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The role of NGF and IL-2 serum level in assisting the diagnosis in first episode schizophrenia

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ABSTRACT

Development of reliable diagnostic bio-markers for schizophrenia remains a diagnostic challenge. Serum NGF and IL-2 were analyzed to examine the diagnostic efficiency and predictive capability of these two biomarkers in relation to schizophrenia diagnosis. Thirty neuroleptic naïve subjects with first-episode schizophrenia, thirty patients with major depressive disorder (MDD) and twenty-eight healthy control subjects participated in the study. One-way ANOVA demonstrated significantly lower serum IL-2 and NGF among schizophrenic patients and patients with MDD compared with healthy controls. Receiver operating characteristic (ROC) curve analysis was used to ascertain diagnostic efficiency of serum IL-2 and NGF levels. Area under the ROC curve (AUC) revealed a high level of differentiation between schizophrenic patients and healthy controls for both IL-2 and NGF serum concentrations. Diagnostic efficiency of combined NGF and IL-2 serum levels was also high in schizophrenic patients compared with healthy controls. Serum NGF and IL-2 are promising as potential screening or diagnostic biomarkers for schizophrenia and may be a useful adjunct for clinical assessment.

1. Introduction

Approximately one percent of the general population is affected by schizophrenic psychoses (Durany and Thome, 2004). Current diagnostic criteria for schizophrenia are largely based on clinical assessment of psychosis. In many areas of the world, cost and availability of clinicians trained in diagnostic interviewing limit patient access to quality mental health care. Inter-rater reliability and cultural bias are among several factors that further confound schizophrenia diagnosis. These limitations and discordance between operational criteria and clinician diagnoses are a challenge for researchers (Cradock et al., 2001; Young et al., 2004; Niv et al., 2007). A key issue in psychiatry management and research is the lack of reliable biomarkers to increase diagnostic accuracy (Le-Niculescu et al., 2009).

Recent gene expression profiling and other studies have suggested several potential biological markers. The neurotrophin, nerve growth factor (NGF), has a likely role in the pathophysiology of several CNS disorders, including schizophrenia and depression (Perez-Polo et al., 1978; Merali, 2003; Parikh et al., 2003; Angelucci et al., 2004; Durany and Thome, 2004; Shoval and Weizman, 2005; Tsuang et al., 2005; Buckley et al., 2007). NGF influences the responsiveness of immune-competent cells (Levi-Montalcini et al., 1995). Evidence indicates that NGF may be an autocrine/paracrine factor in the development and regulation of immune cells (Thorpe and Perez-Polo, 1987; Otten et al., 1989; Brodie and Gelfand, 1992; Torica et al., 1996). Other findings suggest autoimmune mechanisms in the pathogenesis of at least some schizophrenia cases (Strous and Shoenfeld, 2006). The most widely studied are cytokines (Müller, 1997; Drzyzga et al., 2006). In particular, abnormalities of interleukin-2 (IL-2) serum levels or interleukin-2 production were found in acute schizophrenia cases (Ganguli et al., 1987, 1989, 1995; Theodoropoulou et al., 2001). Studies found lower NGF expression in plasma of schizophrenic patients compared with controls (Kale et al., 2009; Lee and Kim, 2009). However Jockers-Scherübl et al. (2003, 2006) demonstrated earlier disease onset and significantly higher serum NGF concentrations in drug-naive schizophrenic patients with previous long-term cannabis abuse than in schizophrenics without cannabis abuse. Other studies demonstrated altered expression of IL-2 (Nassberger and Traskman-Bendz, 1993; Maes, 1995; Capuron et al., 2001; Anisman et al., 2005; Hernández et al., 2008) and NGF (Aloe et al., 2002; Nestler et al., 2002; Dwivedi et al., 2005; Duman and Monteggia, 2006) in patients with major depressive disorder (MDD).

