Study on Proteomic and Microbiome Changes in Patients With Hepatic Encephalopathy

Undertaking unit: the First Affiliated Hospital with Nanjing Medical University

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Informed consent form

Dear Sir/Madam,

We invite you to participate in the research project "Clinical Study on Metabolomic and Microbiome Changes in Patients with Hepatic Encephalopathy" approved by Jiangsu Provincial People's Hospital (source of the project). It is expected that 32 subjects will voluntarily participate in this study, which will be divided into two groups: the liver cirrhosis group without HE and the HE (hepatic encephalopathy) group. This study has been reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Jiangsu Provincial People's Hospital), with the ethics review number: 2020-SR-459.

Why is this research carried out? Hepatic encephalopathy (HE) is a neuropsychiatric syndrome of varying degrees of severity based on metabolic disorders, caused by severe acute or chronic liver function disorders or various portal veno-systemic shunt (hereinafter referred to as portal veno-systemic shunt) abnormalities. The main clinical manifestations are consciousness disorders, behavioral abnormalities and coma. At present, there are over 7 million patients with liver cirrhosis in China, and the number of patients is constantly increasing. Due to its poor therapeutic effect and high mortality rate, it has increasingly attracted the attention of many researchers. The ammonia poisoning theory and the inflammatory response theory are the classic theories of HE. At present, the treatment mainly aimed at reducing ammonia has an unsatisfactory effect on some patients. Further exploration of the pathogenesis of HE may help to better improve the prognosis of patients.

Metabolomics is an important component of systems biology and a new type of "omics" that emerged after genomics, proteomics, and transcriptomics, aiming to quantitatively describe the multi-parameter changes of metabolites in organisms. Its technical means can be used to study the types, quantities and variation patterns of all metabolites and their intermediates in living organisms.

The metabolites in the body fluids of patients with HE and healthy individuals were detected and analyzed in order to identify the biomarker group of HIS metabolites, which is helpful for guiding clinical diagnosis and treatment, as well as conducting in-depth research on the pathogenesis of HE. The changes of intestinal flora and intestinal microenvironment in patients with liver cirrhosis are closely related to the occurrence of HE. For instance, excessive growth of small intestinal bacteria, changes in the composition and function of the intestinal flora, and damage to the intestinal mucosal barrier can lead to pathological flora transmigration, causing an increase in the concentration of enterogenic toxins such as ammonia, lipopolysaccharides, and indole in the peripheral circulation, and intensifying systemic inflammatory responses. On the one hand, this aggravates liver damage, reduces the liver's metabolism of enterogenic toxins, and increases the amount of ammonia entering the brain tissue. On the other hand, it disrupts the blood-brain barrier, aggravating neuroinflammation and brain tissue edema. In addition, regulating the intestinal flora through lactulose, rifaximin and probiotic preparations can effectively improve the cognitive function

of patients with HE.

At present, there are few studies on gut microbiota and HE, and different studies have reached different conclusions due to differences in detection methods, study populations, and intervention approaches. Studies on the intestinal flora of patients with hepatic encephalopathy have shown that the numbers of Lactobacillus and Enterococcus in the non-hepatic encephalopathy group are significantly lower than those in the healthy population. The number of bifidobacteria in the hepatic encephalopathy group was significantly lower than that in the non-hepatic encephalopathy group and healthy people, while the number of Escherichia coli was significantly higher than that in the non-hepatic encephalopathy group and healthy people. Maintaining the balance of intestinal microecology is an important strategy for the treatment of hepatic encephalopathy.

For this purpose, this study intends to compare the differences in the gut microbiome, serum proteome and metabolome among the healthy population group, the non-HE group of liver cirrhosis and the HE group through 16s rDNA sequencing and non-targeted metabolomics analysis, and screen out the characteristic metabolome and microbiome of patients with hepatic encephalopathy to guide clinical diagnosis and treatment. Meanwhile, mass flow cytometry was used to analyze the immune cell typing of patients in different groups and evaluate the inflammatory response status, so as to search for cell markers in the pathogenesis of HE and conduct in-depth research on the pathogenesis of HE.

What do I need to do if I participate in the research? If you are willing to participate in this study, your blood (3ml) and fecal (2g) samples will be collected for 16s rDNA sequencing and non-targeted metabolomics analysis. The test specimens will be destroyed immediately after completion. The testing institution is Jiangsu Aoyin Medical Technology Co., LTD. The test report has no definite clinical significance. Do not inform the patient. The testing fee shall be paid by the researcher. That is, if you do not participate in this study, you do not need to undergo this examination/treatment.

