

# L-carnitine and acetyl-L-carnitine: potential novel biomarkers for major depressive disorder

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## Research article

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# Abstract

## Background

The lack of biomarkers to identify the target population has greatly limited the precise medical prospects for major depressive disorder (MDD). The endogenous L-carnitine (LC) and its derivative acetyl-L-carnitine (ALC) play different roles in depression, from metabolism and neurochemistry to epigenetic roles. The levels of ALC in people and rodents with depression are significantly reduced. It is necessary to determine whether serum LC and ALC might be used as novel noninvasive biomarkers for the diagnosis of MDD.

## Methods

Ultra-performance liquid chromatography–electrospray–tandem mass spectrometry (UPLC–ESI-MS/MS) was used to determine the concentration of LC and ALC in the serum of healthy controls and patients with MDD; among the latter, in patients who were responsive (effective group) and those who were nonresponsive (ineffective group) after 2–4 weeks of treatment. The diagnostic value of serum LC and ALC for MDD was assessed.

## Results

Serum LC and ALC concentrations were significantly lower in patients with MDD than in healthy controls. The Pearson correlation analysis showed that the HDRS-24 score was positively associated with serum ALC. The ROC analysis revealed an AUC of 0.6943 with 61.0% sensitivity and 74.0% specificity for LC and an AUC of 0.8496 with 67.0% sensitivity and 93.0% specificity for ALC, differentiating patients with MDD from healthy controls. Furthermore, the concentration of LC and ALC in patients with depression was significantly increased in the effective-treatment group and no significant change was observed in the ineffective-treatment group.

## Conclusion

These results suggest that serum LC and ALC might be potential novel biomarkers for the diagnosis of MDD.

## 1. Introduction

Major depressive disorder (MDD) is a common mental disorder that is a cause of illness and disability worldwide [1, 2]. According to a recent survey, the lifetime and 12-month prevalence rates of depression in China are 3.4% and 2.1%, respectively [3]. The pathogenesis of the disease is relatively complex, which is generally considered to be related to genetics, sex, neuroendocrine, psychosocial environment, immunity, intestinal microorganisms, and other factors [4–6]. At present, clinical diagnosis is mainly based on the

description of symptoms by the patient, mental state examination, and clinical behavior observation, lacking objective diagnosis indicators and treatment methods [7–9], which greatly increases the misdiagnosis rate. The effect of drug therapy is not obvious in some patients, which is related to misdiagnosis [10]. Therefore, it is of great significance to explore the pathogenesis of depression and search for biomarkers for the diagnosis and diversified treatment of clinical depression.

Studies have shown that neuroplasticity impairment may be the core pathophysiological mechanism of depression [11, 12]. In addition, studies demonstrated that acetyl-L-carnitine (ALC) has multiple functions related to neuroplasticity [13] and is an antidepressant substance with significant potential. ALC is a natural form of L-carnitine (LC). LC and ALC are naturally occurring substances in the body and have antidepressant effects. High concentrations of carnitine, including free L-carnitine or acylcarnitine besides ALC, are present in biological tissues and cells [14]. Acetyl-L-carnitine (ALC) is an endogenous compound synthesized in the human body, which is mainly present in brain, kidney, and liver. Its main physiological function is to promote coenzyme A to enter into the mitochondria, thus promoting  $\beta$ -oxidation of long-chain fatty acids [15]. In addition to improving mitochondrial function and energy, and enhancing antioxidant activity [16], ALC has been shown to be effective in a variety of neuropsychiatric disorders, such as age-related mental retardation, Alzheimer's disease [17, 18], attention deficit hyperactivity disorder [19], depression [20] and depressive symptoms in the course of fibromyalgia [21], multiple sclerosis [22], and alcohol dependence [23]. It has been demonstrated that ALC can promote rapid antidepressant response in a rodent model [28]. The clinical research results showed that serum ALC levels in patients with severe depression are significantly lower than those in healthy people, and ALC supplementation may lead to an antidepressant response that lasts for 14 days after 3 days of drug withdrawal (Nasca C et al., 2018).

In this study, ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to: (1) determine the concentration of LC and ALC in serum; (2) compare the differences in LC and ALC levels between patients with MDD and healthy controls; (3) analyze the correlations between levels of LC and ALC and degree of depression; and (4) explore the role of ALC and LC levels in the onset and diagnosis of depression.

## 2. Materials And Methods

### 2.1 Participants

A total of 261 individuals with a first depression episode (according to the Diagnostic and Statistical Manual for Psychiatric Disorders, fourth edition) hospitalized at the Anhui Mental Health Center from November 2018 to January 2020, were scanned in this study; 85 patients with MDD were selected. The inclusion criteria were as follows: (1) the age ranged from 18 to 70 years; (2) a primary diagnosis of MDD in a current major depressive episode; (3) the reduction rate of the 24-item Hamilton Depression-Rating Scale (HDRS-24) score in the treatment-effective group was  $\geq 50\%$  and the reduction rate of HDRS-24 score in the treatment-ineffective group was  $< 25\%$ . The exclusion criteria were as follows: (1) alcohol and

drug abusers; (2) use of immunomodulators, various antidepressants, or lithium salts in the last half year; (3) history of serious heart, liver, kidney, and other serious body diseases, metabolic diseases, and infectious diseases such as cold and fever in the last two weeks; (4) combination with other mental illness or nervous system disease history; (5) pregnancy and lactation. The healthy control (HC) individuals were recruited from the Anhui Medical University Health Checkup Center. HC were free of lifelong mental and major illnesses and metabolic diseases. The subjects had no active infections and systemic diseases confirmed by the medical history at the time of the study evaluation. Blood samples were collected from the vein through standard techniques before the evaluation meeting and all subjects were fasting. Tubes with a 5 mL capacity were used to collect the blood. Samples were centrifuged at 3000 r/min for 5 min at 4 °C and the serum was collected. The serum was stored at - 80 °C for further use. All subjects provided informed written consent before participating, according to the principles of the Declaration of Helsinki. This study was approved by the Ethics Committee of the Anhui Provincial Mental Health Center.

Clinical assessment consisted of a physical examination, including measures of height, weight, body mass index (BMI), and biochemical index. Additional information was collected, including demographics, current drug use, Hamilton Anxiety Scale (HAMA) score, and HDRS-24 score. Regarding drug use, 85 patients with a first depression episode did not take any antidepressants while they participated in the study. In addition, we evaluated the LC and ALC levels of 38 patients after treatment. Blood samples were collected through the vein 4–8 weeks after treatment, and were divided into treatment-effective group and treatment-ineffective group according to the HDRS-24 score reduction rate.

## **2.2 UPLC–ESI-MS/MS determination of LC and ALC**

A simple, rapid, and selective UPLC–ESI-MS/MS method for the determination of LC and ALC in human serum was developed. The UPLC-MS/MS instrumentation was composed of a Waters Acquity ultra performance liquid chromatography (UPLC) I Class Binary Solvent Manager, Acquity UPLC Sample Manager-FTN coupled to a Waters Xevo TQ-S mass spectrometer (Waters, Massachusetts, USA). Acetyl-L-carnitine- $d_3$  (ALC- $d_3$ ) was selected as the internal standard (IS). After protein precipitation with acetonitrile-water (1 mL, 2:1, v/v), the analytes and IS were separated on a 2.5  $\mu$ m Waters XSelect HSS T3 C18 column through gradient elution with methanol-water (containing 0.01% aqueous ammonia) as the mobile phase at a flow rate of 0.2 mL/min. Analytes were detected with multiple reaction monitoring using a positive scan mode with electrospray ionization (ESI). The ratios of signal intensities for the transitions  $m/z$  162.10 > 102.97 (LC),  $m/z$  204.14 > 85.03 (ALC), and 207.19 > 85.03 (ALC- $d_3$ ) were converted to concentration using a calibration curve. ALC hydrochloride (> 98% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). L-carnitine·HCl (> 98% purity) and ALC- $d_3$ ·HCl (> 98% purity) were purchased from Toronto Research Chemicals Inc. (TRC, North York, Canada). All the other analytical reagents and solvents were above chromatographic purity level. A quality control was conducted for each experiment to ensure the accuracy of the results.

