Altered serous levels of monoamine neurotransmitter metabolites in patients with refractory and non-refractory depression

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Abstract
The study examined plasma metabolite changes of monoamine neurotransmitters in patients with treatment-resistant depression (TRD) and non-TRD before and after therapy. All 30 TRD and 30 non-TRD patients met the diagnostic criteria for a depressive episode in accordance with the International Classification of Diseases, Tenth Revision. Before treatment, and at 4, 6, and 8 weeks after treatment, the plasma metabolite products of monoamine neurotransmitters in TRD group, including 5-hydroxyindoleacetic acid, 3-methoxy-4-hydroxyphenyl ethylene glycol and homovanillic acid, were significantly lower than those in the non-TRD group. After two types of anti-depressive therapy with 5-serotonin and norepinephrine reuptake inhibitor, combined with psychotherapy, the Hamilton Depression Rating Scale scores were significantly reduced in both groups of patients, and the serous levels of 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenyl ethylene glycol were significantly increased. In contrast, the homovanillic acid level exhibited no significant change. The levels of plasma metabolite products of peripheral monoamine neurotransmitters in depressive patients may predict the degree of depression and the therapeutic effects of treatment.

Key Words
treatment-resistant depression; monoamine neurotransmitter; 5-hydroxyindoleacetic acid; 3-methoxy-4-hydroxyphenyl ethylene glycol; homovanillic acid

Abbreviations
TRD, treatment-resistant depression; 5-HIAA, 5-hydroxyindoleacetic acid; MHPG, 3-methoxy-4-hydroxyphenyl ethylene glycol; HVA: homovanillic acid; HAMD, Hamilton Depression Rating Scale

INTRODUCTION
Treatment-resistant depression (TRD) has unclear etiology and pathogenic mechanisms. Li et al.[1] reported that there were significant differences in the levels of adrenocorticotropic hormones between TRD patients and non-TRD patients, indicating more severe damage to the hypothalamus-pituitary-adrenal cortex in TRD patients. In terms of the etiology of depression, the monoamine neurotransmitter hypothesis has been confirmed by many studies. According to this hypothesis, depression is closely related to a reduction in monoamine neurotransmitters[2-5]. Most currently used anti-depressive drugs aim to improve the levels of monoamine neurotransmitters[6]. Many previous studies have focused on changes in the plasma levels of monoamine neurotransmitters in depressive patients who were successfully treated for the first time[7]. However, there is little evidence of a correlation between the serous level of monoamine neurotransmitter metabolites and the degree of depression in TRD patients.
and antidepressant treatment in TRD patients after treatment with drugs promoting monoamine neurotransmitter levels.

The present study examined changes in the plasma metabolite levels of monoamine neurotransmitters in TRD patients and non-TRD patients before and after antidepressant treatment, and the correlation with depression severity.

RESULTS

Quantitative analysis of subjects
A total of 60 depressive patients, including 30 TRD and 30 non-TRD patients, were included in this study, for an 8-week treatment period. All patients entered the final analysis.

Baseline information
Among 60 depressive patients, there were 19 males and 41 females. There were no significant differences in gender, age, educational level or marital status between the two groups of patients ($P > 0.05$; Table 1).

| Item                  | TRD group ($n = 30$) | Non-TRD group ($n = 30$) | $t$-value | $P$-value |
|-----------------------|----------------------|-------------------------|-----------|-----------|
| Sex [n(%)]            |                      |                         | 0.693     | 0.405     |
| Male                  | 8 (26.7)             | 11 (36.7)               |           |           |
| Female                | 22 (73.3)            | 19 (63.3)               |           |           |
| Age (x±s, yr)         | 46.53±11.45          | 42.73±15.94             | 1.060     | 0.293     |
| Education [n(%)]      |                      |                         | 1.667     | 0.644     |
| Primary school and lower | 10 (33.3)            | 6 (20.0)                |           |           |
| Middle school         | 6 (20.0)             | 9 (30.0)                |           |           |
| Senior high school and secondary technical school | 7 (23.3) | 7 (23.3) |           |           |
| College and higher    |                      |                         | 4.949     | 0.176     |
| Marital status [n(%)] |                      |                         |           |           |
| Unmarried             | 1 (3.3)              | 6 (20.0)                |           |           |
| Married               | 26 (86.7)            | 20 (66.7)               |           |           |
| Divorced              | 1 (3.3)              | 1 (3.3)                 |           |           |
| Widowed               | 2 (6.7)              | 3 (10.0)                |           |           |

TRD: Treatment-resistant depression.

