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Brain-derived neurotrophic factor and inflammatory markers in patients with early- vs. late-stage bipolar disorder

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Abstract

Bipolar I disorder (BD) has a poorer longer-term outcome than previously thought, with persistent cognitive impairment and functional decline. The neurobiological underpinnings that might underlie these changes remain unknown. Changes in brain-derived neurotrophic factor (BDNF) levels and cytokines are potential candidates. The aim of this study was to examine both cytokine and BDNF levels and their relationship in BD patients in the early and late stages of the disorder. We measured serum BDNF, TNF-α, IL-6 and IL-10 levels in a total of 60 patients with BD I and we compared those in early stages of illness with those in late stages of illness and also compared both groups with 60 matched healthy controls. BDNF was decreased only in those patients in the late stage of bipolar disorder. Moreover, BDNF levels were negatively correlated with length of illness. In contrast, all interleukins and TNF-α were increased in the early stages of BD, compared to controls. While TNF-α and IL-6 continued to be significantly higher than controls at late stages of BD, IL-10 did not. When levels were compared between patients at early and late stages of illness, there was a significant decrease in BDNF and IL-6 in the later stage of BD compared to the early stage. Inversely, TNF-α showed a significant increase at the later stage. Failure of inflammatory defences in the late stage of the disorder may account for reduction in BDNF and continued elevations in cytokines; thus these may have the potential to serve as markers of illness progression in BD.

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Key words: Bipolar disorder, BDNF, cytokines, first episode, interleukins, TNF-α.

Introduction

Bipolar disorder (BD) is among the most disabling of all medical disorders and is associated with high mortality rates due to suicide and other medical illnesses (Yatham et al., 2005). BD has a much poorer long-term outcome than previously thought. Patients frequently demonstrate sub-threshold symptoms with persistent cognitive impairment and functional decline (Kapczinski et al., 2008). There is some evidence that patients at early stages of the illness have a much better clinical outcome than those with multiple episodes (Schuepbach et al., 2008; Tohen et al., 1990). For instance, patients with a longer duration of the illness or those that had more than three previous episodes are less likely to respond to treatment, particularly to lithium (Gelenberg et al., 1989; Swann et al., 1999). The duration of inter-episode period shortens as the number of episodes increases (Kessing et al., 1998). Based on these observations, a medical staging model has been proposed for BD recently (Berk et al., 2007).

These clinical observations are consistent with differences reported in brain structure and cognitive function between patients with early and late stages of

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BD. For instance, morphometric studies have shown that patients with BD have changes in many brain structures in comparison to controls (Lopez-Larson et al., 2002; Lyoo et al., 2004, 2006). Notably, some authors reported that such neuroanatomical changes tend to be more pronounced with repeated episodes and correlate with length of illness (Lyoo et al., 2006; Strakowski et al., 2002). Consistent with this, recent studies suggest that those that recently had their first manic episode have minimal alterations in brain structures (Yatham et al., 2007). Furthermore, there is evidence that patients with BD have persistent cognitive impairment and that the extent of impairment is greater in those with multiple episodes compared with those that had a first manic episode of BD (Torres et al., 2007).

The neurobiological underpinnings of poorer clinical response, more pronounced cognitive impairment and neuroanatomical brain changes in multiple-episode patients with BD remain unknown. Among those neurobiological markers that appear to be involved in the pathophysiology of BD, the neurotrophins and the inflammatory cytokines stand out as potential mechanisms for neurodegeneration and cognitive impairment (Duman and Monteggia, 2006; Hashimoto et al., 2004; Kim et al., 2007; Post, 2007b). In particular, the brain-derived neurotrophic factor (BDNF) has received attention due to its role in regulating neuronal survival, structure, and function. BDNF is highly expressed in brain areas that are known to regulate complex cognitive functions (Duman and Monteggia, 2006; Phillips et al., 2003; Post, 2007b). Studies suggest that abnormalities in the BDNF-signalling system might be involved in the cognitive decline observed in certain neuropsychiatric disorders, such as multiple sclerosis (Caggiula et al., 2005), Alzheimer's disease (Laske et al., 2006), Parkinson's disease (Imamura et al., 2005) and mood disorders (Shaltiel et al., 2007). In addition, preclinical and clinical studies have suggested a role for BDNF in BD (Cunha et al., 2006; Frey et al., 2006). BDNF levels were negatively correlated with the severity of mood symptoms in bipolar patients (Cunha et al., 2006; Machado-Vieira et al., 2007; Shimizu et al., 2003). However, it is unknown if there are differences in BDNF levels and other neurobiological markers in BD patients in the early and late course of the disorder.

