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CLINICAL STUDY

# An magnetic resonance-based plasma metabonomic investigation on abnormal Savda in different complicated diseases

Batur Mamtimin, Mawlanjan Hizbulla, Nazuk Kurbantay, Li You, Xinghai Yan, Halmurat Upur

**Batur Mamtimin, Mawlanjan Hizbulla, Nazuk Kurbantay, Halmurat Upur,** College of Pharmacy, Xinjiang Medical University, Urumqi 830011, China
**Li You, Xinghai Yan,** College of Traditional Chinese Medicine, Xinjiang Medical University, Urumqi 830011, China
**Supported by** China National Natural Science Foundation Program (No. 81160459)
**Correspondence to: Prof. Halmurat Upur** and **Prof. Batur Mamtimin,** College of Pharmacy, Xinjiang Medical University, Urumqi 830011, China. halmurat@263.net; batur72@163.com
**Telephone:** +86-991-4365034
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## Abstract

**OBJECTIVE:** To provide potential evidence for the existence of abnormal Savda, we assessed host metabonomic responses and dynamic changes occurring in various diseases using $^{1}$H nuclear magnetic resonance (NMR)-based metabonomics.

**METHODS:** Plasma samples taken from patients with complicated diseases with abnormal Savda ($n$= 140, including 35 cases each of diabetes, asthma, breast cancer, and cervical carcinoma) and from healthy controls ($n$=35) were analyzed by $^{1}$H NMR (600 MHz), and the spectral profiles were analyzed by multivariate analysis using orthogonal projection to latent structure with discriminant analysis.

**RESULTS:** Supervised modeling of the data provided very good discrimination between patients and healthy controls. Compared with the healthy controls, the patient groups with different disease conditions displayed similar metabolic changes, characterized by lower creatine, creatinine, lactate, and amino acid levels (including isoleucine, leucine, valine, alanine, and 1-methylhistidine) and higher lipid levels (very low-density lipoproteins and unsaturated lipids). Additionally, cancer patients (breast and cervical) showed decreased myo-inositol, α-glucose, and β-glucose, and increased pyruvate and carnitine in plasma.

**CONCLUSION:** The data indicate that decreased oxidative defense, liver function abnormalities, amino acid deficiencies, and energy metabolism disorders are common characteristics of complicated diseases, which may be related to the formation of abnormal Savda.



**Key words:** Metabonomics; Magnetic resonance spectroscopy; Abnormal Savda

## INTRODUCTION

Traditional Uighur medicine holds that there are four kinds of humors (body fluids) in the human body, namely Savda, Belghem, Sapra and Kan, which are associated with the fundamental elements of earth, fire, water and air, respectively. Humors are in balance when a person is healthy. However, excess or deficiency of one of the four humors, called "abnormal humor," may result in disease. With a history spanning 2500 years, traditional Uighur medicine has developed a unique system for the diagnosis and treatment of illnesses. The body fluid theory in traditional Uighur medicine suggests that body fluid is the basic substance of vital life and physiological activity. Physiological activity is produced and used continuously, which provides energy for vital activities. In addition, there is a

corresponding balance, inter-restriction, and complementary system between the different kinds of body fluids. When abnormal changes occur in the body fluids, the normal body fluids in turn have certain abnormal characteristics and are termed "abnormal body fluids," forming the basis of various diseases.[1,2] According to the degree of the abnormal change and disease symptoms, abnormal body fluid is divided into abnormal Savda, abnormal Belghem, abnormal Sapra, and abnormal Kan. Uighur medicine considers diseases with abnormal Savda as a special syndrome, which is the foundation of complicated diseases, such as tumors, diabetes, and asthma.[3-5] Abnormal Savda is a pathological product resulting from the combustion of different body fluids, namely Belghem, Sapra and Kan. Abnormal Savda often occurs in complicated diseases; therefore, diseases caused by abnormal Savda are usually intractable, and generally considered a special syndrome.[1,3,6] According to the theory of traditional Uighur medicine, the complicated diseases may have similar biochemical foundations, and this is the key to disease prevention and treatment.[1,3,7]