Plasma metabolite products of monoamine neurotransmitters in two groups
Levels of 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenyl ethylene glycol (MHPG) and homovanillic acid (HVA) were determined with enzyme-linked immunosorbent assay. Compared with non-TRD patients, serous levels of 5-HIAA, MHPG and HVA were significantly reduced in TRD patients before treatment and at 4, 6, and 8 weeks after treatment ($P < 0.01$). As treatment time proceeded, plasma metabolites of monoamine neurotransmitters, including 5-HIAA, MHPG and HVA, gradually increased in all patients. After treatment, levels of 5-HIAA and MHPG were significantly higher ($P < 0.01$), while HVA levels were not significantly higher than those before treatment ($P > 0.05$; Table 2).

| Group                         | Before treatment | 4 weeks after treatment | 6 weeks after treatment | 8 weeks after treatment |
|-------------------------------|------------------|-------------------------|-------------------------|-------------------------|
| Treatment-resistant depression | 12.8±1.1ab       | 92.7±10.1a             | 20.3±2.4a               |                         |
| Non-treatment-resistant depression | 14.7±1.0ab     | 94.8±10.2ab            | 20.8±2.6ab              |                         |
| Treatment-resistant depression | 17.4±1.5ab      | 131.4±14.1ab           | 21.8±2.6ab              |                         |
| Non-treatment-resistant depression | 21.4±2.2ab     | 151.7±15.4ab           | 22.7±2.9ab              |                         |

Data were expressed as mean ± SD, for 30 patients in each group.

| Group                          | Before treatment | 4 weeks after treatment | 6 weeks after treatment | 8 weeks after treatment |
|-------------------------------|------------------|-------------------------|-------------------------|-------------------------|
| Treatment-resistant depression | 23.9±1.7       | 108.3±19.6              | 45.7±10.6               |                         |
| Non-treatment-resistant depression | 28.9±9.7b     | 134.3±23.7b            | 46.6±9.7               |                         |
| Treatment-resistant depression | 33.7±9.9b      | 172.0±25.1b            | 47.6±8.6               |                         |
| Non-treatment-resistant depression | 39.7±9.6b     | 221.1±26.6b            | 48.8±7.5               |                         |

Data were expressed as mean ± SD, for 30 patients in each group.

Hamilton Depression Rating Scale (HAMDS) scores in two groups before and after treatment
The HAMDS results revealed that TRD patients exhibited significantly higher scores than non-TRD patients before treatment and at 4, 6, and 8 weeks after treatment ($P < 0.01$). In addition, HAMDS scores in all patients gradually decreased as treatment progressed ($P < 0.01$; Table 3).

| Group                          | Before treatment | 4 weeks after treatment | 6 weeks after treatment | 8 weeks after treatment |
|-------------------------------|------------------|-------------------------|-------------------------|-------------------------|
| Treatment-resistant depression | 34.4±4.4a       | 20.6±4.0ab              |                         |                         |
| Non-treatment-resistant depression | 28.3±3.6       | 17.6±4.4b              |                         |                         |

Data were expressed as mean ± SD, for 30 patients in each group.

Comprehensive treatment effects in two groups
The rate of decrease in mean HAMDS score was used to assess treatment effects. Excellent treatment response was defined as a reduction rate of greater than or equal to 50%, while effective treatment was defined as a reduction rate greater than or equal to 25% [8]. After a
4-week period of treatment and psychological intervention, the reduction rate of mean HAMD scores was 45.41% in the TRD group, and 44.81% in the non-TRD group, suggesting that comprehensive therapy was effective in the two groups (Table 4).

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**Table 4** Reduction rate of Hamilton Depression Rating Scale scores in two groups before and after treatment

| Time                          | Treatment-resistant depression | Non-treatment-resistant depression |
|-------------------------------|--------------------------------|-----------------------------------|
| 4 weeks after treatment       | 20.57±4.04                     | 17.63±4.45                       |
| 8 weeks after treatment       | 11.23±3.22                     | 9.73±3.06                        |
| Difference                    | 9.34                           | 7.9                              |
| Reduction rate (%)            | 45.41                          | 44.81                            |

Data were expressed as mean ± SD, for 30 patients in each group. Reduction rate = (mean before intervention – mean after intervention)/mean before intervention.

**Correlation analysis on plasma metabolites of monoamine neurotransmitters in two groups before treatment**

Results of a correlation analysis revealed that there was no correlation between depression and levels of 5-HIAA, MHPG, or HVA in either group ($P > 0.05$; Tables 5, 6).

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**Table 5** Correlation with 5-HIAA, MHPG, HVA levels in treatment-resistant depression group ($r$)

| Plasma monoamine neurotransmitter metabolites | 5-HIAA | MHPG | HVA |
|----------------------------------------------|--------|------|-----|
| 5-HIAA                                       | 1.000  | 0.268| -0.103|
| MHPG                                         | 0.268  | 1.000| -0.066|
| HVA                                          | -0.103 | -0.066 | 1.000|

$P > 0.05$. 5-HIAA: 5-hydroxyindoleacetic acid; MHPG: 3-methoxy-4-hydroxyphenyl ethylene glycol; HVA: homovanillic acid.

**Table 6** Correlation between 5-HIAA, MHPG, HVA levels in non-treatment-resistant depression group ($r$)

| Plasma monoamine neurotransmitter metabolites | 5-HIAA | MHPG | HVA |
|----------------------------------------------|--------|------|-----|
| 5-HIAA                                       | 1.000  | 0.336| 0.136|
| MHPG                                         | 0.336  | 1.000| 0.089|
| HVA                                          | 0.136  | 0.089| 1.000|

$P > 0.05$. 5-HIAA: 5-hydroxyindoleacetic acid; MHPG: 3-methoxy-4-hydroxyphenyl ethylene glycol; HVA: homovanillic acid.

**DISCUSSION**

A large number of studies have reported that the incidence of TRD is closely related to the decline of monoamine neurotransmitter transmission function$^{[9-14]}$. In this study, we observed levels of three plasma metabolites, 5-HIAA, MHPG and HVA, in TRD patients, in an attempt to comprehensively explore the correlation between monoaminergic neurotransmitter concentrations and TRD. The results demonstrated that plasma levels of 5-HIAA, MHPG and HVA were significantly decreased in the TRD group before and after treatment, compared with the non-TRD group. These findings indicate that central 5-HT, NE and DA levels in TRD patients were significantly lower than those in non-TRD patients, and the levels trended to gradually decrease with depression severity and time.

Repeated-measures analysis of variance was used for multiple comparisons of 5-HIAA and MHPG levels before and after comprehensive therapy. The results indicated that 5-HIAA and MHPG levels were significantly increased after treatment in both groups. This finding suggests that long-term antidepressant therapy can increase 5-HIAA and MHPG levels, elevate 5-HT and NE content, and decrease depression severity. Compared with TRD patients, 5-HIAA and MHPG levels in the non-TRD depression group were increased at different time points. This may be due to a lower rate of uptake of 5-HIAA and MHPG in depressive patients, or low levels of 5-HIAA or MHPG, resulting in lower sensitivity to drugs and reduced treatment effects at each stage.

Repeated-measures analysis of variance was also applied for multiple comparisons of the HVA level before and after comprehensive treatment. The results revealed that, as treatment progressed, HVA levels increased in two groups. However, there was no significant difference between 4 weeks post-treatment and before antidepressant treatment, between 6 weeks post-treatment and 4 weeks post-treatment, or between 8 weeks post-treatment and 6 weeks post-treatment. These results indicate that antidepressant treatment had no apparent impact on the sharp increase of HVA levels, consistent with the findings of Xiao $et al.$$^{[15]}$. In the current study, the antidepressant drugs used in TRD patients were weak DA inhibitors, which act slowly and have little effect in the short term.

5-serotonin and norepinephrine reuptake inhibitors are clinically used as antidepressant drugs and have been demonstrated to exhibit good safety and tolerability$^{[16]}$. These drugs are reported to be superior to conventional selective serotonin reuptake inhibitors, especially for severe depression$^{[17-19]}$. In the current study, TRD patients were treated with venlafaxine hydrochloride, a serotonin/norepinephrine reuptake inhibitor. The results showed that 5-HT, NE and DA levels were increased in TRD patients. Comprehensive therapy consisting of individualized serotonin and norepinephrine reuptake
inhibitors and additional psychotherapy was found to effectively improve peripheral monoamine neurotransmitter levels, and reduce the degree of depression severity in TRD patients. Many previous studies in China reported similar results. In the current study, depressive patients were treated with dual antidepressant venlafaxine hydrochloride sustained-release capsules, psychological therapy and acanthopanax root preparations. Changes in patients’ depressive symptoms and plasma monoamine metabolites may serve as appropriate indicators for comprehensive treatment. Further studies are required to conclusively determine the influence of psychological treatment or acanthopanax root preparations on plasma monoamine neurotransmitter metabolites. The concept of TRD is currently contentious, and there are discrepancies in the literature. The pathogenesis of TRD is poorly understood, and there is a variation in the prevalence, period, and onset features of patients. The present study is restricted by the limitations of the study area, small sample size and classification of subtypes. In addition, no healthy control group was tested, because the homogeneity of demographic data is difficult to control. As such, we only conducted a self-control comparison of patients. We plan to use this paradigm to examine healthy participants in future studies.

In the current study, we investigated the levels of plasma metabolites of monoamine neurotransmitters in TRD patients and the influence of antidepressant treatment, in a comprehensive attempt to extend current knowledge of TRD pathogenesis and treatment. However, our results have some inconsistencies, indicating etiological heterogeneity in TRD, and highlighting the need for a comprehensive evaluation of plasma monoamine neurotransmitter metabolites and antidepressant treatment.

SUBJECTS AND METHODS

Design
A clinical case study.

Time and setting
Experiments were performed from March 2010 to September 2011 at Department of Rehabilitation and Psychology, the First Affiliated Hospital of Shihezi University Medical College, China.

Subjects
Depressive patients who were admitted to the Department of Rehabilitation and Psychology, the First Affiliated Hospital of Shihezi University Medical College, China between March 2010 and September 2011 were included in this study. Major depression was diagnosed according to the diagnostic criteria of the International Classification of Diseases, Tenth Revision. The selected patients were divided into two equal groups. TRD group comprised of 30 cases. Inclusion criteria: (1) Patients were more than 18 years old, irrespective of gender. (2) Cases were ineffective for two or more different types of antidepressants for sufficient doses and durations (more than 6 weeks). (3) HAMD total scores were more than or equal to 24 points. (4) There were no abnormalities in routine blood tests, liver and kidney biochemistry, electrocardiography, electroencephalography, and physical examination. (5) Patients reported no drug or alcohol abuse. Exclusion criteria: (1) Cases with serious heart, brain, liver, kidney, immune disorders, and obesity, poor nutrition, acute and chronic infection. (2) Cases with other psychiatric disorders and mental retardation. (3) Cases received long-term spirit blocker treatment within 2 months prior to recruitment. (4) Patients received thyroxine treatment within 3 months prior to recruitment. (5) Patients received hormone therapy. (6) Pregnant and lactating women.

The non-TRD group comprised of 30 patients, in which HAMD total scores were more than or equal to 24 points. The criteria were the same as those in TRD group except for the TRD performance. The study complied with the ethical requirements in the Declaration of Helsinki, and all subjects or their guardians signed informed consents.

Methods
Research process
General subject data were collected on the day of recruitment. A fasting blood test was conducted via the elbow vein in the next morning and plasma samples were collected for determination. All patients were assessed with the HAMD within 2 days after recruitment. All the questionnaires were examined by two individuals, and eligible questionnaires were input into a psychological test software system (Adult Psychological Test System 5.0 edition; Shanghai Huicheng Consulting Company, China). Patients were given antidepressant treatment for 8 weeks, and received adjuvant psychological therapy and other drug treatments. The plasma samples were collected and determined at 4, 6, and 8 weeks after antidepressant treatment, and patients were again examined with the HAMD rating scale.

