Analysis of Vaginal Microecology in Women at 6-8 Weeks Postpartum

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Introduction. The vaginal microecological condition of women at 6-8 weeks postpartum was analyzed to provide scientific guidance for the prevention and treatment of vaginitis in postpartum women.

Methods. The clinical data of 160 cases of women who delivered at term from January to December 2023 and returned to the hospital for reexamination after 6-8 weeks were retrospectively analyzed, They were listed as the study subjects of puerperium group, and 160 women with non-menstrual health examination were selected as the control group in the same period. All 320 cases were tested for vaginal microecology status by multiple targeted amplification combined with high-throughput sequencing technology, and then delivery related information was collected to analyze the overall status of vaginal microecology and the incidence of vaginitis in the two groups.

Results. The positive rate of GBS, C. albicans, MG, TV, CT, UU and UP were higher than that of the control group ($P < 0.05$).

Conclusion. Multiple targeted amplification combined with high-throughput sequencing technology can accurately analyze the vaginal microecological status of women at 6-8 weeks postpartum, strengthen clinical monitoring, actively cultivate vaginal beneficial bacteria, and improve the vaginal health status of postpartum women.

Keywords. 6-8 weeks postpartum; vaginal microecology; multiple targeted amplification; high-throughput sequencing; vaginitis

INTRODUCTION

Delivery is a normal physiological process for women, and 6-8 weeks after delivery is a key period for women's physiological recovery. During this period, paying attention to the change of women's vaginal microecological conditions has a positive significance for the protection of women's reproductive health [1].Vaginal microbial community is a complex ecosystem, microbial composition diversity, and microbial competition and restriction, jointly maintain the stability of the vaginal environment. Once the vaginal microecological balance is unbalanced, it will not only cause abnormal vaginal flora and abnormal vaginal pH, but also lead to the reduction of vaginal resistance to pathogenic microorganisms and increase the risk of postpartum infection [2].Usually, maintaining a normal vaginal microecology, on the one hand, can maintain vaginal health, on the other hand, also conducive to prevent infection and inflammation. However, after childbirth, due to certain fluctuations in the body hormone level and the relative decline in autoimmunity, such physiological changes and the postpartum recovery period may affect the stability and diversity of vaginal microbial communities, and increase the risk of gynecological diseases.In the past, the microscopic examination method was often used to detect the vaginal microecological condition, but its experience requirement was high, the objectivity was low, and the standards were different. It is easy to miss and miss the mixed vaginitis, and there are certain limitations in the clinical application of [3-4].In this study, multiplex targeted amplification combined with high-throughput sequencing was used to analyze the vaginal microecological status of women at 6-8 weeks postpartum, timely prevent and treat related diseases, and improve the vaginal health status of postpartum women.

1. DATA AND METHODS

1.1 General information

The clinical data of 160 women who were delivered at term in January to December 2023 and returned to the hospital for reexamination after 6-8 weeks postpartum were listed as the study subjects of the puerperal group, and 160 women with non-menstrual health examination were selected as the control group in the same period. This study has been approved by the ethics committee of our hospital.Inclusion criteria:（1）Lochia was clean, and no antibiotics were applied 1 week before enrollment;（2）24-48h before sampling, no bath, no vaginal lavage;（3）There were no missing clinical data for the 320 subjects.Exclusion criteria:（1）Previous tumor history or cervical disease;（2）Serious postpartum complications;（3）Liver and kidney and other organ insufficiency.

1.2 General methods

All 320 subjects underwent multiple targeted amplification and high-throughput sequencing technology to deeply understand the composition and function of the microbial community in the vagina of postpartum women. （1）Main instruments and reagents: gene sequencing instrument (manufacturer: Guangzhou Jinqirui Biotechnology Co., Ltd.; model KM MiniSeqDx-CN), nucleic acid quantitative analyzer (manufacturer: Invitrogen; model Qubit3.0/4. 0), Nucleic acid extraction instrument (manufacturer: Thermo Fisher; model KingFisher flex), nucleic acid extraction or purification reagent (manufacturer: Guangzhou JinQirui; model KS 118-BYTQ-24), automatic nucleic acid protein analyzer (manufacturer: Guangding Biotechnology; model Qsep100), etc.（2）Sample collection:Guide the object to be examined to take the stone position, separate the legs to fully expose the genitals and perineum, and then open the vagina with a disposable voeurum. Under the naked eye, doctors rotate the side wall of the vagina to collect vaginal secretions to ensure that there are secretions attached to the swab.After collection, keep it at 4℃. If trichomonas vaginalis testing is needed, pay attention to moisturizing and insulation.（3）test method:①Sample processing: For swab samples without preservation