In this study, we used the receiver operating characteristic (ROC) analysis to investigate the potential of serum NGF and IL-2 as

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diagnostic biomarkers for schizophrenia. Specificity, sensitivity, and percentage of correctly classified patients were evaluated using serum NGF and IL-2 levels of patients with first episode of schizophrenia, major depressive disorder and healthy control subjects. Combined serum NGF and IL-2 levels were also evaluated to determine impact on the percentage of correctly classified patients.

2. Methods

2.1. Subjects

All patients were recruited from the Department of Psychiatry, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China. Control subjects were recruited via advertisements at Kunming Medical University. After a description of the study, written informed consent was obtained from each participant. The study protocol was approved by the Ethics Committee at The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China. Each participant received a detailed history and physical examination in which systemic diseases and comorbid psychiatric diagnoses were noted. All subjects were free of chronic and acute diseases (i.e., infectious or allergic) and other physical conditions associated with abnormal cell-mediated immunity for at least 2 weeks prior to joining the study. Standard laboratory testing was normal for all participants.

Schizophrenia and MDD diagnoses were determined by experienced psychiatrists using the Structured Clinical Interview for DSM-IV (SCID) and DSM-IV criteria for MDD. Thirty first episode, neuroleptic- and benzodiazepine-naïve inpatients diagnosed with schizophrenia and thirty antidepressant-naïve inpatients or outpatients with MDD participated in the study. The baseline clinical state of patients was evaluated independently by the two authors at baseline. For schizophrenic patients, findings were recorded as PANSS-general psychopathology (GPS), PANSS-positive (PSS) and PANSS-negative (NSS) symptom scores (Kay et al., 1987), and the Hamilton Depression Rating Scale (HAM-D) was used to assess depressive symptoms for MDD patients (Hamilton, 1960). Control subjects were matched with schizophrenia and MDD patients for age, sex, education level, number of cigarettes smoked per day, and pattern of smoking.

2.2. NGF and IL-2 ELISA

A fasting venous blood sample (5 ml) was collected from all subjects and allowed to clot at room temperature. Serum was obtained by centrifugation at 3000×g for 10 min, then aliquoted and stored at -70 °C until analysis. Double antibody sandwich enzyme-linked immunosorbent assays (ELISA) were employed to quantify human serum NGF and IL-2. Human β-NGF and human IL-2 were assessed using the DuoSet ELISA Development System according to the manufacturer's instructions (R and D Systems, Minneapolis, MN, USA). All measurements were performed in duplicate and expressed as ρg/mL. Assays that were compared directly were measured within the same assay. Intra-assay variation of standards was less than 5%. Analytical range of the serum NGF assay was 31.2 to 2000 ρg/ml, with a 5.3% intra-assay coefficient of variation. Cross-reactivity to related neurotrophins (NT-3, NT-4) was less than 3%. Analytical range of the IL-2 assay was 0 to 2000 ρg/ml, with a 4.3% intra-assay coefficient of variation. The minimum detectable amount of IL-2 is typically <7.0 ρg/ml and the assay recognizes both natural and recombinant human IL-2. No significant cross-reactivity or interference was observed.

2.3. Statistical analysis

All parameters were analyzed using the Shapiro-Wilk test for normality. All distributions were found to be normal, permitting parametric multivariate analysis. Demographic and smoking variables were evaluated statistically using the χ^2 test. Schizophrenic, MDD, and control groups were compared using one-way analysis of variance (ANOVA). We also applied linear discriminate analysis (LDA) to further separate study groups into one or more linear combinations of independent variables. The general principles of LDA are described elsewhere (Huberty and Hussein, 2003).

In this study, a discriminant analysis was performed on serum NGF and IL-2 levels to classify subjects as patients or controls. The discriminant model has the form: $D = B_0 + B_1X_1 + B_2X_2$, where D, the discriminant score, is the dependent variable, while X_1 and X_2 represent serum NGF and IL-2 levels. Those variables, together with calculated coefficients B_0, B_1, and B_2, were selected to maximize the distance between the three groups. We evaluated the utility of different combinations of serum NGF and IL-2 levels to discriminate among control groups, schizophrenia patients, and MDD patients. Receiver operating characteristic curves (ROCs) were constructed as plots of the percentage of true-positives (sensitivity) versus false-positives (100-specificity) of serum IL-2 and NGF concentrations in schizophrenia and MDD groups, and the area under each curve was calculated.

For all statistical tests, a two-tailed $P<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS15.0 for Windows (SPSS Inc., Chicago, IL, USA) and SAS 9.0 (SAS Institute Inc. Raleigh, NC, USA).

3. Results

3.1. Study Population

The demographic characteristics of the three groups are presented in Table 1. Thirty patients with schizophrenia [male/female = 17/13; mean age (SD) = 22.83 (4.07) years; age range = 18-33 years], thirty patients with MDD [male/female = 13/17; mean age (SD) = 25.63 (6.41) years; age range = 18-38 years], and 28 healthy control subjects [male/female = 15/13; mean age (SD) = 23.11 (3.21) years; age range = 18-29 years] participated in the study. No statistical differences were found in age, gender, body mass index (BMI), smoking patterns, or duration of illness, and serum IL-2 and NGF levels did not differ within three groups ($P>0.05$). Therefore, data were pooled for subsequent analysis.

3.2. IL-2 and NGF serum levels

Figs. 1 and 2 show the levels of circulating IL-2 and NGF in first episode schizophrenics, MDD patients and healthy subjects. One-way ANOVA exhibited significant differences in IL-2 and NGF serum levels among groups (IL-2: F = -14.279, $P<0.001$; NGF: $F = 13.116$, $P<0.001$). IL-2 and NGF serum levels were significantly lower in the schizophrenic (IL-2: $P<0.001$; NGF: $P<0.001$) and MDD groups (IL-2: $P<0.001$; NGF: $P<0.001$) compared with healthy subjects. However, the differences between schizophrenic and MDD groups were not statistically significant.

3.3. Diagnostic efficiency of NGF and IL-2 serum levels

Area under the ROC curve (AUC) was used to evaluate diagnostic efficiency of serum IL-2 and NGF levels (Table 2). In schizophrenic patients, a serum IL-2 diagnostic cutoff value of 73.95 pg/ml was chosen to maximize sensitivity at 76.7% with a corresponding specificity of 96.4% (95% CI: 0.761–0.971). The serum NGF diagnostic cutoff of 55.25 pg/ml gave a maximal sensitivity of 60.0% with a specificity of 89.3% (95% CI: 0.661–0.901). The areas under the serum IL-2 and NGF ROC curves were 0.866 and 0.781, respectively. The serum IL-2 diagnostic cutoff value of 72.72 pg/ml maximized ability to discriminate between schizophrenia and MDD at a sensitivity of 90.0%, with a specificity of 76.7% (95% CI: 0.662–0.927). The area under the ROC curve was 0.794. In comparison of MDD and schizophrenic groups, serum IL-2 had a high level of differentiation, while the area under the NGF ROC curve was only 0.580.

3.4. Stepwise discriminant function analysis

To explore the diagnostic efficiency of combination of NGF and IL-2 serum levels, stepwise discriminant analysis (SDA) was used to create discriminant scores, which were then analyzed by ROC for the three experimental groups. SDA (Wilkes method) was performed

Table 1
Demographic and clinical characteristics of subjects.

	Control	Schizophrenia	Major Depressive Disorder
Sex	15 male/13 female	17 male/13 female	17 male/13 female
Age (years)	23.11 (±3.21)	22.83 (±4.07)	25.63 (±6.41)
Age range (years)	18 - 29	18 - 33	18 - 38
BMI(body mass index)	22.45(±1.23)	23.01(±1.67)	22.63(±1.29)
Smoker	9 male	8 male	9 male
Illness duration (months)	—	8 13 (±1.65)	7.09 (±1.93)
Total PANSS score	—	91.63 (±7.6)	—
HAMD score	—	—	20.13 (±2.09)

Mean (±SD).