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Application of Metabonomics Methods in Determination of Metabolites of Vaginal Microbiota

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Abstract

The bacterial composition of the vaginal microbiome is thought to be related to women's health and disease status. The microbiome of vagina is dynamic, with components changes related to life status and diseases such as pregnancy, menstruation, bacterial vaginosis, and HPV infection. With understanding of the reproductive tract microecology, people began to pay more attention to eco-diagnosis and eco-therapy for the purpose of understanding and adjusting the microbiota. With the understanding of the microecology of the reproductive tract, people began to pay more attention to the detection of changes in the microbiota to understand disease status. The new eco-therapy improves the disease state by adjusting the microbiota. Metabolites are an important feature of the vaginal microenvironment. The differences in some metabolites are related to the functional variation of the vaginal microbiota. The metabolomics method can also carry out quantitative and qualitative analysis of metabolites. From this we get an inspiration, we can use metabolomics methods to detect metabolites in the vagina, find their correlation with gynecological diseases, and provide an idea for future women to solve the "embarrassing" problem. This summarizes several common metabolomics methods used to measure vaginal metabolites in recent years, and uses common mass spectrometry, chromatography and spectroscopy instruments to analyze metabolites of vaginal microbiota.

Keywords: metabolomics; vagina; gynecological diseases;1H-NMR; LC-MS; GC-MS; MS; HPLC

Introduction

The vaginal microbiota plays a vital role in maintaining the function of the female reproductive tract and female health, preventing sexually transmitted infections and the colonization of genitourinary tract pathogens. Unlike most other parts of the human body, vaginal microbiome is highly dynamic and characterized by temporal perturbations, which are influenced by sexual development, sexual intercourse, menstruation, hormone levels, and personal hygiene [1]. Because of this dynamic change in vaginal, microorganisms and compositions may be unstable at different times, even for a particular woman.

The development of high-throughput DNA sequencing technology has greatly improved the ability to study the

human microbiota. New genetic technology methods used in previous studies, such as 16S rRNA gene sequencing, have examined the composition of the vaginal microbiota relative to various health and disease state [2-4]. The use of new generation molecular sequencing techniques has revealed that the majority of vaginal bacterial communities are led by Lactobacilli [5,6]. Ravel et al. analyzed the vaginal microbiota from non-pregnant, fertile and asymptomatic groups and summary the vaginal bacterial communities were grouped into five main types of Community-Sate Types (CSTs). Four CSTs were dominated by different species of Lactobacillus (L. crispatus, CST I; L. gasseri, CST II; L. iners, CST III; and L. jensenii, CST V). The other communities (CST IV) were varied and formed by a great proportion of obligate anaerobic bacteria including Gardnerella, Atopobium, Prevotella spp. and other bacterial species [7]. The high risk of CST IV was relative the diseases of persistent HPV infection, sexually transmitted diseases (STDs), HIV infections, preterm birth, adverse pregnancy outcomes such as post-abortion sepsis, early, late and recurrent abortions, etc [8-14].

With the gradual deepening of the understanding of the human microecosystem, people began to pay more attention to eco-diagnosis and eco-therapy for the purpose of understanding and adjusting the microflora. Recent studies on the interaction between vaginal microecology and vaginal epidermal cells suggest that vaginal health can be maintained by improving the composition of metabolites in the vagina. Many studies have shown that vaginal flora and its metabolites have a very important indication of whether women suffer from gynecological diseases [3]. Unlike genotyping techniques that have been validated and consolidated in microbiological research, there is little information about the application of metabolomics in microbial identification. Metabolites are an important feature of the reproductive tract microenvironment. The differences in some metabolites are related to the functional variation of the vaginal microbiota. The metabolites of the flora may become a signal molecule for the diagnosis of gynecological diseases. Future research based on genomics, proteomics and metabolomics technologies may eventually have a significant impact on women's reproductive health and disease treatment.

Metabonomics is a basic and powerful tool. It can obtain different information from the host's health and disease status, including the changes of bacterial types and the correlation of microenvironment, so as to comprehensively understand the overall situation of growth and development or disease development process. The following is a summary of four methods of metabolomics to measure vaginal metabolites including Proton Nuclear Magnetic Resonance (1H-NMR)High Performance Liquid Chromatography HPLC Mass spectrometry MS), Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS).

Proton Nuclear Magnetic Resonance Spectroscopy

Metabolomics uses high-flux analysis methods such as magnetic resonance (1H-NMR) spectroscopy to analyze complex systems to reliably and sensitively identify metabolites produced by microorganisms and host cells. This tool has the great advantage of quantitative and qualitative analysis of thousands of metabolites [15].