## **2.3. Statistical analysis**

All statistical analyses were conducted using the SPSS version 17.0 (SPSS, Chicago, Illinois, USA). Two-tailed t-tests and  $\chi^2$  analysis were used to compare the continuous and classified demography and clinical characteristics of HC and patients with MDD. The serum LC and ALC concentrations of all the individuals in the HC and MDD groups were compared with an independent-sample t-test and the serum LC and ALC concentrations of MDD before and after treatment were compared with a paired-sample t-test. The relation between HDRS-24 scores and the other variables were analyzed with Pearson correlation tests and the independent relationships were determined by multivariate linear regression analysis. The receiver operating characteristic (ROC) curve analysis was used to determine the area under the curve (AUC) and cut-off values of serum LC and ALC.  $P < 0.05$  was considered statistically significant. Unless otherwise specified, the data are expressed as mean  $\pm$  SD.

### 3. Results

#### 3.1 LC and ALC levels differ between healthy controls and patients with MDD

There were no differences in age, BMI, gender, and other demographic characteristics between the HC (n = 85) and patients with MDD (n = 85) (Supplemental materials, Table S1). All the patients were in an acute depressive episode during study participation. The level of ALC in the MDD group was significantly lower than that in the HC group (Fig. 1A,  $P < 0.0001$ ,  $t = 8.64$ , effect size = 1.33, Power = 1.00, HC:  $2.23 \mu\text{g/mL} \pm 0.65$ , MDD:  $1.43 \mu\text{g/mL} \pm 0.54$ ). Similarly, the level of LC in the MDD group was lower than that in the HC (Fig. 1B,  $P < 0.0001$ ,  $t = 4.46$ , effect size = 0.68, Power = 0.99, HC:  $6.16 \mu\text{g/mL} \pm 1.19$ , MDD:  $5.31 \mu\text{g/mL} \pm 1.30$ ). In addition, the covariance analysis showed that the differences of serum ALC and LC between the MDD and the HC groups were not influenced by age and sex (Supplemental materials, Tables S2–S5).

In patients with mild MDD, there was no correlation between ALC level and disease severity using the Hamilton Depression Scale (HDRS-24). In patients with moderate to severe MDD, there was a significant negative correlation between ALC level and the severity score: the higher the severity, the lower the ALC concentration ( $P = 0.04$ ,  $r = -0.326$ ) (Fig. 2A). Multivariate linear regression analysis also showed that the HDRS-24 score was positively associated with serum ALC (Table 1).

Table 1  
Multiple linear regression analysis between HDRS-24 scores (dependent variable) and serum ALC and LC (independent variables)

Variables	B	<i>t</i>	<i>P</i> -value	95% CI	
				Lower bound	Upper bound
ALC	-4.986	-2.233	.031*	-9.486	-.486
LC	-.654	-.814	.420	-2.273	.965

Note: \**P* < 0.05 was considered statistically significant.

In all the patients with depression, there was no correlation between the LC level and the disease severity. In addition, there was a significant negative correlation between LC and ALC levels in patients with depression ( $r = -0.31$ ,  $P = 0.004$ ) (Figure S1), but no correlation in the HC group.

In addition, the ROC curve analysis showed potential diagnostic values of serum ALC and LC (Fig. 3). The AUC of ALC and LC were 0.8496 and 0.6943, respectively. When the critical value of ALC was 1.54  $\mu\text{g/mL}$ , the sensitivity and specificity of MDD patients and HC were 67.0% and 93.0%, respectively. At the LC critical concentration of 5.54  $\mu\text{g/mL}$ , the sensitivity was 61.0%, and the specificity was 74.0%. When the LC and ALC results were considered together, the ROC analysis showed that the AUC of patients with MDD and HC was 0.7814, the sensitivity was 67.0%, and the specificity was 80.0%.

### 3.2 ALC and LC levels differ before and after treatment

From June 2019 to January 2020, sera were collected from 30 patients with effective treatment and 8 patients with ineffective treatment. In the effective-treatment group, the ALC levels were significantly higher after treatment compared to those before treatment ( $t = -2.09$ ,  $P = 0.045$ , size effect = 0.38) (Table 3). Similarly, the LC levels were significantly higher after treatment than those before treatment ( $t = -3.08$ ,  $P = 0.004$ , size effect = 0.56) (Table 1). There was no significant change in LC and ALC levels before and after treatment in the ineffective-treatment group (detailed results are shown in Table 2).