Who is suitable (not suitable) to participate in the research?

(1) Liver cirrhosis without HE group. Inclusion criteria: Liver cirrhosis without HE, referring to the "Consensus on Diagnosis and Treatment of Hepatic Encephalopathy in China". No history of anti-cancer treatment and no other serious diseases. Exclusion criteria: Use of antibiotics, prebiotics, probiotics and proton pump inhibitors within three months; Those with unstable vital signs; Those who are unwilling to cooperate.

(2) HE group, inclusion criteria: CHE patients with liver cirrhosis, referring to the "Consensus on Diagnosis and Treatment of Hepatic Encephalopathy in China"; No history of anti-cancer treatment, no severe heart, lung, brain or kidney diseases, or severe diabetic complications. Exclusion criteria: Use of antibiotics, prebiotics, probiotics and proton pump inhibitors within three months; Those with unstable vital signs; Those who are unwilling to cooperate.

What are the risks of participating in a study? During the blood drawing process, the

vascular characteristics and coagulation functions of different individuals vary. Moreover, for patients taking anticoagulants, if the pressing time and Angle are inappropriate after puncture, it may lead to subcutaneous congestion, cyanosis, subcutaneous induration and other conditions. If any discomfort or adverse reactions occur, please contact the research doctor in a timely manner.

What are the benefits of participating in research? This study did not directly benefit the participants.

What other treatment options are there if one does not participate in this study? 1. General treatment: Eliminating the triggers of hepatic encephalopathy is the fundamental principle of its general treatment and also the basis for other drug treatments, including the following measures. (1) Adjust the diet structure. (2) Use sedatives with caution. (3) Correct electrolyte and acid-base balance disorders. (4) Stop bleeding and clear intestinal blood accumulation. (5) Others: If the patient has hypoxia, oxygen inhalation should be given. For those with hypoglycemia, hypertonic glucose can be injected intravenously. If there is an infection, it should be controlled in time. 2. Drug treatment: (1) Reduce the production and absorption of ammonia in the intestine: lactulose (β -galactose-fructose) (2) Promote the metabolism of ammonia in the body: L-ornithine - L-aspartic acid (3) GABA/BZ (γ -aminobutyric acid/benzodiazepine) complex receptor antagonist flumacinib (4) reduces or antagonizes the pseudoneurotransmitter branched-chain amino acids (BCAA) 3. Symptomatic treatment.

Do I need to pay any relevant fees to participate in the research? The costs of 16s rDNA sequencing and non-targeted metabolomics analysis in this study were paid by the researchers. If you also have the treatment and examination required for other diseases, as well as the cost of switching to other treatments due to ineffective treatment, it will not be covered by the free service. This study focuses on inpatients and does not provide compensation for transportation expenses. In the event of any damage related to the test, corresponding treatment and compensation will be provided in accordance with relevant national regulations.

Is personal information confidential? Your medical records will be kept at the hospital, and researchers, research authorities, and ethics committees will be permitted to access your medical records. Any public reports regarding the results of this study will not disclose your personal identity. We will make every effort to protect the privacy of your personal medical information within the scope permitted by law.

Must I take part in the research? Participation in this study is entirely voluntary. You can refuse to join the study or withdraw from it at any time during the study process, and this will not affect the treatment provided by your doctor. If you decide to withdraw from this study, please contact your doctor. You may be required to undergo relevant examinations, which are beneficial for protecting your health.

If you have any questions related to your personal rights and interests, you can contact

the Ethics Committee of this court. The contact number is 025-68306360.

Subject Declaration: I have read the above introduction about this study and have a full understanding of the risks and benefits that may arise from participating in this study. I voluntarily participate in this research. I will receive a copy of this informed consent form signed with my name and date.
I agree or refuse that other studies except this one may use my medical records and pathological examination specimens.
Subject signature: Date: __ __ __ __ year _ _ month _ _ day
Contact phone number of the subject: Mobile phone number

(Where applicable) Signature of legal representative/Witness: Date: __ __ __ __ year _ _ month _ _ day
Contact number of the legal representative/witness: Mobile phone number:

Researcher's statement: I confirm that I have explained the details of this study to the patient, especially the risks and benefits that may arise from participating in this study, and answered all relevant questions from the subject, who voluntarily agreed to participate in this study. This informed consent form is in duplicate. The researcher and the subject each keep one signed informed consent form.
Study physician Signature: Date: __ __ __ __ year _ _ month _ _ day
Research doctor's working phone number: Mobile phone number: