital endometriosis can help patient complaints, clinical examination, ultrasound examination, sometimes magnetic resonance imaging.
At the present stage, much has been done to understand the mechanisms of endometriosis, but still the "gold standard" of diagnosis is invasive methods, especially laparoscopy. The search for possible biomarkers for the diagnosis of endometriosis continues.
Among non-invasive methods, the most promising for future study and research is the micro-RNA. In patient with endometriosis the micro-RNA profile of blood and eutopic endometrium may provide important information in confirming diagnosis.
Molecular genetic methods, while achieving good sensitivity and specificity results, will be able to improve the results of infertility treatment associated with endometriosis through earlier treatment, including surgery. In addition, various research groups have high expectations regarding the role of microRNA in evaluating the effectiveness of drug therapy and microRNAs treatments have provided promising results for some chronic diseases and cancers.

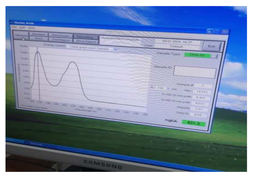
Materials and methods

In our study we have chosen microRNA let-7 and mir-9 as prominent representatives, because there are data on the prospect of using let-7 directly for the treatment of diseases, and mir-9 as microRNA that thereby induce apoptosis, which plays a special role in the pathogenesis of endometriosis.

We used RNA-GO to obtain high quality total RNA from cell samples (by NanoDrop-1000). Next step was to carry out reverse transcription for microRNA let-7 and mir-9 and control U6 (we used Tap Man microRNA Reverse Transcription Kit). We explored samples from control group and endometriosis group, which we divided into subgroups according to the classification ASRM I-II stage and III-IV stage, and also we evaluated the severity of pain syndrome in patients by Visual analog scale in millimeters (0-no pain; 100mm-worst possible pain).

Results.

The results obtained were investigated using the software "7500 Fast Real-time PCR" ("Applied Biosystems", USA). All data are presented as arithmetic data (mean values ± SEM). The results of the analysis in all groups were tested with respect to assessing the normality of the statistical distribution. Statistical analysis was performed using SPSS Statistics 17.0 (IBM Corporation, USA).
We got the first results from 2 groups of patients : 1- control, patients without endometriosis (n=20); 2- patients with endometriosis but without prior treatment (n=40,this group is divided into subgroups depending on the degree of spread of endometriosis assessed by laparoscopic intervention).
p-value for let-7 0.04644; p-value mir-9 0.2985.
We did not find a clear correlation between the severity of pain by VAS and the severity of endometriosis by ASRM, but at the IV stage of endometriosis, the average indicators were still higher than at the I respectively. Confidence interval for let-7 was (54.64-204.41) for control group, endometriosis group - (2.56-11.35) and for mir-9 respectively (0-15.54) and (0-13.30).



	Age	microRNA_let-7	mir-9
mean	33,7377	41,11262	4,995636
std	6,549559	94,21912	25,82021
min	20	0,03	0,00094
max	49	517,24	186,8633

Conclusions.

We continue the study and the set of research samples in the hope of finding informative non-invasive biomarkers for diagnosis and assessment of the effectiveness of treatment of patients with external genital endometriosis and want to check the correlation between the concentration for let-7 in endometrial samples and blood plasma samples. The ultimate goal of the work is to create an algorithm for diagnostics and tactics for choosing of treatment method, taking into account micro-RNA and patient complaints, which will help to identify the disease at an earlier stage and select an effective therapy.

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