Table 2  
Comparison of serum concentrations of LC ( $\mu\text{g}/\text{mL}$ ) and ALC ( $\mu\text{g}/\text{mL}$ ) before and after treatment (mean  $\pm$  SD)

	Variables	Before treatment	After treatment	Statistics	P-value	Effect size	Power
Effective-treatment group (n = 30)	LC	1.45 $\pm$ 0.50	1.71 $\pm$ 0.68	-2.09	0.045*	0.38	0.83
	ALC	5.41 $\pm$ 1.27	6.31 $\pm$ 1.26	-3.08	0.004**	0.56	0.99
	LC + ALC	6.86 $\pm$ 1.57	8.02 $\pm$ 1.89	-3.06	0.005**	0.52	0.98
Ineffective-treatment group (n = 8)	LC	2.14 $\pm$ 0.81	1.64 $\pm$ 0.46	1.34	0.22	0.47	0.42
	ALC	5.64 $\pm$ 1.39	6.17 $\pm$ 0.97	-0.85	0.42	0.3	0.2
	LC + ALC	7.77 $\pm$ 1.29	7.80 $\pm$ 1.36	-0.04	0.97	0.01	0.05

Note: \*\* $P < 0.01$ , \* $P < 0.05$  was considered statistically significant.

## 4. Discussion

In this study, we demonstrated that the serum LC and ALC concentrations were significantly lower in patients with MDD than those in the HC group. The concentration of ALC was negatively correlated with the severity of MDD in patients with moderate to severe MDD. The results of the ROC analysis revealed an AUC of 0.6943 with 61.0% sensitivity and 74.0% specificity for LC and an AUC of 0.8496 with 67.0% sensitivity and 93.0% specificity for ALC in differentiating patients with MDD from those in the HC group. In the effective-treatment group, the concentration of LC and ALC in patients with depression was significantly increased after treatment with antidepressants. However, there was no significant change in the ineffective-treatment group. These results suggest that ALC and LC may be diagnostic and therapeutic markers of depression.

LC is a nonessential amino acid derived from the essential amino acids lysine and methionine, whose balance is maintained through dietary intake and endogenous formation followed by renal excretion [29]. In the brain, LC and its derivative ALC are present in high concentration, reducing nerve damage by regulating mitochondrial permeability and preventing excitatory toxicity [30]. LC and ALC may play different roles in depression, from metabolism [31] and neurochemistry [32–33] to epigenetic roles [34–36]. In agreement with the results obtained using a depression rodent model and clinical MDD samples studied by Nasca et al. [28], the ALC level in patients with MDD was significantly lower than that of the age- and gender-matched HC group, and a negative correlation between the ALC levels and HDRS-24 scores was found in the present study. A previous contribution reported a positive correlation between

ALC concentrations in peripheral blood and the central nervous system [37]. Based on the ROC analysis, the serum ALC cut-off point of 1.54 µg/mL showed a 67.0% sensitivity and a 93.0% specificity, indicating that serum ALC has a superior diagnostic value (AUC = 0.8496) in MDD.

In contrast to the above clinical study [28], we found that there was a significant difference in LC levels between patients with MDD and HC. This difference may be due to race, sample size, and eating habits. The Pearson correlation analysis showed that there was no correlation between HDRS-24 scores and serum LC concentrations, and the AUC for LC was less than 0.7. These results suggest that LC is closely related to depression, but may not be suitable as a biomarker of MDD. About 75% of the human carnitine is derived from exogenous carnitine (mainly from meat and dairy products), and further research is needed to assess whether the decrease of LC and ALC levels in patients with MDD is sensitive to unhealthy lifestyle choices, such as lack of exercise, poor diet, smoking, and lack of adequate sleep.

