“Promising non-invasive biomarker for diagnosis and treating external genital endometriosis”.

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Key words: external genital endometriosis, non-invasive biomarkers, micro-RNA, let-7, mir-9, Fast Real-time PCR.

Objectives

Introduction.

Endometriosis characterized by the proliferation of endometrial tissue outside of the uterine cavity that causes pelvic pain and infertility. At the present stage, much has been done to understand the mechanisms of disease, but still the "gold standard" of diagnosis is invasive methods, especially laparoscopy. 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 may provide important information in confirming diagnosis.

Materials and methods.

We’ve conducted the study among patients with genital endometriosis, in which we aimed to find a link between the level of circulating mir-RNA let-7 and mir-9 in serum samples and stage of endometriosis by using genome-wide microarray. We explored samples of 61 cases : control group and endometriosis group, which we divided into subgroups according to the classification ASRM I-II stage and III-IV stage. All patients had laparoscopic surgical treatment in one medical center with confirmation of the histological diagnosis after a complete clinical and laboratory examination. The assessment of the prevalence of endometriosis was carried out by the same surgical team according to ASRM requirements. Control group 24 patients , they did not have signs of endometriosis when examining the pelvic organs and peritoneum by laparoscopic intervention.

MicroRNA determination technique.

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). The results obtained were investigated using the software “7500 Fast Real-time PCR” (“Applied Biosystems”, USA).

Results.

To determine the correlation (r) we used the Spearman method (used for non normal distributed data). And we used Mann-Whitney-Wilcoxon method with Bonferroni correction (used for non normal distributed data) to determine the significance of the difference between the mean values 3 groups – control and 2 groups of endometriosis. 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 75 ±5mm and 45 ±5mm. 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). Both indicators of 'microRNA let-7' and 'ASRM points' reliably negative correlation (-0.64), and also indicators of 'Endometriosis (+/-)' and 'microRNA let-7' correlate to the nearest strong (-0.67) due to a high degree of reliability and also a negative character. So, on a given number of samples, we see a difference in indicators between the control groups and endometriosis groups, while for let-7 there is a correlation depending on the degree of prevalence of the process, but no correlation for mir-9.

	Age	microRNA_let-7	mir-9
count	61	61	61
mean	33,7377	41,11262	4,995636
std	6,549559	94,21912	25,82021
min	20	0,03	0,00094
25%	28	0,84	0,041
50%	33	4,27	0,101761
75%	38	18,18	0,557
max	49	517,24	186,8633

Table 1. Analysis for basic research indicators.

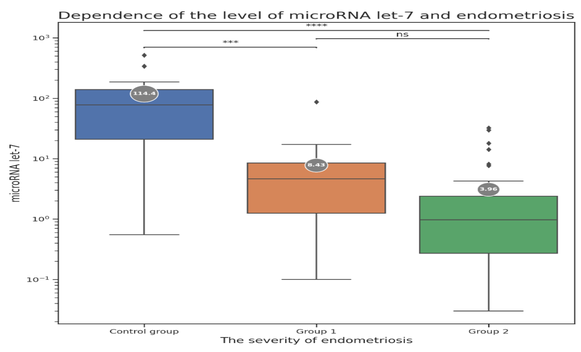

Table 2. Dependence of the level of microRNA let-7 and endometriosis.

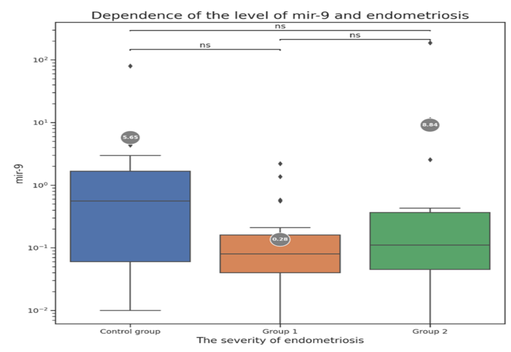

Table 3. Dependence of the level of microRNA mir-9 and endometriosis.

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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