solution, add 3ml sterile saline, mix well, take 1.3ml sample eluate, add 1.5ml sterilized centrifuge tube for backup; for swab samples containing preservation solution, vortex with vortex mixer for 30s, and then take 1.3ml sample for backup. After that, the sampling tube was vortexed on the vortex mixing machine for 30s, speed: 12000rpm, time: 5min, high speed centrifugation, supernatant was removed, 500 μ L sample was retained for wall breaking. After wall breaking, the sample was centrifuged again, and 250 μ L was automatically extracted for nucleic acid extraction.②Nucleic acid extraction: automatic extraction or manual purification reagent extraction on the automatic extraction workstation, and then determine the nucleic acid concentration by the nucleic acid analyzer, according to the instructions, record the nucleic acid concentration.③Library preparation:Thawed multiple targeted amplification premix (S50), mixed well, centrifuged, sampled according to the nucleic acid concentration, 10 ng / μ L, added 11 μ L nuclease water to 100 ng, then added S50 to complete the library; <10 ng / μ L, took 11 μ L directly and added to S50; set the product purification orderly according to the target region enrichment targeted amplification program, and then completed library amplification and library purification according to the process.④ sequencing ：The cartridge was removed and thawed at room temperature at 19-25℃ for 90min, keeping the water surface of the tank no more than the cartridge. The new sequencing chip was removed and left at room temperature for 30min, and denatured before adding the library to the reagent cartridge.According to the theoretical sequencing flux ratio, mix and dilute the library in proportion, and measure the general order: mixed volume =1:1. The sequenced library was mixed into one tube with 0.1mol / L sodium hydroxide dilution, 100 μ L was added into a low adsorption centrifuge containing 900 μ L nuclease water, instantaneous centrifugation; then 5 μ L 0.1mol / L sodium hydroxide and 5 μ L raw mixture library, instantaneous centrifugation, empty for 5min, denaturation, dilute to 5 pmol / L, and make sequencing mixture library according to the formula, and add the 16 well according to the schematic. Based on the self-test analysis of MiniSeq Control Software software database, data quality requirements: Q3075%, minimum raw reads

50k, the number of internal reference amplification uniform reads 50. One instrument cleaning was performed, both before and after each sequencing run.

1.3 Observational indicators

The test results of two groups of pathogenic microorganisms were counted to determine the status of vaginal microecology.

1.4 Statistical method

All data of this study were processed by SPSS 27.00 software, count data were expressed in [case (%)], $\chi 2$ test; measurement data were expressed in (), line t-test, $P < 0.05$ was statistically significant.

2.RESULTS

2.1 Two groups of pathogenic microbial results

See Table 1-2 for more details.

Table 1 Test results of pathogenic microorganisms in puerperal group (n=160)

classification	pathogenic microorganism	positive	pathogenic microorganism	positive	pathogenic microorganism	positive
Bacteria	Streptococcus agalactiae (GBS)	14	Haemophilus duklei (HD)	0	Neisseria gonorrhoeae (NG)	0
virus	Herpes simplex virus type 1 (HSV-1)	0	Varicella-zoster virus (VZV)	0	Cytomegalovirus (CMV)	0
	Herpes simplex Virus Type 2 (HSV-2)	0				
fungus	C. albicans	12	candida glabrata	0	Kudri Azwei (Candida)	0
	Oidium tropioale	0	Candida parapsilosis	0	Candida Dublin	0
Mycoplasma / Chlamydia / Spirochetes	Mycoplasma hominis (NH)	0	Ureaplasma Minor (UP)	5	Ureaplasma Minor Type 6 (UP 6)	0
	Mycoplasma genitalium (MG)	7	Ureaplasma Minor Type 1 (UP 1)	0	Ureaplasma Minor Type 14 (UP 14)	0
	Chlamydia trachomatis (CT)	6	Ureaplasma Minor Type 3 (UP 3)	0	Microspironema pallidum (Microspironema pallidum)	0
	Ureaplasma urea (UU)	7				
parasite	Trichomonas vaginalis (TV)	10	toxoplasma gondii	0		

Table 2 Test results of pathogenic microorganisms of the control group (n=160)

classification	pathogenic microorganism	positive	pathogenic microorganism	positive	pathogenic microorganism	positive
Bacteria	Streptococcus agalactiae (GBS)	2	Haemophilus duklei (HD)	0	Neisseria gonorrhoeae (NG)	0
virus	Herpes simplex virus type 1 (HSV-1)	0	Varicella-zoster virus (VZV)	0	Cytomegalovirus (CMV)	0
	Herpes simplex Virus Type 2 (HSV-2)	0				
fungus	C. albicans	1	candida glabrata	0	Kudri Azwei (Candida)	0
	Oidium tropioale	0	Candida parapsilosis	0	Candida Dublin	0
Mycoplasma / Chlamydia / Spirochetes	Mycoplasma hominis (NH)	0	Ureaplasma Minor (UP)	0	Ureaplasma Minor Type 6 (UP 6)	0
	Mycoplasma genitalium (MG)	1	Ureaplasma Minor Type 1 (UP 1)	0	Ureaplasma Minor Type 14 (UP 14)	0
	Chlamydia trachomatis (CT)	0	Ureaplasma Minor Type 3 (UP 3)	0	Microspironema pallidum (Microspironema pallidum)	0
	Ureaplasma urea (UU)	1				
parasite	Trichomonas vaginalis (TV)	1	toxoplasma gondii	0		

2.2 Contrast the status of vaginal microecology vs there was no statistical difference in age and body quality between the two groups (P> 0.05). The positive rates of GBS, C. albicans, MG, TV, CT, UU and UP were higher than that of the control group, which was significant (P <0.05). See Table 2.