Similarly, increased pro-inflammatory cytokines were associated with impairment in spatial learning in preclinical studies (Larson and Dunn, 2001). Of these cytokines, increased TNF-α was associated with cognitive impairment in those with HIV (Seilhean et al., 1997) and Alzheimer's disease (Tobinick et al., 2006). In parallel, there is substantial evidence for the involvement of immune and inflammatory responses in BD (Brietzke and Kapczinski, 2008). It is of note that inflammation plays a role in many systemic disorders that are associated with long-term bipolar illness, such as diabetes and cardiovascular disease and increased allostatic load (Kapczinski et al., 2008). Studies have indicated that a pro-inflammatory state is associated with mania (Brietzke and Kapczinski, 2008). A number of recent reports have shown increased levels of the pro-inflammatory cytokine TNF-α in acutely manic or depressed patients with BD (Kim et al., 2007; O'Brien et al., 2006; Ortiz-Dominguez et al., 2007). Some studies have shown increased IL-6 during depression (Ortiz-Dominguez et al., 2007), while others show increased IL-6 during mania (Kim et al., 2007). Furthermore, it has been suggested that a balance between cytokine levels and neurotrophin levels is associated with programmed cell death (apoptosis) (Nagatsu et al., 2000). There is evidence from post-mortem studies that BD pathophysiology may involve apoptotic cell death and neuronal and glial cell loss (Benes et al., 1998), which may go along with the persistent inter-episode cognitive impairment and reduced brain morphometry in patients with BD. However, no study to date has examined both cytokine and BDNF levels and their relationship in the same subjects with BD.

Therefore, we measured serum BDNF, TNF-α, IL-6 and IL-10 levels in patients with BD during early stages of BD (patients who recently had their first manic episode) and during late stages of BD (patients with a minimum of 10 yr of diagnosed BD), and compared these with a matched healthy control groups.

Methods

Sixty patients with BD I and 60 healthy controls, matched for age, gender and level of education, were recruited. The double case-control design consisted of 30 patients at early stage of BD (within first 3 yr of a first manic episode); 30 patients at a late stage of BD (minimum 10 yr after diagnosis of BD) and their respective matched control groups. Early-stage patients were recruited from University of British Columbia (UBC) and Vancouver General Hospitals through the First-episode Mania Program, Vancouver, Canada. Inclusion criteria were age 15–35 yr, and having experienced a first manic episode within the 3–36 months prior to the blood draw. Late-stage patients were recruited from Bipolar Disorders Program, Hospital de Clinicas de Porto Alegre (HCPA), Porto Alegre, Brazil. Inclusion criteria were

age 18–65 yr and having a formal diagnosis of BD I for >10 yr (retrospectively assessed through medical records).

All subjects had a comprehensive clinical interview by a board-certified psychiatrist. The diagnosis of BD I was established based on all the available clinical information and confirmed with Mini International Neuropsychiatric Interview (MINI; Sheehan et al., 1998) at the UBC site or with the Structured Clinical Interview for DSM-IV – Axis I (SCID-I) at the HCPA site (APA, 2000). The comorbidities were diagnosed according to DSM-IV-TR. Subjects enrolled in both programmes received open-label maintenance treatment for BD from clinicians with expertise in management of mood disorders and familiar with the most recent clinical guidelines (Yatham et al., 2005, 2006). Patients did not have significant comorbid medical conditions and they were not on medication other than those prescribed for their psychiatric condition. Psychiatric status was assessed within 1 wk of blood draw with clinical rating scales: the Young Mania Rating Scale (YMRS; Young et al., 1978) and the Hamilton Depression Rating Scale, 21-item version (HAMD-21; Williams, 1988). Functioning was assessed with the Global Assessment of Functioning scale (GAF; APA, 2000). Clinical variables were collected with a standardized protocol. The variable, length of illness, includes the time in years from the first mood episode to the time the patient had a blood draw for BDNF and cytokines. Despite having a recent first manic episode, many patients from the early-stage group have had a previous depressive episode, which was accounted for in the length-of-illness variable.

Controls, matched for age, gender and level of education, were recruited from both centres and screened to rule out any history of psychiatric disorder, neurodegenerative disorder, mental retardation, cancer or chronic/acute infection. Controls were non-smokers and were not on any medication. All of the procedures described in this study received approval from the local clinical research ethics committees. Written informed consent was obtained from all patients and healthy subjects prior to conducting any study procedures.

Biochemical assays

Ten milliliters of blood were drawn from each subject by venepuncture into a free-anticoagulant vacuum tube. The blood was immediately centrifuged at 3000 *g* for 5 min, and serum was kept frozen at −80 °C until assayed.

