

The combination of serum BDNF, cortisol and IFN-gamma can effectively diagnose major depressive disorder

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Primary research

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Abstract

Background Misdiagnosis and ineffective treatment are common in major depressive disorder (MDD) in current clinical practice, while the combination of various proteins involved in the pathogenesis of MDD may assist the correct diagnosis. The study aimed to explore whether the combination of serum inflammatory, stress, and neurotrophic factors could be helpful for the diagnosis of MDD, and to investigate the predictors associated with early symptom improvement. Methods Baseline serum levels of C-reactive protein (CRP), interleukin (IL)-6, IL-10, IL-1beta, tumor necrosis factor (TNF)-alpha, interferon (INF)-gamma, cortisol and brain-derived neurotrophic factor (BDNF) were detected in 30 MDD patients and 30 age- and gender-matched healthy controls. 17-item Hamilton Depression Rating Scale (HAMD-17) and Hamilton Anxiety Rating Scale (HAMA) were applied to assess depressive and anxious symptoms both at baseline and 2 weeks after antidepressant treatment. Stepwise multiple linear regression was employed to identify the early efficacy predictors; while a logistic regression model was built with above proteins and area under receiver operating characteristic (AUC) curve was calculated to evaluate the model's diagnostic power. Results Multiple linear regression revealed that baseline scores of retardation ($\beta = -0.432$, $P = 0.012$) and psychological anxiety ($\beta = -0.423$, $P = 0.014$) factors were negatively associated with the reduction rate of HAMD-17. A simple and efficient diagnostic model was established by forward stepwise logistic regression and the model achieved an AUC of 0.884, with 86.7% sensitivity and 83.3% specificity. Conclusions The results showed that combining serum BDNF, cortisol and IFN-gamma could aid the diagnosis of MDD, while baseline retardation and psychological anxiety may negatively predict the early symptom improvement.

Background

Major depressive disorder (MDD) is one of the most common mental disorders characterized by depressed mood, loss of interests, reduced drive, and associated with sleep disturbance, diminished appetite, cognitive dysfunction, and motor symptoms. The lifetime prevalence of MDD is approximately 17% [1], affecting approximately 350 million people worldwide [2]. It has been predicted that MDD would rank first in the global total burden of healthcare by 2030 [3]. According to the latest data in 2019, the lifetime prevalence rate of MDD in China has reached 3.4%, and it is estimated that about 44 million people suffer from MDD [4]. More importantly, MDD often recurrent, and the suicide rate is as high as 15%-25% [2]. It has become a major mental disorder that has troubled people's physical and mental health, bringing heavy burdens to patients, families and society.

Accurate diagnosis and early efficacy prediction of MDD are prerequisite for the effective treatment and good prognosis. Unfortunately, as MDD is a complex disease with high heterogeneity, the diagnostic consistency is only 28% [5]. Studies also report that less than a half MDD patients received effective treatment and only approximately 1/3 obtained remission [6,7]. That is because the pathogenesis of MDD is still unclear, and lack of objective biological diagnostic indicators. Current diagnoses still rely heavily on the subjective identification of depressive symptom clusters by psychiatrists. In addition, the antidepressant efficacy depends on the clinicians' assessments of the symptom changes over 6-8 weeks

in clinical practice, which may lead to MDD patients taking ineffective drugs for long and delaying optimal treatment time. Happily, report has been found that early improvement after 2 weeks antidepressant treatment may be a predictor for eventual efficacy [8]. Therefore, it is extremely important and urgent to develop objective tests for accurate diagnosis and early efficacy predictors for MDD.

Although the brain tissue or cerebrospinal fluid (CSF) is an ideal resource for studying biomarkers of MDD, it is difficult to obtain CSF of MDD patients in clinical practice, and brain tissue is even more difficult to obtain. Therefore, peripheral blood proteins became more and more favored by researchers for they are easy to obtain and can reflect the function of the central system to some extent in recent years. Quite a few studies revealed that a platform composed of multiple peripheral blood proteins may be a promising objective diagnostic tool with a much higher diagnostic value than a single protein [9-14]. Nevertheless, which peripheral blood proteins should be selected to construct the objective diagnostic platform? This is a question worth considering.

Since the monoamine transmitter hypothesis has been questioned, the neurotrophic hypothesis, the inflammatory hypothesis as well as the hypothalamic-pituitary-adrenal (HPA) axis dysfunction hypothesis have received more and more attention. Studies have found specific variations in inflammatory factors [15], cortisol [16] and neurotrophic factors [17] in peripheral blood of MDD patients. After conducting a comprehensive survey of the literature, we selected eight serum candidate markers based on the above three hypotheses. They respectively were C-reactive protein (CRP), interleukin (IL)-6, IL-10, IL-1beta, tumor necrosis factor (TNF)-alpha, interferon (INF)-gamma, cortisol and brain-derived neurotrophic factor (BDNF).