Many clinical studies [38–39] have confirmed that ALC can significantly reduce the symptoms of depression compared with the established antidepressants and has similar effectiveness. However, no studies have reported the changes of LC and ALC levels in patients after treatment with antidepressant drugs. To verify the correlation between the level of LC and ALC and the outcome of depression, we compared the changes of LC and ALC in patients with depression before and after treatment. In the treatment group with a HDRS-24 score reduction rate greater than 50%, the content of ALC and LC increased significantly after treatment with antidepressant drugs. There was no significant change in the concentration of ALC and LC in the ineffective-treatment group. These results show that the concentration of LC and ALC are related to the therapeutic effect of depression. Due to the impact of the new coronavirus pneumonia, we stopped collecting blood samples from patients with MDD, and this resulted in a very small sample size after treatment, especially in the ineffective-treatment group. We expect to further expand the sample size and compare the effects of different types of antidepressants on LC and ALC concentrations in patients with depression.

LC and ALC are transformed into each other in the body. Carnitine acetyltransferase catalyzes the synthesis of acetyl-CoA (CoA) and L-carnitine on the mitochondrial inner membrane, and enters the mitochondrial matrix through the carnitine/acetylcarnitine acyltransferase. Carnitine penetrates the mitochondrial matrix and there, the carnitine acetyltransferase converts acylcarnitine to carnitine and acetyl CoA [40]. Since LC and ALC are transformed into each other in the body, they are obviously negatively correlated in depression patients, and their contents are closely related to the onset and outcome of depression. LC and ALC might be considered as potential biomarkers of depression at the same time. Unexpectedly, LC and ALC showed a significant positive correlation in patients with MDD, but there was no such correlation in HC. The correlation in patients with MDD may be related to the onset of depression, and further research is needed to confirm this assumption.

This study has several limitations. First, the study is a single-center study with a small sample size. Second, the effects of diet, exercise, sleep, and other external factors on LC and ALC concentrations were

not considered. Third, the effects of different drugs on the LC and ALC concentrations after treatment were not considered.

## **Conclusion**

In conclusion, the detection of serum LC and ALC may prove to have clinical value in the diagnosis of MDD. Multicenter and longitudinal studies are clearly needed to validate the potential of LC and ALC as novel biomarkers for MDD.

## **Abbreviations**

AUC, area under curve; MDD, major depressive disorder; ROC, Receiver operating characteristic

## **Declarations**

### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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### **Ethics declarations**

Ethics approval and consent to participate

All study procedures and materials were approved by Anhui Mental Health Center. Written informed consent to participate was obtained from all study participants.

### **Consent for publication**

Not applicable.

### **Competing interests**

The authors declare that they have no competing interests.

## Authors Contributions

Q-RX, JL and L-JN designed the study, wrote the protocol, and they both performed the statistical analysis. Q-RX carried out the study and collected important background information. L-JN performed the experiments and drafted the manuscript. FS, Y-YX, carried out the concepts, design, definition of intellectual content, literature search, data acquisition, data analysis and manuscript preparation. Y-YM and X-HZ provided assistance for data collection and performed the experiments. All authors have read and approved of the version to be published. All authors Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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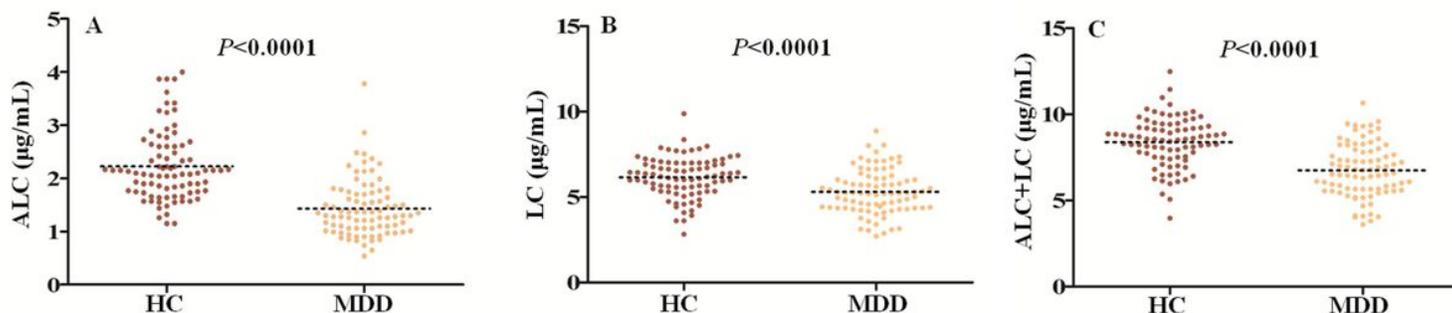
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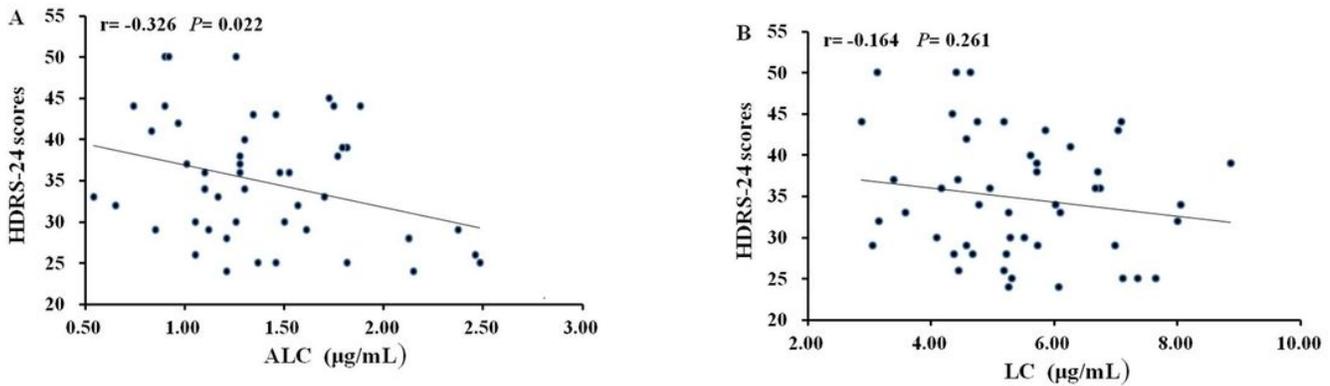
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## Figures