HAMD assessment
The 24-item HAMD scoring system, including five grades of 0–4 points, was used. Total score < 8 points: normal; total score 8–20 points: suspected depression; total score 20–35 points: definite depression; total score > 35...
points: severe depression. The scale was assessed by a well-trained experimenter and a psychological consultant, and the Kappa value for consistency was more than 0.80.

**Antidepressant drug treatment**
The two groups were treated with antidepressant drugs for 8 weeks. If the reduction rate of HAMD symptom scores was < 25% in the TRD group after 6-week treatment, the drug was regarded as ineffective. First, paroxetine hydrochloride tablets (Paxil; Sino-American Tianjin SmithKline and French Lab., Ltd., Tianjin, China) were given at a dose of 20–60 mg per day for 6 weeks. The HAMD reduction rate was < 25%, so this drug treatment was considered ineffective. Duloxetine HCl in enteric-coated capsules (Cymbalta; Eli Lilly and Company, Indianapolis, Indiana, USA) was then given at a dose of 30–60 mg per day for 6 weeks, and an ineffective curative effect was defined by a HAMD score reduction rate < 25%. Finally, patients were treated with sustained release capsules of venlafaxine hydrochloride (Efexor XR; Wyeth Medica, Kildare, Ireland) at a dose of 75–150 mg per day prior to recruitment in TRD group. In contrast, the non-TRD group was only treated with venlafaxine hydrochloride (sustained release capsules at a dose of 75–150 mg per day).

**Adjuvant therapy**
The two groups received the same psychological treatment and basic drug treatment, including cognitive behavioral therapy and acanthopanax root injection. During hospitalization, patients were interviewed once or twice a week, for 50 minutes each, and intravenously injected with acanthopanax (Heilongjiang, Wusuli River Pharmaceutical Company Limited, Wusuli River City, Heilongjiang Province, China), 300 mg once a day.

**Sample collection**
We collected 3 mL elbow vein blood from all patients between 8:00 and 9:00 a.m., while women were in the non-menstrual period. Blood samples were taken for 30 minutes, then centrifuged at 2 000 r/min for 10 minutes. The blood plasma was isolated and packaged, and stored at –70°C in a refrigerator. All specimens were collected for testing.

**Examination of plasma monoamine neurotransmitter metabolites**
The metabolites were identified according to an enzyme-linked immunosorbent assay kit of 5-HIAA, MHPG, HVA (Shanghai Hora Biotechnology Co., Ltd., Shanghai, China). Absorbance was read with a microplate reader (ELx-800 type; BioKit Company, Massachusetts, USA) at 450 nm wavelength. 5-HIAA, MHPG, HVA concentrations were calculated with a standard curve.

**Statistical analysis**
All data were analyzed with SPSS 13.0 software (SPSS, Chicago, IL, USA) and expressed as mean ± SD. Data were compared using two independent samples t-tests, one-way analysis of variance, and Pearson correlation analysis. A value of P < 0.05 was considered statistically significant.

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**Author contributions:** Guiqing Zhang had full access to the study concept and design, drafted and supervised the manuscript, and coordinated the funding. Yanxia Zhang, Xia Liang, and Jianxia Yang were in charge of data analysis and statistical processing. Min Hu and Yueqi Zhang were responsible for the selection of clinical cases, experimental observation and recordings.

**Conflicts of interest:** None declared.

**Ethical approval:** This pilot was approved by Ethics Committee in the First Affiliated Hospital of Shihzei University Medical College in China.

**REFERENCES**

[1] Li Z, Sun XL, Huang Y, et al. Correlation between neuroendocrine and cognitive function in patients with refractory depression. Zhongguo Xunzheng Yixue Zazhi. 2007;7(8):575-579.

[2] Zhou DF. Action mechanism of antidepressive agents and clinical preponderance of selective 5-serotonin and norepinephrine reuptake inhibitors. Zhongguo Xinyao yu Linchuang Zazhi. 2005;24(9):673-675.