In the present study, the main aims were (1) to explore the predictive factors may associated with the early depressive symptom improvement; (2) to establish a logistic regression model which built with above proteins and to explore whether the combination could be helpful for distinguishing MDD patients from HC subjects with relatively good accuracy, specificity and sensitivity.

Methods

Study design and participants

This study was a case-control study. Thirty MDD inpatients were recruited for the study from Zhongda Hospital Affiliated to Southeast University and thirty gender- and age-matched healthy controls (HC) with no psychiatric or medical disorders were recruited through socially-oriented advertising. The study was carried out with the approval of the Ethics Committee of the Affiliated ZhongDa Hospital of Southeast University, and the study was registered in the Chinese Clinical Trial Registry (Registration number: ChiCTR-DOC-16010081). All participants provided written informed consent after given a full explanation of the study in accordance with the Declaration of Helsinki.

The diagnosis was made with a semi-structured clinical interview for DSM-Axis 1 Disorders diagnostic criteria according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) by

two senior psychiatrists. Patients' inclusion criteria were as follows: (1) age \geq 18 years, (2) met the diagnostic criteria of DSM-IV with MDD, (3) 17-item Hamilton Depression Rating Scale (HAMD-17) score \geq 17; while HC group that had a HAMD-17 score $<$ 7 were included. The exclusion criteria included: (1) in pregnancy or the puerperium, (2) with a comorbid axis I diagnoses (including bipolar depression), (3) with psychoactive substance including alcohol, tobacco or drug abuse and dependence, (4) with physical diseases (e.g., tumor, organic brain diseases, cardiovascular diseases, endocrine disease and autoimmune diseases), and (5) with a history of acute or chronic infections or nonsteroidal anti-inflammatory drugs used in the previous six weeks.

Clinical assessment

A self-made general information questionnaire was used to collect the sociodemographic data of both patients and HC subjects as applicable, including age, gender, education years, marital status, body mass index (BMI), alcohol, tobacco or substance use, familial history, physical or psychiatric illness, antidepressants and psychotropic medication history, number of episodes, and duration of recent disease. HAMD-17 and Hamilton Anxiety Rating Scale (HAMA) were applied to assess depressive and anxious severity of all the participants, respectively. HAMD-17 contains factors of anxiety/somatization, retardation, cognitive disorder, sleep disorder, and weight; while HAMA includes psychological anxiety factor and physical anxiety factor.

Sample collection and serum proteins measurement

Five milliliters fasting blood were taken from the antecubital vein of each subject between 06:30 and 08:00 a.m., and the serum was obtained after centrifuging at 3500 rpm for 10 minutes. Then, serum samples were divided into equal parts of 0.5 ml and preserved at -80°C until further analysis. Measurement of CRP, IL-6, IL-10, IL-1beta, TNF-alpha, INF-gamma, cortisol and BDNF were performed by enzyme-linked immunosorbent assay (ELISA) method using commercially available kits (CRP, IL-6, IL-10, IL-1beta, TNF-alpha, INF-gamma and BDNF kits from RayBiotech, Norcross, GA, USA; cortisol kit from R&D Systems, Mutlukent Mah, Arda Sk, USA). The detection limits of CRP, IL-6, IL-10, IL-1beta, TNF-alpha, INF-gamma, cortisol and BDNF ELISA kits were 34 pg/mL, 3 pg/mL, 1 pg/mL, 0.3 pg/mL, 0.384 pg/mL, 15 pg/mL, 0.2 ng/mL and 80 pg/mL, respectively.

Statistical analysis

SPSS statistical software (version 22, IBM, Armonk, NY, USA) was used to analyze the clinical and assay data. Continuous variables were presented as mean \pm standard deviation ($M \pm SD$) and discrete variables were expressed as number and percentage. The abnormal data determined by $M \pm 3 SD$ were excluded, and subsequently, replace the missing values with the average value of the adjacent points. To examine whether the continuous variables were normally distributed, the Kolmogorov-Smirnov test was used. The Independent samples *t*-test or Chi-square test was applied to compare demographic and assay data between MDD patients at baseline and HC subjects as applicable. One-way analysis of variance (ANOVA) and Bonferroni post hoc test were performed to compare HAMD-17 and HAMA scores among pre-

treatment and post-treatment MDD patients and HC subjects, while paired-samples *t*-test was employed to examine the five factors (anxiety/somatization, retardation, cognitive disorder, sleep disorder, and weight) of HAMD-17 and the two factors (psychological and physical anxiety) of HAMA. Pearson's correlation analysis was also used to test the correlations among different proteins, demographic, and clinical data. Stepwise multiple linear regression analysis was employed to identify and quantify the relationships of the reduction rate of HAMD-17 with the factor scores of HAMD-17 and HAMA, and the proteins levels at baseline. Forward logistic stepwise regression analysis was performed to find the most appropriate multi-protein combination model for distinguishing MDD patients from HC subjects. The stepping criteria applied for entry and removal were based on the significance level of the F-value and set at 0.05 and 0.10, respectively. The receiver operating characteristic (ROC) curve analysis was employed to evaluate the model's differential capacity by calculating the area under the curve (AUC: 0.9-1 = excellent; 0.8-0.9 = good; 0.7-0.8 = fair; 0.6-0.7 = poor; 0.5-0.6 = fail). Statistical significance was set at a 2-tailed $P < 0.05$ for all statistical tests.