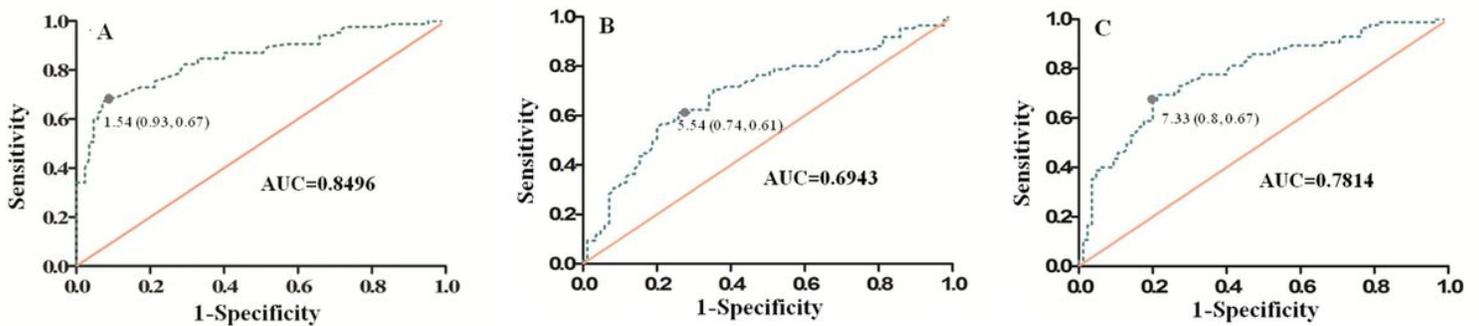
**Figure 1**

Decreased L-carnitine (LC) and acetyl-L-carnitine (ALC) levels in patients with MDD compared to those in the HC group. (A) Serum ALC concentrations in HC and patients with MDD; (B) LC concentrations in HC and patients with MDD; (C) sum of serum concentrations of ALC and LC in HC and patients with MDD. Note: \*\*\* $P < 0.001$  was considered statistically significant. Dashed bars indicate group mean.



**Figure 2**

Correlation between HDRS-24 scores and serum concentrations of ALC (A) and LC (B) in patients with MDD. Note:  $P < 0.05$  was considered statistically significant



**Figure 3**

ROC curve for serum ALC and LC in the identification of patients with MDD. (A) ROC curve for ALC; (B) ROC curve for LC; (C) ROC curve for ALC and LC.

## Supplementary Files

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