Informed Consent Form

Official Title: The Mechanism of Vaginal Flora and Its Metabolites in the Pathogenesis of Cervical Cancer

Application Official：The Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University

Version：1.0

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Dear madam

After the doctor checks, you meet the inclusion conditions of our project, we will invite you to voluntarily participate in the study named “The Mechanism of Vaginal Flora and Its Metabolites in the Pathogenesis of Cervical Cancer”. The purpose is to determine the general situation of individuals, vaginal microorganisms and their metabolites, to explore the correlation between vaginal flora and its metabolites and cervical cancer, and to build a risk assessment model of cervical cancer pathogenesis, and to screen the biomarkers related to the incidence of cervical cancer and therefore provide new ideas for the cervical cancer control.

1. Research Background

Cervical cancer is the fourth most common gynecological malignancy in the world, caused by carcinogenic human papillomavirus[1]. According to the statistics of the World Health Organization in 2018, there are nearly 570, 000 new cases of cervical cancer and about 311, 000 deaths worldwide every year. There are 106, 000 new cases of cervical cancer and about 48, 000 deaths in China every year. In recent years, a lot of data show that the incidence rate of cervical cancer in young women is increasing gradually. Cervical cancer is becoming younger and younger[2], which poses a great threat to the health of women.

At present, the etiology of cervical cancer is not clear. Studies at home and abroad shew that the occurrence of cervical cancer is related to the age of first sexual intercourse, the number of sexual partners, the age and frequency of first delivery, personal hygiene conditions, smoking, oral contraceptives and other factors[3-5]. It has been recognized that the persistent infection of high-risk human papillomavirus (HR-HPV) is the necessary conditions for cervical intraepithelial neoplasia (cervical intraepithelial neoplasia, CIN) and carcinogenesis. The progression from HPV infection to cervical cancer is slow and complex, which takes about 5-10 years, and only few patients eventually develop cervical precancerous or cancerous. Therefore, there may be other carcinogenic factors or cooperate with HPV in this process[6-7]. The factors increase the susceptibility of cervical epithelial cells to HPV, and increased expression of HPV virus. Therefore ensure the persistence of HPV infection, and finally lead to cancer. As an important factor to maintain the stability of vaginal microenvironment, whether vaginal flora plays a certain role in the process of HR-HPV continuous

infection has always been concerned.

2. Research Purpose (with clear objectives)

2.1 To explore the correlation between vaginal flora and its metabolites and cervical cancer, and to build a risk assessment model of cervical cancer pathogenesis;

2.2 Explore the mechanism of the development and conversion of vaginal flora and its metabolites in cervical cancer, and construct a prediction model of cervical cancer outcome;

2.3 Screen the biomarkers related to the incidence of cervical cancer and therefore provide new ideas for the screening and treatment of cervical cancer.

3. Introduction of the Clinical Research Project

This project is a clinical observational study, with no additional medication or surgical intervention for the study subjects. Only vaginal secretions and blood samples will be collected. The collection will be conducted simultaneously during the medical activities of the study subjects.

Vaginal secretions and blood sample will be collected in this study for the following tests: (1) Genital tract inflammation score: ELISA kit is used to detect the expression levels of 7 cytokines (IL-1α, IL-1β, IL-8, MIP-1β, CCL20, RANTES and TNF-α, etc.) in the vaginal secretions, and determine a cumulative score according to the level of each cytokine. (2) Blood inflammatory factors: Use ELISA kit to detect 7 kinds of inflammatory factors (IL-1α, IL-1β, IL-8, MIP-1β, CCL20, RANTES and TNF-α.) in the blood sample. (3) 16sDNA sequencing and biological information analysis: Extract DNA with a total bacterial DNA extraction kit, using bacterial DNA as a template, bacterial 16S rDNA V3~V4 variable regions as targets, and barcode-equipped universal primers for PCR amplification. The PCR products will be sequenced using Illumina NovaSeq sequencing technology. (4) The metabolite composition and content in vaginal secretions: The non-targeted metabolomics method is used to detect the metabolite composition and content in vaginal secretions.

This study intends to carry out a cross-sectional study and a cohort study. The cross-sectional study intends to recruit 300 premenopausal non-pregnant women, dividing them into five groups, with 60 in each group: HPV negative [Ctrl HPV (-)], HPV positive [Ctrl HPV (+)], low-grade squamous Intraepithelial lesion (LSIL group),

high-grade squamous intraepithelial lesion (HSIL group) and newly diagnosed invasive cervical cancer (ICC group). Obtain basic information through the questionnaire, and collect vaginal secretion and blood samples every time the patients review the clincal department as scheduled. At the same time, patients who are diagnosed with cervical cancer for the first time will be included in the cohort study. Collect the same kind of information. The follow-up period is set to be 3 years, and samples will be collected every six months. If any condition changes within the 3 years, samples should be collected. If new treatments are taken, samples should be taken before and after treatment. And if the lesion turns negative after treatment within the 3 years, complete the follow-up.

4. Clinical Research Process:

4.1 Sign the informed consent form

4.2 Conduct enrollment screening

4.3 During the study period, you also have some corresponding responsibilities, such as visiting the hospital and receiving examination on time. At the same time, you are also responsible for reporting to your doctor any changes in your physical and mental conditions during the study. Please be sure to inform your doctor of any other drugs you are currently using or used during the study. During the study, please do not use any other drugs for treatment. If you need other treatment, please contact your doctor in advance to obtain formal medical guidance.