Results

Demographic and clinical data

The demographic and clinical data of all participants are presented in Table 1. No statistically significant differences were observed between the MDD and HC groups with respect to age, gender, education years, marital status, and BMI (Table 1, all $P > 0.05$). There were significant differences in HAMD-17 and HAMA scores among pre-treatment MDD, post-treatment MDD and HC groups. Post hoc multiple comparisons showed that HAMD-17 and HAMA scores of both the pre-treatment MDD group (mean difference I-J = 20.167, $P < 0.001$ and mean difference I-J = 18.200, $P < 0.001$; respectively) and post-treatment MDD group (mean difference I-J = 6.382, $P < 0.001$ and mean difference I-J = 5.508, $P < 0.001$; respectively) were significantly higher than the HC group. After 2 weeks of antidepressant treatment, HAMD-17 and HAMA scores in MDD patients significantly declined, but still much higher than those in HC subjects.

Serum proteins measurements

Among the eight selected serum proteins, serum levels of IL-6 in eight MDD patients and 15 HC, IL-1beta in 20 MDD patients and 22 HC, and TNF-alpha in 10 MDD patients and 14 HC were below the detectable limits of the ELISA kits. These three proteins were removed from subsequent analysis for the missing values exceeded 1/3 of the measurement values. Serum IFN-gamma levels were below the minimum detectable concentration of the kit in seven participants and higher than 3 SD in two participants. Serum levels of BDNF, cortisol, CRP and IL-10 were respectively higher than 3 SD in one, two, one and three participants. Since the missing values in these four proteins were less than 20% of the total measurement values, we replaced the deletions with the average value of adjacent points. Serum levels of IFN-gamma and cortisol in the baseline MDD group were higher than those in the HC group, while serum BDNF levels were lower in MDD patients than those in HC (Figure 1, $P < 0.05$). However, there were no significant

differences in serum levels of IL-10 and CRP between the two groups, were only found the trends to decline and raise, respectively (Figure 1, $P > 0.05$).

Correlation analysis in MDD patients

In MDD group, baseline HAMD-17 scores were significantly positively correlated with serum IFN-gamma levels ($r = 0.378$, $P = 0.039$); while the reduction rate of HAMD-17 was negatively correlated with baseline retardation factor ($r = -0.399$, $P = 0.032$) and psychological anxiety factor scores ($r = -0.390$, $P = 0.037$). In addition, significant positive correlations between serum levels of IFN-gamma and CRP ($r = 0.497$, $P = 0.005$), and negative correlations between serum levels of CRP and BDNF were also observed ($r = -0.377$, $P = 0.040$).

Multiple linear regression analysis and logistic regression analysis

Stepwise multiple linear regression analysis revealed that, in patients with MDD, scores of retardation factor and psychological anxiety factor at baseline were negatively associated with the reduction rate of HAMD-17 scores ($\beta = -0.432$, $P = 0.012$ and $\beta = -0.423$, $P = 0.014$, respectively). However, no association was found between the baseline levels of the remaining five proteins and the reduction rate of HAMD-17 scores.

Forward logistic stepwise regression analysis was employed to determine a regression model to maximize the identification ability with the minimum numbers among the remaining proteins. Three independent variables, BDNF, cortisol and IFN-gamma, were entered into the logistic regression equation. The partial regression coefficients were 0.005, -0.045 and -0.006, respectively. Their corresponding p -values were 0.005, 0.002 and 0.013, respectively. The corresponding regression equation based on the 3 proteins was **see equation 1 in the supplementary files**.

Performance of the proteins in the differentiation of MDD and HC subjects

As described in Table 2, ROC curve analysis revealed that the AUCs of single BDNF, cortisol and IFN-gamma were 0.759 with a sensitivity of 70.0% and a specificity of 83.3%, 0.766 with a sensitivity of 96.7% and a specificity of 50.0%, and 0.716 with a sensitivity of 63.3% and a specificity of 76.7%, respectively (Figure 2A-C). While the AUCs of the combination of any two proteins increased to larger than 0.8 (Figure 2D-F). Unfortunately, however, sensitivity or specificity of both single protein and combination of two proteins was poor. Interestingly, when combined with all of these three proteins, the AUC reached to 0.884, with the sensitivity and specificity of 86.7% and 83.3%, respectively (Figure 3).