Foschi et al. used 1H-NMR spectroscopy to study the metabolic characteristics of Lactobacillus from 40 strains of various origin (intestinal, vaginal, food, probiotics). The results identified a panel of metabolites whose variations in concentration were associated with the taxonomy, but also revealed a high intra-species variability that did not allow a species-level identification. The metabolic characteristics of related species may be significant differences, and several metabolic pathways and molecules are related to specific Lactobacillus species. Although 1H-NMR spectroscopy analysis cannot be proposed as a reliable tool for identification of Lactobacillus species or strains, it can help to study the metabolites of different strains of Lactobacillus. Later, Camilla et al. described the composition of vaginal metabolites in detail based on 1H-NMR spectroscopy. The change of bacterial community that occurs during genital infection is a major change in the composition of vaginal metabolites, and the reduction of lactic acid is a common sign of all pathological conditions. The data revealed that the vaginal secretions of BV-infected women have higher content of biogenic amines and short-chain organic acids than normal women. In conclusion, according to the metabolomics analysis, we found that different infections of vaginitis have unique vaginal metabolome fingerprints by 1H-NMR analysis [16]. In the future, using 1H-NMR spectroscopy to analyze the metabolites of different strains can be used to evaluate the effect of intervention or treatment of vaginal infection.

At the same time, Parolin et al. used 1H-NMR spectroscopy to evaluate the metabolic profile of Chlamydia trachomatis (CT) infection women. Regarding the metabolome, 4-aminobutyrate showed significant different concentrations in asymptomatic ($2.08 \times 10^{-3} \pm 1.21 \times 10^{-2}$) and symptomatic CT women ($1.01 \times 10^{-2} \pm 2.72 \times 10^{-2}$) ($P = 0.027$). In addition, vaginal swabs were collected and analyzed by 1H-NMR, which provides a new perspective

for future metabolomics research [17].

Vitali et al. recruited vaginal fluid of bacterial vaginosis (BV)-affected patients and healthy controls to analyze the metabolites by 1H-NMR spectroscopy. There were significant differences in the concentrations of 32 metabolites between BV and non-BV women ($P < 0.05$). They proposed a molecular combination method to detect the niacin, malonic acid and acetate as biomarker for BV infection women by combining qPCR and 1H-NMR spectroscopy [18].

High Performance Liquid Chromatography

High-efficiency liquid chromatography (HPLC) is a powerful technique for analyzing specific metabolites, including amino acids. HPLC has the advantages of fast, quantitative and easy access to a variety of metabolites. In addition, it is highly adaptable because any compound that is soluble in liquid samples can be separated, identified and quantified by an HPLC. Sungur et al. studied the inhibitory and immunomodulatory effects of extracellular polysaccharides (EPSs) of L. gasseri strain (from normal vagina) on the growth of cervical cancer cells (Hela) by HPLC [19]. Additionally, monosaccharide composition of the L-EPSs produced by L. gasseri strains was determined by HPLC. In conclusion, diversity in sugar composition of EPS might contribute to adhesion and proliferation properties. This shows that the metabolites of vaginal flora may be used as targets for the treatment of gynecological diseases in the future.

Subsequently, Welch et al. used HPLC to study the inhibitory effect, potential therapeutic role, for an analogue of reutericyclin (glycerol monolaurate; GML), against microbial pathogens including human immunodeficiency virus type 1 (HIV-1). The results showed that HPLC-purified reutericyclin and reutericyclin secreted by Lactobacillus inhibit HIV-1. These data emphasize the importance and protective nature of the normal vaginal flora during viral infections and provide insights into the antiviral mechanism of GML during HIV-1 infection and, morebroadly, to other enveloped viruses [20].

Polat et al. used HPLC to study the expression of vaginal metabolites in women diagnosed with microbial invasion of the amniotic cavity of preterm women. The HPLC data showed that the concentrations of taurine, lysine and cysteine in women with invasion of amniotic cavity were significantly higher than those in women without infection. However, the ratio of vaginal glutamic acid, aspartic acid and aspartic acid to aspartic acid in vagina of infection women was significantly lower than that in uninfected women [21].

Mass Spectrometry (MS)

Mass spectrometry (MS) produces gas phase ions directly from the sample for rapid and cost-effective metabolic analysis of unretouched and complex biological materials, including bacteria. It does not require chromatography separation or sample preparation for direct processing and rapid analysis of complete biological fluids. BV is a common but highly enigmatic condition that is associated with adverse outcomes for women and their neonates. Srinivasan et al. used MS compared the metabolomics characteristics in cervicovaginal lavage (CVL) fluid obtained from 40 women with BV to profiles in 20 women without BV. In their study, there was a significant difference in the level of 62% of the metabolites between women with BV and those without BV. Higher levels of the signaling eicosanoid 12-hydroxyeicosatetraenoic acid (12-HETE), a biomarker for inflammation, were noted in BV. Lactobacillus crispatus and Lactobacillus jensenii exhibited similar metabolite correlation patterns, which were distinct from correlation patterns exhibited by BV-associated bacteria. Insights from this study provide opportunities for developing new diagnostic markers of BV and novel approaches for treatment or prevention of BV [22].