BDNF assay

BDNF serum levels were measured with sandwich-ELISA, using a commercial kit according to the manufacturer's instructions (Chemicon, Temecula, CA, USA). Briefly, 96-well, flat-bottomed microtitre plates were coated for 24 h with the samples diluted 1:2 in sample diluents and the standard curve ranged from 7.8 to 500 pg BDNF. Plates were then washed four times with wash buffer, monoclonal anti-BNDF rabbit antibody was added (diluted 1:1000 with sample diluents) and incubated for 3 h at room temperature. After washing, a second incubation with peroxidase-conjugated anti-rabbit antibody (diluted 1:1000) for 1 h at room temperature was performed. After the addition of streptavidin enzyme, substrate and stop solution, the amount of BDNF was determined (absorbance set at 450 nm). The standard curve demonstrates a direct relationship between optical density (OD) and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin (BSA) as a standard. The assay sensitivity for BDNF was 7.8 pg/ml (range 7.8–500 pg/ml).

Cytokine assay

The serum concentration of cytokines was measured using enzyme-linked immunosorbent assay (ELISA). Briefly, polystyrene high-binding, 96-well microtitre plates (Nunc-Immuno Plate; Maxisorp, Rochester, NY, USA) were coated with capture antibody. After overnight incubation at 4 °C, the plates were washed (as in subsequent steps) with PBS containing 0.05% Tween-20 and 0.4 M NaCl and then incubated in dilution buffer (PBS containing 1.0% BSA; 100 μl per well) for 2 h at room temperature to block non-specific binding. After washing, the diluted (1:10 in PBS buffer; pH 7.4) samples (100 μl per well), or the serially diluted standards of each cytokine were added to the plates and incubated overnight at 4 °C. After washing the plates, the 100 μl of peroxidase-conjugated anti-rabbit IgG (Abcam, Cambridge, UK) was added to each well and the plates were incubated for 1 h at room temperature. After further washing, colour development was initiated by the addition of substrate and was allowed to develop for up to 30 min at room temperature and terminated by the addition of 4 M sulphuric acid. Antibody binding was determined from absorbance at 490 nm, and the concentration of cytokines was calculated from a standard curve. The sensitivity and range for cytokine assays, respectively, were: TNF-α (<2 pg/ml, 2.08–60 pg/ml), IL-6 (<10 pg/ml, 15.6–250 pg/ml), and IL-10 (<1.30 pg/ml, 1.56–50 pg/ml). Laboratory procedures

Table 1. Sociodemographic variables in patients with bipolar disorder (BD) and controls

Variable	Early stage			Late stage		
	Patients with BD ($n=30$)	Controls ($n=30$)	p	Patients with BD ($n=30$)	Controls ($n=30$)	p
Sex (male)	43.3% (13)	33.3% (10)	0.596[a]	30% (9)	36.7% (11)	0.785[c]
Age (yr) mean (S.D.)	22.4 (3.9)	22.1 (3.6)	0.734[b]	41.4 (8.4)	43.2 (6.4)	0.376[b]
Education (yr) mean (S.D.)	13.5 (2.0)	12.9 (2.8)	0.331[b]	9.3 (3.8)	10.6 (1.6)	0.086[b]
Ethnicity (Caucasian)	73.3% (22)	83.3% (25)	0.532[a]	90% (27)	93.3% (28)	1.0[a]

[a] Fisher's exact test.
[b] t test.
[c] χ^2 test.

were performed in a double-blind fashion. All assays were performed in duplicate.

Statistics

Statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics are used to report sociodemographic and clinical characteristics of the sample. Association between dichotomous variables was assessed with χ^2 test or Fisher's exact test when appropriate, as indicated. Continuous demographic/clinical variables were compared between patients and their respective controls using t test, as indicated. Main comparisons were performed including the four groups (patient and controls, early and late stage) in a model with age, gender and education as covariates (ANCOVA), followed by post-hoc Tukey tests when appropriate. BDNF and cytokine levels were compared between groups of patients using a general linear model that included gender, age, education, medication use and mood rating scales as covariates. A correlation (Pearson coefficient) matrix was performed to examine the relationship of BDNF levels with age, age of onset, length of illness, education, YMRS and HAMD scores, number of mood episodes, TNF-α, IL-6 and IL-10. Based on these correlations, significant associations were entered as covariates in the models. We used a linear regression model to examine the association between BDNF levels and length of illness, including age, HAMD and YMRS as covariates. The same model was used to examine the association of TNF-α, IL-6 and IL-10 with length of illness.

All statistical tests were two-tailed and were performed using a significance level of $\alpha=0.05$. Data are presented as means $\pm$ standard deviation (S.D.), median, or percentage, as indicated.

Results

Sociodemographic variables are shown in Table 1. There is no difference between patients and their respective control groups regarding gender, age, years of education and ethnicity. Early- and late-stage patients have expected differences in age, which was accounted for in the study design by including two control groups.