Discussion

To date, the current diagnosis of MDD still depends on clinical symptomatologic criteria, like commonly used the International Statistical Classification of Diseases (ICD) and DSM diagnostic systems, and there are no objective diagnostic tools for clinical use. Consequently, the misdiagnosis of MDD is very common, which may lead to inappropriate treatment of patients. Furthermore, clinicians need at least 6-8

weeks to estimate the antidepressant treatment effect, which may result in patients receiving inappropriate treatment for long. Therefore, a convenient, effective objective diagnostic tool and reliable early efficacy predictions could help to reduce misdiagnosis, choose medication and treatment, even improve prognosis. In this study, we innovatively combined the representative or pivotal proteins involved in different hypotheses of the pathogenesis of MDD and demonstrated that a logistic regression model based on serum BDNF, cortisol and IFN-gamma can show a better differential efficacy between MDD and HC. In addition, early depressive symptom improvement was found to be associated with retardation and psychological anxiety degree of MDD patients at baseline.

As is well-known, the neurotrophic hypothesis, the HPA axis dysfunction hypothesis and the inflammatory hypothesis are the currently highly accepted pathogenesis hypotheses of MDD, and many of the involved proteins have specific changes, so as to be considered as strong candidates for being biomarkers of MDD. The neurotrophic hypothesis suggests that MDD is closely related to the decreased expression of BDNF and other neurotrophic factors in the limbic system [18]. A meta-analysis including 9484 subjects indicated that serum BDNF levels of MDD patients decreased significantly compared to HC [17], which was consistent with our current result. Previous studies showed that there was a robust relationship between stress and MDD [19], stress was positively correlated with cortisol levels and these two factors interacted with each other to predict depressive behaviors [20]. Therefore, increased cortisol levels were observed in MDD patients [16], so as the results of our present study. Moreover, there is growing evidence that MDD is correlated with the upregulation of the inflammatory factors/ cytokines [15,21-24]. Simon et al. [25] assessed 20 cytokines simultaneously in both MDD and HC individuals, and found that multiple pro-inflammatory cytokines, including IFN-gamma, IL-1beta, IL-6, IL-10 elevated significantly in MDD, while there was no significant difference in TNF-alpha levels between MDD and HC. In the present study, serum IFN-gamma levels were significantly higher in MDD than those in HC, which was consistent with prior studies [26,27]; while there were no significant differences in serum levels of IL-10 and CRP between MDD and HC groups, and serum levels of IL-6, IL-1beta and TNF-alpha even lower than the minimum detectable concentration of the ELISA kits in more than 1/3 participants, which was inconsistent with several meta-analysis studies [15,23]. We speculated that there were several possible reasons for these conflicts in the results. First, the ELISA kits we used in this study were different from those used in previous studies. Second, MDD itself is a highly heterogeneous complex disease. Third, unlike inflammatory diseases, the inflammatory response of MDD is low-grade and the levels of some cytokines are below the minimum detectable limits of the common commercial ELISA kits not only in HC but also in MDD patients.

In the present study, only serum IFN-gamma levels were found significantly positively correlated with HAMD-17 scores representing the severity of depressive symptoms in the MDD group. Schmidt and his colleagues [28] found no significant correlations between CRP, IFN-gamma and HAMD-17 total scores, but found IFN-gamma significantly negatively correlated with “work and activities (item 7)” and “genital symptoms (item 14)”, which to some extent also reflected the severity of MDD. We also observed that CRP was significantly positively correlated with IFN-gamma and negatively correlated with BDNF. This may be because CRP is a nonspecific inflammatory marker of a systemic inflammatory response, which

is often associated with the occurrence and severity of inflammation including MDD. Furthermore, the inflammatory factors lead to abnormal synapses and reduce the synthesis of neurotrophic factors like BDNF through attacking neuronal cells [29].

In addition, scores of retardation factor and psychological anxiety factor at baseline were observed to negatively predict the reduction rate of HAM-D-17 scores in MDD patients. That is, the more severe an MDD patient's baseline retardation and psychological anxiety were, the less improvement of the symptom was after antidepressants treatment for 2 weeks. These findings are in line with results of some studies and contrary to others [30,31]. Retardation is one of the basic features of MDD [32], results of several clinical studies suggested that MDD patients with severer baseline retardation should be treated with electroconvulsive therapy (ECT) as early as possible [33-35].

