Postural Orthostatic Tachycardia Syndrome with Increased Erythrocytic Hydrogen Sulfide and Response to Midodrine Hydrochloride

Jinyan Yang, MM[1], Juan Zhao, MD[1], Shuxu Du, MD[2], Die Liu, MD[1], Chunhin Fu, MM[3], Xueying Li, MM[4], Stella Chen, MM[5], Chaoshu Tang, PhD[6], Junbao Du, PhD[1,7], and Hongfang Jin, PhD[1]

Objectives To evaluate the use of erythrocytic hydrogen sulfide (H_2S) in predicting the therapeutic efficacy of midodrine hydrochloride for children with postural orthostatic tachycardia syndrome (POTS).

Study design Fifty-five children were included in this study, involving 28 children with POTS (POTS group) and 27 healthy children (control group). Children in the POTS group received midodrine hydrochloride treatment. Erythrocytic H_2S production was measured; a receiver operating characteristic curve was used to assess if erythrocytic H_2S could predict the therapeutic response to midodrine hydrochloride treatment.

Results H_2S production from erythrocytes was significantly higher in the POTS group than in the control group ($P < .01$). H_2S production was also significantly higher in responders to midodrine hydrochloride than in nonresponders ($P < .05$). The change in symptom score and baseline erythrocytic H_2S production had a positive linear relationship ($P < .01$). There was also a positive correlation with the change in heart rate ($P < .05$). The receiver operating characteristic curve showed an area under curve value of 0.813. Erythrocytic H_2S production yielded a sensitivity of 78.9% and a specificity of 77.8% in predicting the efficacy of midodrine hydrochloride therapy for children with POTS.

Conclusion Erythrocytic H_2S could serve as a useful predictor of therapeutic response to midodrine hydrochloride in children with POTS. *(J Pediatr 2013;163:1169-73)*.

Postural orthostatic tachycardia syndrome (POTS),[1-3] which is a form of chronic orthostatic intolerance (OI), is often associated with a significant tachycardia after rising to an upright position, and has a serious impact on a patient's quality of life.[4] Stewart reported that more than 500 000 Americans suffer from POTS.[5]

The exact cause of POTS is unknown, which makes clinical treatment difficult. Excessive reduction of blood volume is considered to be one of the mechanisms responsible for the symptoms of POTS.[6] Previous studies suggested that POTS may include abnormal vascular relaxation.[7] In 2011, Chen et al showed that midodrine hydrochloride, an alpha-1 adrenoreceptor agonist that constricts peripheral arteries and veins, was effective in the treatment of children with POTS,[8] but it was only effective in 68% of patients. Therefore, a clinical method that can predict the responsiveness of patients to midodrine hydrochloride would be helpful.

Hydrogen sulfide (H_2S) is a new vasodilating gasotransmitter.[9,10] Because excessive vasodilation is thought to be one of the mechanisms for POTS, a possible link between H_2S and POTS may exist. Zhang et al reported that the plasma content of H_2S increased noticeably in children with POTS.[11] Geng et al reported that endogenous H_2S was primarily released from erythrocytes and that erythrocytic H_2S production and serum H_2S content were positively correlated in hypertensive patients.[9] This suggests that erythrocytic H_2S production could contribute to the serum H_2S level. The H_2S production by erythrocytes may mirror changes in the body's internal environment. The present study was designed to examine if erythrocytic H_2S production could serve as a predictor for the therapeutic response to midodrine hydrochloride in children with POTS.

Methods

Fifty-five children were included in the study, of whom 28 children suffered from POTS (POTS group) and 27 were healthy children serving as a control group. The 28 children in the POTS group consisted of 12 boys and 16 girls (age 11.5 ± 2.5 years) who were admitted to the Department of Pediatrics, Peking

AUC	Area under curve
HR	Heart rate
H_2S	Hydrogen sulfide
HUT	Head-up test
OI	Orthostatic intolerance
POTS	Postural orthostatic tachycardia syndrome

From the [1]Department of Pediatrics, Peking University First Hospital; [2]Department of Pediatrics, the Capital Medical University, Shijitan Hospital, Beijing, PR China; [3]Department of Biochemistry, Hong Kong University of Science and Technology, Hong Kong, PR China; [4]Department of Statistics, Peking University First Hospital, 100034, Beijing, PR China; [5]Department of Biochemistry and Cellular Biology, University of California, San Diego, La Jolla, CA; [6]Department of Physiology and Pathophysiology, Peking University Health Sciences Centre; and [7]Key Laboratory of Molecular Cardiology, Ministry of Education, Beijing, PR China

Supported by the National Twelfth 5-Year Plan for Science and Technology Support (2012BAI03B03), the Major Basic Research Project of China (2012CB517806 and 2011CB503904), and the National Natural Science Foundation of China (81121061). The authors declare no conflicts of interest.

 http://dx.doi.org/10.1016/j.jpeds.2013.04.039

University First Hospital between July 2011 and February 2012. The 27 children in the control group (17 boys and 10 girls; age 10.4 ± 1.8 years) were classified as healthy on the basis of their medical history, physical examination, and electrocardiogram. The study protocol conformed to the criteria of the Ethics Committee of Peking University First Hospital.