Table 2 The vaginal microecological conditions of the two groups (n,%)

project		Control group (n=160)	Puerum group (n=160)	t/χ^2 value	*P* value
Age (year)		29.67±4.21	28.94±4.51	1.499	0.135
Body mass (kg/m²)		22.63±1.48	22.69±1.52	0.358	0.721
GBS	negative	158（98.75）	146（91.25）	9.474	0.002
	positive	2（1.25）	14（8.75）		

C. albicans	negative	159（99.37）	148（92.50）	9.702	0.001
	positive	1（0.63）	12（7.50）		
MG	negative	159（99.37）	153（93.75）	4.615	0.032
	positive	1（0.63）	7（6.25）		
TV	negative	159（99.37）	150（93.75）	7.626	0.006
	positive	1（0.63）	10（6.25）		
CT	negative	160（100.00）	154（96.25）	6.115	0.013
	positive	0（0.00）	6（3.75）		
UU	negative	159（99.37）	153（93.75）	4.615	0.032
	positive	1（0.63）	7（6.25）		
UP	negative	160（100.00）	155（96.87）	5.079	0.024
	positive	0（0.00）	5（3.13）		

3.DISCUSSION

There are also differences in the composition of vaginal flora in women [6-7].Pregnancy is a special period for women. Due to the huge changes of pregnancy and estrogen in the body, it has a great influence on the autoimmune status.According to the analysis of existing studies, drugs, infection, sexual life, and number of abortion are the main factors affecting vaginal microecology [8].Moreover, changes in the vaginal flora of postpartum women are a strictly continuous process that may have long-term effects on the health of the female reproductive tract.In the traditional microscopic examination, due to the different standards and the low positive detection rate, the proportion of the obtained test results is relatively high.At present, with the development of molecular biology, bioinformatics and other technologies, it has laid a good foundation for clinical in-depth analysis of vaginal microecology [9].

Puerperium group patients after pathogenic microorganism detection, a total of 14 cases detected positive GBS, 12 cases of S. albicus positive, 7 cases of MG positive, 6 positive CT positive, 7 UU positive, 10 TV positive, 5 UP positive, the rest are negative, the total positive rate is much higher than the control group, the puerperium population is more susceptible to bacteria, fungi and other pathogenic microorganisms, make 6-8 weeks postpartum women vaginal micro ecological condition.By using multiple targeted amplification and high-throughput sequencing

technology, the vaginal microecology can be comprehensively evaluated, and the vaginal microecology can be evaluated according to the detection results of pathogenic microorganisms.By designing specific primers for multiple specific genes, it can simultaneously amplify the selected target sequence within the same reaction system to detect higher [10] throughput.High-throughput sequencing technology can use sequencers to complete massively parallel sequencing of amplified DNA fragments. This combined method can detect gene variations and expression changes of very low abundance, with good sensitivity and accuracy. Through microflora detection, it can provide predictable prompt for clinicians and improve the clinical diagnostic accuracy [11-12].In this context, it can also transition from the past treatment method of killing microorganisms to a new treatment mode with the purpose of increasing probiotics and restoring the normal vaginal microecological environment.In addition, women born 6-8 weeks postpartum are more prone to the occurrence of gynecological diseases such as vaginitis due to vaginal microecological imbalance, and the reason of which may be related to the decreased proportion of Lactobacillus.As one of the important bacteria in the vagina, Lactobacillus is to maintain acidic environment, kill pathogenic bacteria, form protective bacterial membrane, and is the core [13-14] of vaginal microecological balance [13-14].By producing an acidic environment, it can inhibit the reproduction of other harmful microorganisms and maintain vaginal health.Related studies also showed that in [15], postpartum women have relatively low Lactobacillus abundance in the vaginal flora, and its stability also decreased with the transition from inert Lactobacillus to CST type.According to the research results, provide individualized vaginal microecological maintenance program for postpartum women, which can help them maintain a healthy vaginal microecological environment, prevent the occurrence of vaginitis and other diseases, and provide scientific basis for disease treatment [16-17].At the same time, in the actual testing process, the standard process should be constantly improved, and the quality control management should be strengthened to get more accurate results.

To sum up, the vaginal microecology of women from 6 to 8 weeks after delivery

is easy to imbalance, which is easy to cause gynecological diseases such as vaginitis. Strengthening the analysis and monitoring of the vaginal microecological conditions of women during this period, and actively cultivating vaginal beneficial bacteria are of positive significance for the prevention and treatment of related diseases and the improvement of the vaginal health status of postpartum women. However, based on the current small sample size, the collection of vaginal microecological distribution of women in 6-8 weeks postpartum.

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