For a useful diagnostic biomarker or test to be used in everyday clinical practice, it needs to be reproducible, reliable, inexpensive and non-invasive. The serological examination is a convenient and simple method to evaluate the changes of different proteins involved in the pathogenesis of MDD in the body. As been reported before, to be a clinically useful biomarker or test for diagnosing and classifying a disorder correctly, at least 80% sensitivity and specificity must be provided [36]. Schneider and Prvulovic [36] pointed out that there were still no useful diagnostic biomarkers for MDD because none of the candidate markers fulfilled criteria as diagnostic biomarkers and a multivariable approach might be feasible/helpful to develop diagnostic biomarkers. In the present study, we detected serum levels of multiple proteins and assessed the diagnostic power of each protein and different combinations of these proteins to find a suitable diagnostic model of MDD for clinical use. Despite all AUCs were larger than 0.7, either sensitivity or specificity was lower than 80% when BDNF, cortisol or IFN-gamma was used to distinguish MDD from HC alone in the study. In other words, single BDNF, cortisol or IFN-gamma cannot be used to diagnose MDD for lacking sufficient sensitivity and specificity. Gratifyingly, the model based on these three proteins met the criteria of diagnostic tests for clinical practice, achieved AUC of 0.884 with a sensitivity of 86.7% and specificity of 83.3%. Although the sensitivity, specificity and diagnostic efficacy of the diagnostic platform for MDD in our present study are slightly less than those in some previous studies [9,12], we only need to combine three proteins to obtain the relatively superior diagnostic sensitivity, specificity and diagnostic efficacy, which is less than five or nine different indicators required in those previous studies. Therefore, the diagnostic biomarker platform we proposed is more valuable for clinical application, both in terms of economy and simplicity.

Besides, to explore the possibility of existing a more economical diagnostic platform, we also compared the combinations of any two in BDNF, cortisol and IFN-gamma. The results revealed that the diagnostic effectiveness of the combination of cortisol and BDNF was highest (AUC=0.834), and it also had the highest sensitivity of 90%. Unfortunately, however, the specificity of the combination was only 66.7%. Consequently, the combination of BDNF and cortisol is suitable for screening, while the combination of BDNF, cortisol and IFN-gamma conduce to diagnose for MDD. The clinicians may select the most appropriate one according to different needs.

Certain limiting factors should be considered in this study. Firstly, only several proteins involving in the neurotrophic hypothesis, the HPA axis dysfunction hypothesis and the inflammatory hypothesis were included in the study, given levels of proteins involving in other MDD pathogenesis hypotheses were also abnormal, proteins involved in more hypotheses testing would be helpful to build an objective diagnostic platform with much higher diagnostic effectiveness, sensitivity and specificity. Secondly, the sample size was relatively small which might result in false positives or false negatives. Thirdly, there were more female participants in this study, which might lead to gender bias in this diagnostic model. Fourthly, although the results of the study were very good, we might be missing the opportunity to obtain an objective diagnostic platform for MDD with much better accuracy, sensitivity and specificity because the ELISA kits we used to detect cytokines were not the hypersensitive kits, three cytokines (TNF-alpha, IL-6 and IL-1beta) were excluded from the forward logistic stepwise regression analysis. In future studies, we should use the hypersensitive ELISA kits to detect cytokines levels in larger samples with different mental disorders to validate the current preliminary results, or even to obtain a much better objective diagnostic biomarker platform for MDD.

Conclusion

In conclusion, the current study demonstrates that serum BDNF, cortisol and IFN-gamma levels changed specially in MDD patients, and combing these three proteins assist to distinguish MDD patients from HC subjects. Baseline degrees of retardation and psychological anxiety could negatively predicted patients'symptom improvement after 2 weeks of antidepressant treatment. These findings could help improve the accuracy of the diagnosis and switch eariler to another effective therapeutic regimen.

Declarations

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Author's contributions

Yuan Y was the principal investigator, designed the study protocol, and was involved in the recruitment of subjects and the revise of the manuscript. Chen S collected the samples of the subjects, performed the experiment, analyzed the statistical analysis, and wrote the manuscript. Zhang Y also participated in the collection of the subjects' samples and revised the manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This research was approved by the Ethics Committee of the Affiliated ZhongDa Hospital of Southeast University and corresponding informed consents were signed by all the participants.

Consent for publication

All of the authors have consented to publication of this research.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Table 1. Demographic and clinical data of participants.

	Controls (n=30)	MDD (n=30)		<i>F/t/x²</i>	<i>P</i>
		Pre-treatment	Post-treatment		
Age (years)	36.40±14.04	35.63±15.10	NA	-0.204	0.806 ^a
Age of onset (years)	NA	33.93±15.38	NA	NA	NA
Gender (M/F)	8/22	7/23	NA	0.089	0.766 ^b
Education (years)	13.77±4.26	11.63±4.17	NA	-1.958	0.055 ^a
BMI	22.88±2.97	22.22±3.57	NA	-0.784	0.436 ^a
Marital status (Single/Married/Divorce)	15/14/1	11/18/1	NA	1.115	0.573 ^b
Total duration of illness (months)	NA	21.90±27.36	NA	NA	NA
duration of illness this time (months)	NA	13.80±22.75	NA	NA	NA
Episodes	NA	1.17±0.38	NA	NA	NA
HAMD-17	1.07±1.55	21.23±3.52 [*]	7.45±4.16 ^{*#}	299.47	<0.001 ^c
HAMD-A/S		6.37±1.65	2.38±1.30	14.242	<0.001 ^d
HAMD-R		6.80±1.67	2.97±1.55	16.250	<0.001 ^d
HAMD-CD		2.97±1.65	0.97±1.32	7.620	<0.001 ^d
HAMD-SLD		3.87±1.87	0.86±0.88	10.649	<0.001 ^d
HAMD-W		0.53±0.78	0.00±0.00	3.794	0.001 ^d
HAMA	0.73±1.02	18.93±4.73 [*]	6.24±4.11 ^{*#}	194.699	<0.001 ^c
HAMA-PSA		13.13±2.95	4.93±2.75	18.029	<0.001 ^d
HAMA-PHA		5.80±3.30	1.31±1.82	8.830	<0.001 ^d