[3] Khan A. Vilazodone, a novel dual-acting serotonergic antidepressant for managing major depression. Expert Opin Invest Drugs. 2009;18(11):1753-1764.

[4] Skolnick P, Krieter P, Tizzano J, et al. Preclinical and clinical pharmacology of DOV 216,303, a "triple" reuptake inhibitor. CNS Drug Rev. 2006;12(2):123-134.

[5] Liu J, Sun J, Ma AO, et al. Progress in depression. Zhonghua Xiandai Neikexue Zazhi. 2005;1(4):323-325.

[6] Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci. 2006;7(2):137-151.

[7] Yao H, Xiao H, Zhang XB. The study on plasma interleukin monoamine metabolites of depressed patients plasma interleukin monoamine metabolites of depressed patients. Jingshen Jibing yu Jingshen Weisheng. 2003;3(1):66-67.
[8] Guo NF, Yu JS. Counselors (National Vocational Qualification training course). Beijing: Nationalities Publishing House. 2005:174-178, 239.

[9] Wang YL, Liu Y, Sun QX. A clinical study on ziprasidone combined with amitriptyline in treatment-refractory depression. Zhongguo Shiyou yuyao. 2009;4(16):61-63.

[10] Si XC. Effects of 5-HT2c receptor antagonist on blood pressure and contractility of the aortic rings in hypertensive rats. Zhongguo Linchuang Kangfu. 2003;7(15):2166-2167.

[11] Liu ZM, Xu FM. The research progress on selective 5-HT reuptake inhibitors in the treatment of depression, obsession, anxiety disorders progress. Zhongguo Linchuang Kangfu. 2001;5(2):68-69.

[12] Li X, Cai J, Lu Z, et al. An analysis on the serotonin levels in the platelets of patients with treatment resistant depression. Zhongguo Xingwei yixue kexue. 2007;16(12):1088-1089.

[13] Muck-Seler D, Pivac N, Sagud M, et al. The effects of paroxetine and tianeptine on peripheral biochemical markers in major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2002;26(7-8):1235-1243.

[14] Jay TM, Rocher C, Hotte M, et al. Plasticity at hippocampal to prefrontal cortex synapses is impaired by loss of dopamine and stress: importance for psychiatric diseases. Neurotox Res. 2004;6(3):233-244.

[15] Xiao H, Yao H, Hou G, et al. Plasma metabolite change of monoamine neurotransmitters in patients with depression after treatment. Zhongguo Linchuang Kangfu. 2005;9(16):246-247.

[16] Feng XY, Gong QY. Pharmacology and clinical application of venlafaxine. Zhongguo xinyao yu linchuang zazhi. 2000;19(5):342-344.

[17] Liu J. The report of Venlafaxine treatment for two cases of severe depressive episode. Linchuang yiyao shijian. 2010;19(3B):376-377.

[18] Redrobe JP, Bourin M, Colombel MC, et al. Dose-dependent noradrenergic and erotonergic properties of venlafaxine in animal models indicative of antidepressant activity. Psychopharmacology (Berl). 1998;138(1):1-8.

[19] Wang DS, He XY. The effect of venlafaxin combined with psychological intervention treatment for anxiety emotion in depression disorders. Dangdai yixue. 2010;16(10):26-27.

[20] Fu H, Lin CH, Lin HJ. Control study of effexor and paroxetine in the treatment of depression. Shiyan yixue zazhi. 2009;25(12):2068-2069.

[21] Liu L, Qi SG, Dong XH, et al. Therapeutic effect of quetiapine,aripiprazole combined venlafaxine on treatment refractory depression. Zhongguo Jiankang xinlixue zazhi. 2010;18(5):522-524.

[22] Du B, Gao CG, Wang G, et al. Efficacy and safety of duloxetine with fluoxetine in the treatment of major depressive disorder. Zhongguo Linchuang yaolixue zazhi. 2009;25(2):99-103.

[23] World Health Organization. The International Statistical of Diseases and Related Health Problems 10 Revision(Icd-10), Division of Mental Health. Beijing: Peoples’s Medical Publishing House. 1995:97-100.

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