5. Possible benefits

(1) Dynamically understand your own health status and important health hazard factors;

(2) The researcher will follow up the subjects in combination with laboratory examination, and provide appropriate health guidance to improve or promote the health of the subjects.

(3) The obtained vaginal flora analysis results and a comprehensive understanding of the composition of vaginal microbiota are helpful to guide the health regulation of subjects.

(4) Obtaining the analysis results of metabolites in vaginal secretion and a comprehensive understanding of the metabolites of vaginal microbiota are helpful to guide the health regulation of subjects.

6. Cost information related to this study

Subjects don`t need to pay any additional costs in this study, including purchase of a specimen collector, sample collection and testing.

7. Possible risks

(1) Risk of pregnancy

It is important that you are not pregnant or breastfeeding when you join the study, and pregnancy is not advocated during the study. You may not participate in this study if you are pregnant, planning to be pregnant or breastfeeding. If you are a female subject with fertility, the study doctor will ask you to provide a urine sample for pregnancy test before you start the study.

If you are a female subject with fertility, you must use reliable contraceptive methods during the study. The research doctor will tell you which contraceptive methods are acceptable. The following contraceptive methods are recommended: condoms with or without spermicide (a sperm killing drug), vaginal diaphragms or cervical caps with spermicide, or intrauterine devices (small contraceptive devices installed in women's uterine). Emergency contraceptive measures taken after unprotected sexual intercourse, such as emergency contraceptives, cannot be used as conventional contraceptive methods. If you find a positive pregnancy test result during your participation in the study, you should immediately inform the study doctor and need to agree to receive further follow-up examination. If you are confirmed to be pregnant, you need to terminate and withdraw from the study.

(2) Risk of sample collection

Some adverse reactions may occur during blood collection: subjects have symptoms of needle dizziness; symptoms of hypoglycemia such as dizziness, blurring of vision, weakness; infection caused by the use of contaminated medical supplies, etc.

Possible risks of vaginal secretion collection: local bleeding and secondary infection often accompanied by increased secretion, peculiar smell, lower abdominal pain, endless vaginal bleeding, and even lumbosacral pain.

In case of any adverse reaction caused by collecting blood samples and vaginal secretions, the doctor will provide you with active treatment. If medical event happens, it will be handled according to the medical event procedure.

8. Confidentiality measures

The results of this clinical study are only used for scientific research purposes. Therefore, your participation in the study and your personal data during the study are confidential and will be protected

in accordance with the law. Your name and identity will not be disclosed, and your name will not appear in any research reports and public publications. The hospital ethics committee and researchers have the right to access all your research data, including clinical observation tables, experimental data, etc.

9. Droit

This clinical study has been reviewed and approved by the medical ethics committee of the Sun Yat Sen Memorial Hospital of Sun Yat sen University. The scheme design meets the ethical requirements, which will ensure that your rights and interests will not be infringed in this study.

Your participation in this clinical study is entirely voluntary. You can refuse to participate or withdraw at any time without discrimination or retaliation, and your medical treatment and rights will not be affected. If you withdraw from the clinical study, for safety reasons, you should complete some corresponding medical examinations when you withdraw. If the doctor thinks you are not suitable to continue to participate during the study, the doctor has the right to stop your participation in order to protect your interests. In addition, during the study, you have the right to know the information related to the study at any time.

In case of any discomfort during the clinical study, please inform your research doctor immediately, and we will take corresponding medical measures in time; If you have a study related adverse event due to sampling, the investigator will carry out active treatment.

10. Detailed contact information

If you have any concerns or questions about participating in this study, or if you experience any abnormal reactions while participating in this study, or if there is an emergency, you should contact:

Doctor: Rao Qunxian phone number: 13902250700

If you have any complaints or concerns about the way the research doctors conducted the study or question your rights as a study subject, you can contact the following ethics committee person:

Name: Lin Shuangxiu phone code: 81332587

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Subjects declare

I have read the informed consent carefully, the researchers have informed me and answered my questions, I am fully aware of the following:

(1) As a subject, I will abide by the requirements of the instructions to subjects, voluntarily participate in this study, fully cooperate with the researchers, and truthfully and objectively provide the researchers with the health status and relevant information before participating in this study.

(2) The results of this clinical study are only used for scientific research purposes. Except for the ethics committee and researchers, My personal data participating in the study are confidential and will be protected in accordance with the law.

(3) I voluntarily participate in this study. If there are adverse reactions related to the study during the study period, I will receive proper and active treatment.

(4) My participation in this clinical study is entirely voluntary. I can refuse to participate in or withdraw from the study at any time without discrimination or retaliation, and my medical treatment and rights will not be affected.

(5) If I am a woman of childbearing age or my spouse is a woman of childbearing age, I will take one or more contraceptive measures during the study to avoid pregnancy. If I am pregnant, I will suspend the clinical study and terminate the pregnancy. If I continue to be pregnant, I will bear the consequences.

At the same time, I declare:

(1) I am willing to follow the research medication method;

(2) During the study, I am willing to cooperate with the doctor to visit the doctor within the specified time and do the corresponding examination;

(3) This informed consent has been received.

Subjects Sign: contact information:

Date: year month day

Signature of the Subject's Legal Agent (if necessary): contact information:

Date: year month day

Signature of the Witness Person (if necessary)： contact information:

Date: year month day

Investigator statement

I have fully explained and informed the purpose of the clinical study, study method, procedure and the possible risks and potential interests of the participant in the study and satisfactorily answered all relevant questions to the subject.

Investigator (informing the subject) signature： contact information:

Date: year month day