Abbreviations: MDD, major depressive disorder; HC, healthy control; M/F, male/female; BMI, body mass index; HAMD-17, 17-item Hamilton Depression Rating Scale; HAMD-A/S, Hamilton Depression Rating Scale-Anxiety/somatization factor; HAMD-R, Hamilton Depression Rating Scale-Retardation factor; HAMD-CD, Hamilton Depression Rating Scale-Cognitive disorder factor; HAMD-SLD, Hamilton Depression Rating Scale-Sleep disorder factor; HAMD-Weight, Hamilton Depression Rating Scale-Weight factor; HAMA, Hamilton

Anxiety Rating Scale; HAMA-PSA, Hamilton Anxiety Rating Scale-Psychological anxiety factor; HAMA-PHA, Hamilton Anxiety Rating Scale-Physical anxiety factor; BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; IL-10, interleukin-10; IFN-gamma, interferon-gamma. NA, Not Available.

^aIndependent samples t-test; ^bChi-square test; ^cOne-way ANOVA; ^dPaired-samples t-test.

* $P < 0.001$ compared with HC group; # $P < 0.001$ compared with Pre-treatment MDD group.

Table 2. The comparison of AUCs of single protein and different combinations of proteins.

Different combinations	AUC	Sensitivity	Specificity
IFN-gamma	0.716	63.3%	76.7%
BDNF	0.759	70.0%	83.3%
cortisol	0.766	96.7%	50.0%
BDNF + IFN-gamma	0.818	80.0%	76.7%
IFN-gamma + cortisol	0.834	76.7%	76.7%
BDNF + cortisol	0.841	90.0%	66.7%
BDNF + cortisol + IFN-gamma	0.884	86.7%	83.3%

Abbreviations: AUC, area under the receiver operating characteristic curve; BDNF, brain-derived neurotrophic factor; IFN-gamma, interferon-gamma.