Metabonomics is a quantitative measurement of the dynamic and multiparametric metabolic responses of living systems to pathophysiologic stimuli or genetic modification.[8] Nuclear magnetic resonance (NMR) is a new, efficient, reproducible, and non-destructive method that is widely used in metabonomic studies for acquiring the metabolic profiles of biological specimens without extensive sample preparation. Compared with other analytical techniques, NMR has shown promise in the identification of disease biomarker profiles with diagnostic and prognostic values.[9-12]

The plasma of a patient principally contains all the information about the pathological changes in metabolite content, which in turn reflects abnormalities in the functions of multiple organs and tissues, and provides insight into the underlying molecular mechanisms. We believe that if abnormal Savda results from a metabolic abnormality, abnormal metabolic products will appear in body fluids, such as blood plasma. Hence, in the present study, we investigated metabonomic changes in blood plasma using $^1$H NMR spectroscopy with multivariate data analysis. The main purpose was to define host metabonomic responses to dynamic changes under different disease conditions, and to identify the metabolic signatures of some complicated diseases. This information may prove valuable for finding common characteristics shared by different disease conditions, potentially establish evidence for the existence of abnormal Savda, and aid in better understanding the pathophysiology of abnormal Savda described in traditional Uighur medicine. Plasma samples taken from patients with complicated diseases ($n$=140, including 35 cases each of diabetes, asthma, breast cancer, and cervical carcinoma) and from healthy controls ($n$=35) were analyzed by $^1$H NMR, and the spectral profiles were analyzed by multivariate analysis.

## MATERIALS AND METHODS

### *Reagents*

Analytical grade $Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot 12H_2O$ were purchased from Guoyao Chemical Co., Ltd. (Shanghai, China). Deuterium oxide ($D_2O$) containing 0.05% sodium 3-trimethlysilyl [2,2,3,3-$^2H_4$] propionate acid was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

### *Sample preparation*

A total of 140 cases of complicated diseases with abnormal Savda syndrome, including 35 cases each of diabetes, asthma, breast cancer, and cervical carcinoma, were enrolled in the study. Diagnosis of each disease condition was clinically made at the First Affiliated Hospital of Xinjiang Medical University during the period from June 2010 to June 2011. Abnormal Savda syndrome was confirmed by the theory of traditional Uighur medicine.[1,3] Thirty-five healthy volunteers were selected based on their medical examinations and served as the control group. Patients with cardiovascular disease, hepatic disease, renal disease or inflammatory disease, and pregnant women were excluded. The average age of participants was (46±12) years, ranging from 20-65 years. Blood samples were collected from study participants before breakfast. Plasma was obtained by centrifugation of blood samples at 3500 ×$g$ for 5 min, and then immediately stored at - 80℃ until used for NMR spectroscopy.

The study protocol was approved by the Ethics Committee of Xinjiang Medical University. All participants gave written informed consent to participate in the study.

### *$^1$H NMR spectroscopy of plasma*

Plasma samples for NMR analysis were prepared by mixing 200 μL plasma with 400 μL saline buffer solution (0.045 M $NaH_2PO_4$+0.045 M $K_2HPO_4$ in 20% v/v $D_2O$ and 80% v/v $H_2O$, pH 7.4). The plasma-saline mixture was kept at room temperature for 10 min, and then further centrifuged at 10 000 ×$g$ for 10 min. The clear supernatant (550 μL) was transferred into a 5-mm NMR tube for spectroscopic analysis. The plasma $^1$H NMR spectra of each sample was recorded under the Carr-Purcell-Meboom-Gill pulse sequence [RD − 90° − (τ − 180° − τ)$_n$-AQ] using a Varian Unity Inova600 NMR spectrometer with an ID probe operating at a proton frequency of 599.95 MHz. For each sample, 128 scans were obtained from 32 768 data points over a spectral width of 10 000 Hz, which resulted in an acquisition time of 1.64 s and relaxation delay of 2 s at 298 K. For assignment purposes, selected samples were subjected to two-dimensional NMR, including the $^1$H-$^1$H homonuclear correlation spectroscopy (COSY) test, the total correlation spectroscopy (TOCSY) test, and the J-resolved (J-Res) test.[13-15]

### *Data analysis*

Before Fourier transformation, free induction decays