The diagnostic methods and standard definition of POTS were based on previously published literature.[1-3,6,12-15] The head-up test (HUT) is the standard method for detecting POTS. The examinations were performed in a quiet environment. Heart rate (HR) and blood pressure of the patients were monitored by a Dash 2000 Multi-Lead Physiological Monitor (GE Company, New York, New York). After 10 minutes in the supine position, the patient was brought to a standing position for 10 minutes or until symptoms of OI appeared. The diagnostic criteria of POTS used in this study is an increase in HR of ≥30 bpm or a HR over 120 bpm accompanied by the symptoms of OI during the 10-min HUT.[1-3,6,12-15]

The course of midodrine hydrochloride therapy in this study was 2.5 mg per day for 1.5 to 7 (3.4 ± 1.5) months. The 28 children in the POTS group were followed up in the clinic for a period of 1.5-8 (5.4 ± 1.7) months. To evaluate the therapeutic effect of the drug, the number and frequency of symptoms of each patient were recorded. Also, HUT was repeated. If a patient had a post-treatment HR increase lower than 30 bpm during HUT, he or she was determined to be a responder, whereas a patient with HR increases over 30 bpm was classified as a nonresponder.

Symptom scoring was used to evaluate the therapeutic effect of midodrine hydrochloride.[16-18] The scoring was based on the typical symptoms of OI, including syncope, dizziness or light-headedness, chest tightness, nausea, palpitation, headache, tremor, sweating, blurred vision, and concentration difficulties. A numerical value for each symptom was determined by its frequency, as shown in Table I (available at www.jpeds.com). The total score was the sum of all the symptom scores. The symptom scoring was performed for each participant both at the beginning and at the end of therapy with midodrine hydrochloride.

After 8 hours or more of fasting, blood was collected by venipuncture into a tube with heparin.[19] The blood was centrifuged at 800 × g for 7 minutes at 4°C and the plasma and leukocytic cream were removed. The erythrocytes were washed twice with 0.1 mol/L phosphate buffered saline and centrifuged at 800 × g for 5 minutes at 4°C. Cells were counted and the erythrocytes were stored in the refrigerator until the assay was performed.

Erythrocytes, 1×10^8, were lysed in 900 μL of ice-cold *Tris*-hydrochloric acid (50 mmol/L, pH 7.4), and then ultrasound cracking was performed for 15 seconds.[19] The erythrocyte lysate was transferred to a 25 mL Erlenmeyer flask and 100 μL of beta-mercaptopyruvate (Sigma, St. Louis, Missouri) was added for a final concentration of 2 mmol/L. Central wells contained 0.5 mL of 1% sodium hydroxide as a trapping solution. The flasks were sealed quickly with a double layer of parafilm and incubated in a 37°C shaking water bath for 60 minutes, after which 0.5 mL of 20% trichloroacetic acid was added to stop the reaction. The flasks were sealed again and placed in the shaking water bath for another 1 hour at 37°C to ensure complete trapping of H_2S. Erythrocytic H_2S production was measured by sensitive sulphur electrode. The H_2S production was expressed as unit nmol/min/10^8 erythrocytes.

Statistical Analyses

Statistical analysis was completed by SPSS v. 14.0 software; (SPSS Inc, Chicago, Illinois). Continuous variables are presented as (mean ± SD). Discrete data are presented as cases. Comparisons between the POTS group and the control group were performed using a 2-sample *t* test. The paired *t* test was used for comparing data from before and after treatment. A value of $P < .05$ was considered significant. A receiver operating characteristic curve was used to evaluate the predictive value of erythrocytic H_2S production in assessing the therapeutic effect of midodrine hydrochloride. The area under curve (AUC) indicated the predictive value of erythrocyte H_2S production. That the 95% CI of AUC did not contain 0.5 or a *P* value <.05 confirmed that erythrocyte H_2S production was a reliable predictor of the therapeutic effect of midodrine hydrochloride in treating children with POTS. An AUC 0.5-0.7 indicates a low predictive value; AUC 0.7-0.9 indicates a moderate predictive value, and an AUC > 0.9 indicates a high predictive value.[20-23]

Results

General patient information, including age, sex ratio, height, body weight, supine blood pressure, and HR are listed in Table II (available at www.jpeds.com). The pretreatment H_2S production from erythrocytes was significantly higher in the POTS group than in the control group ($P < .001$, Table II).

In the POTS group, 28 patients received therapeutic doses of midodrine hydrochloride (2.5 mg per day). All of the 28 patients were rescored and HUT was performed again after 3 months of treatment. Their symptom scores and frequency of tachycardia during HUT before treatment were decreased significantly ($P < .001$, Table III).

Of the 28 patients in the POTS group receiving midodrine hydrochloride as therapy, 19 showed HR increases <30 bpm during HUT after treatment and were classified as responders. The erythrocytic H_2S production in responders

Table III. Comparisons of symptom scores, changes of HR in children with POTS treated by midodrine hydrochloride

Treatment	*n*	Symptom score	△HR (bpm)*
Pre-treatment	28	8.3 ± 4.1	39.7 ± 10.1
Post-treatment	28	3.8 ± 4.1	26.2 ± 6.7
Difference†	28	4.5 ± 3.2	13.5 ± 12.3
P	-	<.001	<.001

* △HR, the increased HR during HUT.
†Pre-treatment minus post-treatment.