Prussian collected vaginal swabs from healthy people (n=41) and BV patients (n=21), and directly analyzed the metabolites using desorption electrospray ionization mass spectrometry (DESI-MS). It was found that the swabs of BV patients contained consistently high levels of amino acid metabolites, including polyaminooctane and methylamine. These compounds are the main cause of the fishy smell of the vagina, and it also proves that these metabolites have the potential as biomarkers of BV metabolites. The DESI-MS method was also applied directly to bacterial biomass to confirm the ability to detect intact bacterial species from a swab. These results highlight the potential of direct swab analysis by DESI-MS for a wide range of clinical applications including rapid mucosal diagnostics for microbiology, immune responses, and biochemistry [23].

Gas Chromatography-Mass Spectrometry (Gc-Ms) And Liquid Chromatography-Mass Spectrometry (LC-MS)

A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. The most powerful multi-omic systems-based method combined the advantages of HPLC and MS for metabolome analysis in recent years. LC-MS is a very versatile technology with several different LC modes or MS analyzers and analysis modes. The research of LC-MS-based metabolomics is progressing rapidly, and because of the continuous improvement of histography and mass

spectrometology ability, metabolomics/metabolomics has great potential in the field of biomedical science and has become a key analytical technique of metabolomics [24]. When Yeoman et al. studied 36 BV infected women with GC-MS, 176 different metabolites were found, of which 67 were significantly different. In addition to putrescine, cadaverine and 2-methyl-2-hydroxybutyric acid, etc, most of the metabolites will be reduced in the state of BV infected women. The characteristic odor associated with BV was linked to increases in putrescine and cadaverine, which were both linked to Dialister spp. Additional correlations were seen with the presence of discharge, 2-methyl-2-hydroxybutanoic acid, and Mobiluncus spp., and with pain, diethylene glycol and Gardnerella spp.

Thomas et al [25] used a GC-MS-based metabolomic approach to search for biomarkers that may act as early indicators of spontaneous preterm birth (sPTB). Cervicovaginal fluids were collected at 20 weeks from women who were originally assessed as being at low risk of sPTB. GC-MS analysis revealed a total of 112 compounds were extracted from the cervical fluid that the mainly identified metabolites such as amino acids, organic acids and fatty acids. The GC-MS methyl chloroformate derivatization method is more stable than other derivatization methods in the study. The statistical analysis showed no significant correlation with low risk of sPTB.

Ghartey et al. was to investigate if the cervicovaginal (CV) metabolome was different in asymptomatic women destined to have a pathogenesis of preterm birth (PTB) compared to term birth. A total of 313 biochemicals were analyzed and identified by GC/MS and UPLC MS/MS in CV fluid. 82 biochemicals were different in the 20-24 weeks (V1) in those destined to have a PTB compared to term birth while 48 were different at 24-28 weeks (V2). Changes in the CV metabolome may be observed weeks prior to any clinical symptoms. Understanding the CV metabolome may hold promise for unraveling the pathogenesis of PTB and may provide novel biomarkers to identify women most at risk [26].

Oliver et al. analyzed saliva and urine metabolomes using gas chromatography-time of flight mass spectrometry (GC-TOF MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) lipidomics approaches for samples from mothers and their infants through the first year of life. The data revealed most women had either a simple community with one highly abundant species of Lactobacillus or a more diverse community characterized by a high abundance of Gardnerella. Here, sequencing and metabolomic techniques used to show novel associations between vaginal microbes and metabolites during healthy pregnancy [27].

Borguna et al. conducted GC-MS and LC-MS analysis of metabolites on 39 subjects including13 HPV-negative [HPV (-)], 26 HPV-positive [LR-HPV (+)], and 14 high-risk HPV positive [HR-HPV (+)]. They found that compared with women with LR-HPV, the HR-HPV infected women had lower concentrations of amino acids, lipids and peptides in self-collected mid-vaginal swabs. The vaginal metabolome of HPV (+) women differed from HPV (-) women in terms of several metabolites, including biogenic amines, glutathione, and lipid-related metabolites. If the temporal relation between increased levels of reduced glutathione and oxidised glutathione and HPV incidence/persistence is confirmed in future studies, anti-oxidant therapies may be considered as a non-surgical HPV control intervention [28].

Conclusion and Prospects

Vaginal microbial ecosystem may be affected by many lifestyle and physiological changes, such as smoking, staying up late, menstrual cycle, pregnancy, menopause and other hormone changes. The correlation and interaction of different bacterial communities of vagina play an important role in the state of "health" and "disease" of women. When female vaginal micro-environmental bacteriums are in equilibrium, they can play a vital role in maintaining a healthy vaginal environment. After gynecological diseases occur, vaginal bacteria and vaginal metabolites will change significantly. The observed increased vaginal ecosystem stability during pregnancy may play an important functional role in reducing susceptibility to elevated infections that may lead to intrauterine infections and subsequent preterm birth.

The summary of the research methods shows above that the use of metabonomics to detect vaginal secretions or fluid of vagina become a possible trend to understand the relationship between reproductive tract health and diseases This methodology may be used as diagnostic markers or treatment of gynecological inflammation in the future. The use of metabonomics to explore and analyze reproductive tract diseases is a hot-spot field these years. The use of metabonomics can be seen to explore and analyze vaginal metabolism of gynecological diseases such as bacterial vascular disease, premature women, HPV infection and so on.

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