Figures

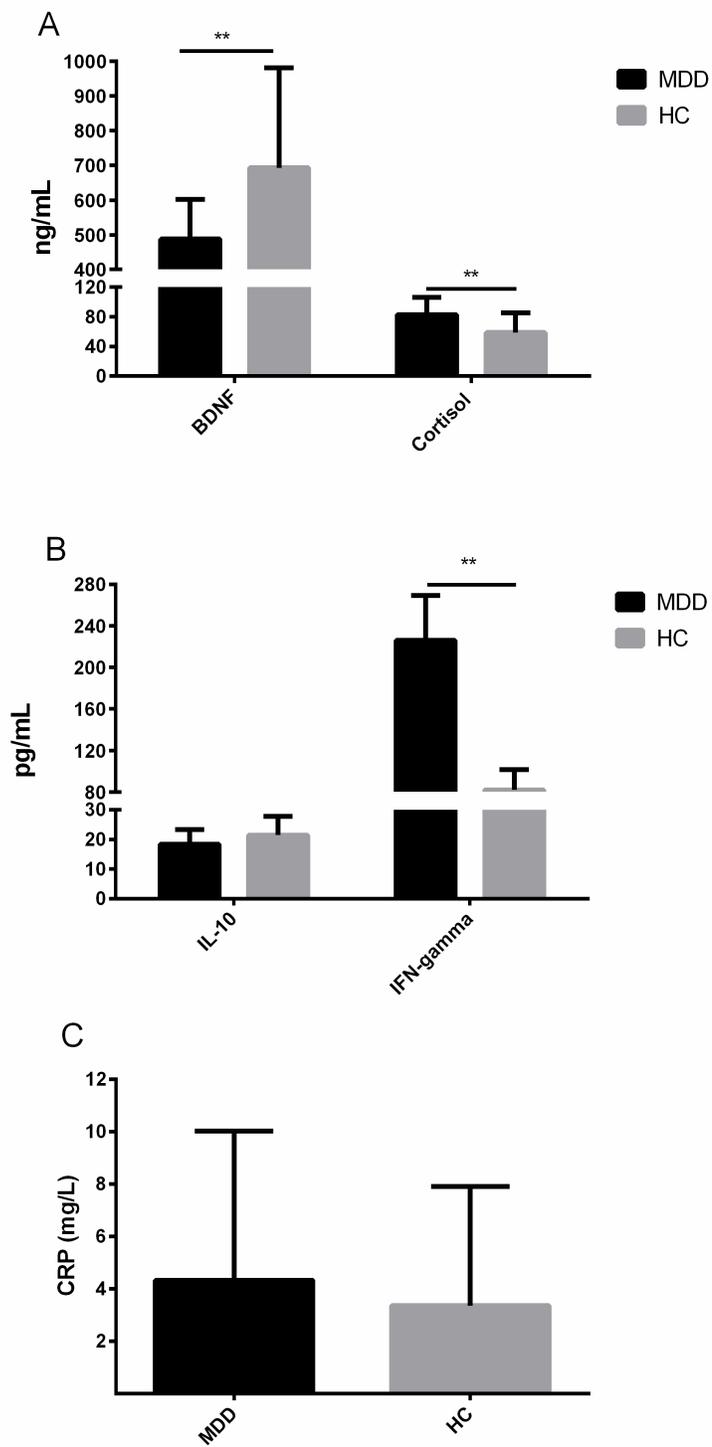


Figure 1

Serum levels of five proteins in MDD patients and HC. Abbreviations: MDD, major depressive disorder; HC, healthy controls; BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; IL-10, interleukin-10; IFN-gamma, interferon-gamma.

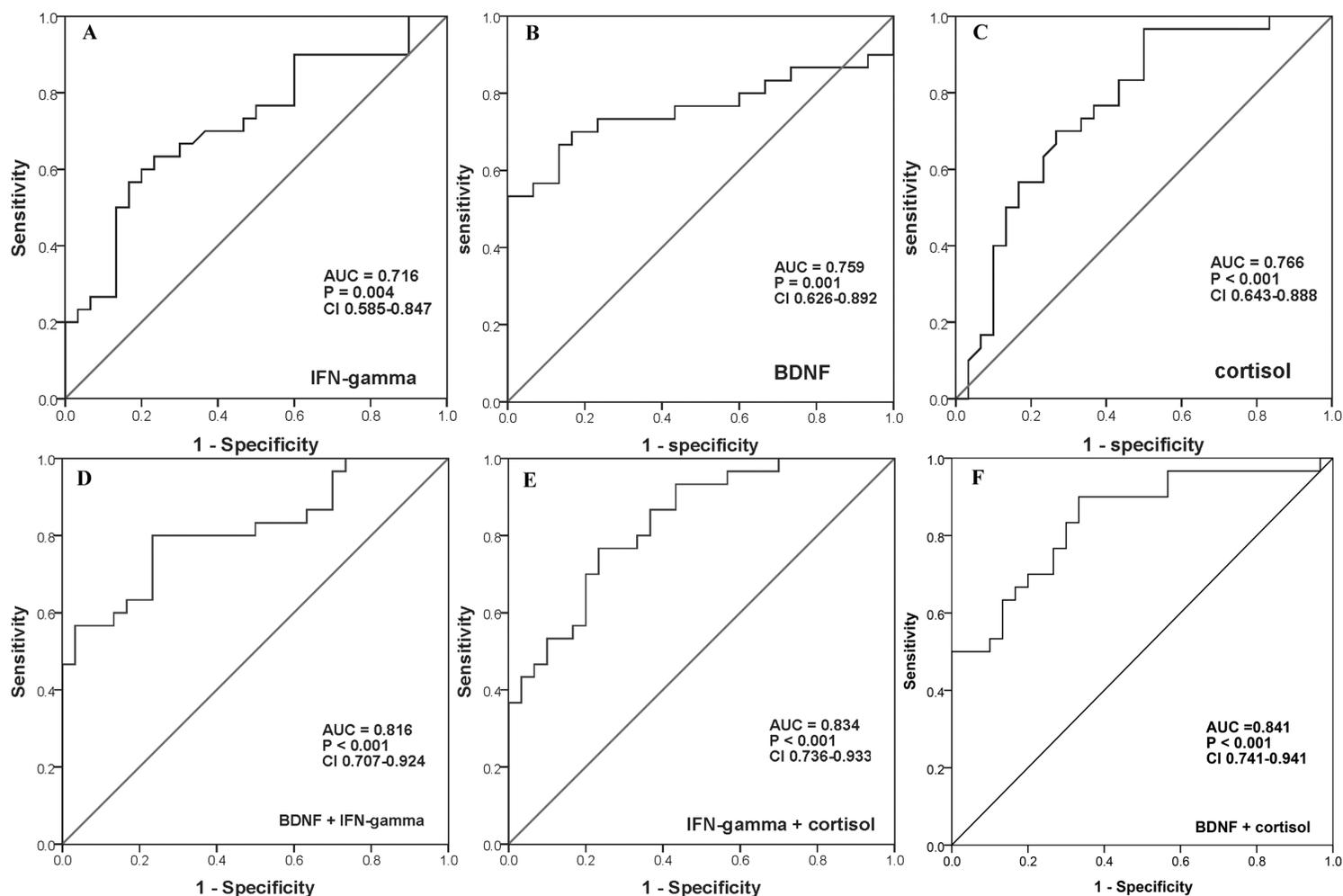


Figure 2

ROC curves of the diagnostic power of each single protein and the different combinations of 2 proteins. (A) ROC curve of IFN-gamma. (B) ROC curve of BDNF. (C) ROC curve of cortisol. (D) ROC curves of the combination of BDNF and IFN-gamma. (E) ROC curves of the combination of IFN-gamma and cortisol. (F) ROC curves of the combination of BDNF and cortisol. Abbreviations: ROC, receiver operating characteristic; IFN-gamma, interferon-gamma; BDNF, brain-derived neurotrophic factor; AUC, area under the receiver operating characteristic curve.

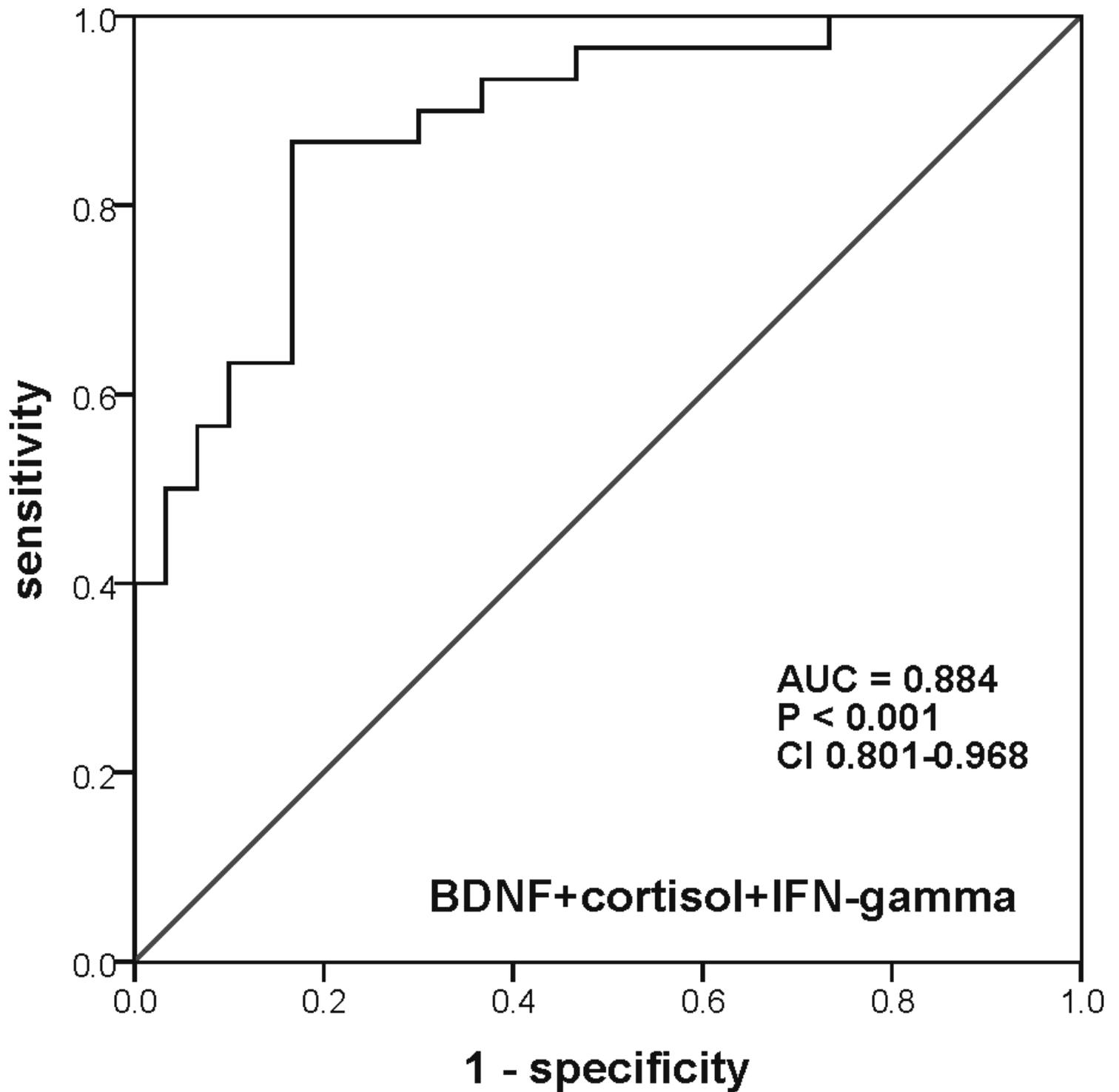


Figure 3

ROC curve of the diagnostic power of the combination of the 3 proteins. Abbreviations: ROC, receiver operating characteristic; IFN-gamma, interferon-gamma; BDNF, brain-derived neurotrophic factor; AUC, area under the receiver operating characteristic curve.

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