Clinical characteristics of patients with BD at early and late stages of illness are shown in Table 2. As expected, patients showed a significant difference in length of illness ($t=10.9$, d.f. $=58$, $p<0.001$). There was also a difference in the number of previous mood episodes (see Table 2). Age of illness onset was significantly younger in the early-stage group ($t=-4.4$, d.f. $=58$, $p<0.001$), which could be in part explained by a recall bias, as the late-stage group was recruited in a cross-sectional manner and the age of onset collected retrospectively. The two groups of patients had differences in mood rating scale scores; patients with >10 yr illness had a higher score on YMRS ($t=2.2$, d.f. $=58$, $p=0.02$) and HAMD ($t=3.1$, d.f. $=58$, $p=0.002$), which is consistent with poorer clinical response and greater likelihood of sub-syndromal symptoms in patients with late-stage BD. There was no difference between the two groups of patients regarding functioning as per GAF scores. Moreover,

Table 2. Clinical characteristics of patients at early and late stages of bipolar disorder

Variable	Early stage ($n=30$)	Late stage ($n=30$)	p
Age onset of illness, mean (S.D.)	20.2 (4.2)	27.2 (7.4)	<0.001[a]
Length of illness, mean (S.D.)	2.1 (2.9)	13.9 (5.12)	<0.001[a]
No. previous depression episode, mean (S.D.)	1.1 (1.5)	6.2 (8.12)	0.007[b]
No. previous hypomania episode, mean (S.D.)	0.8 (2.1)	0.05 (0.2)	0.046[b]
No. previous mania episode, mean (S.D.)	1.0 (0.0)	7.1 (9.1)	0.004[b]
YMRS mean (S.D.)	1.53 (2.8)	3.6 (4.1)	0.026[b]
HAMD mean (S.D.)	3.8 (7.1)	9.2 (6.0)	0.002[b]
GAF mean (S.D.)	63.3 (12.5)	61.4 (17.5)	0.643[b]
Medication			
Mood stabilizer	83.3% (25)	86.7% (26)	1.0[c]
Antipsychotic	76.7% (23)	60% (18)	0.267[c]
Antidepressant	10% (3)	20% (6)	0.472[c]

YMRS, Young Mania Rating Scale; HAMD, Hamilton Depression Rating Scale; GAF, Global Assessment of Functioning scale.
[a] χ^2 test.
[b] t test.
[c] Fisher's exact test.

there was no difference between groups in type of medication use.

Figure 1 shows the results for BDNF and cytokine levels. BDNF levels were similar between early-stage BD patients and matched controls, but were clearly decreased in late-stage BD patients compared to matched controls (Figure 1a). An ANCOVA with BDNF levels as a dependent variable with age, gender and education as covariates showed a significant effect of group ($F=16.1$, d.f. $=1$, 105, $p<0.001$). Post-hoc tests indicated a significant difference between late-stage BD patients and controls ($p<0.001$), but not between early-stage BD patients and controls ($p=0.073$). It should be noted that four patients were excluded from analysis of BDNF levels prior to assay starting due to incorrect sample storage. In order to maintain the homogeneity of sample, their respective matched controls were also excluded from this assay only. The same ANCOVA model was performed with cytokine levels (Figure 1b–d) as dependent variables which showed that there was a group effect for TNF-α levels ($F=76.4$, d.f. $=6$, 113, $p<0.001$), on IL-6 ($F=28.4$, d.f. $=6$, 113, $p<0.001$) and for IL-10 ($F=11.3$, d.f. $=6$, 113, $p<0.001$). Post-hoc tests showed that TNF-α levels were already significantly higher in early-stage BD patients than in controls ($p=0.002$), and continued to be higher than controls in late-stage BD patients ($p<0.001$). Similarly, IL-6 levels were increased in patients with BD at early ($p<0.001$) and late stages ($p=0.028$), compared to the control groups. IL-10 was increased in early-stage BD ($p<0.001$) but not in the late-stage BD ($p=0.121$).

The direct comparison of the levels of neurotrophins and cytokines between early- and late-stage BD showed interesting differences in the direction of the changes. While BDNF ($F=24.6$, d.f. $=5$, 50, $p<0.001$) and IL-6 ($F=6.0$, d.f. $=7$, 52, $p=0.018$) showed a significant decrease from the early to late stage of BD (Figure 1a, c), TNF-α ($F=12.1$, d.f. $=7$, 52, $p=0.001$) significantly increased from early to late stage (Figure 1b). These results were controlled for age, gender, education, medication use and mood symptoms, as per scores in YMRS and HAMD. IL-10 (Figure 1d) showed a significant decrease from early to

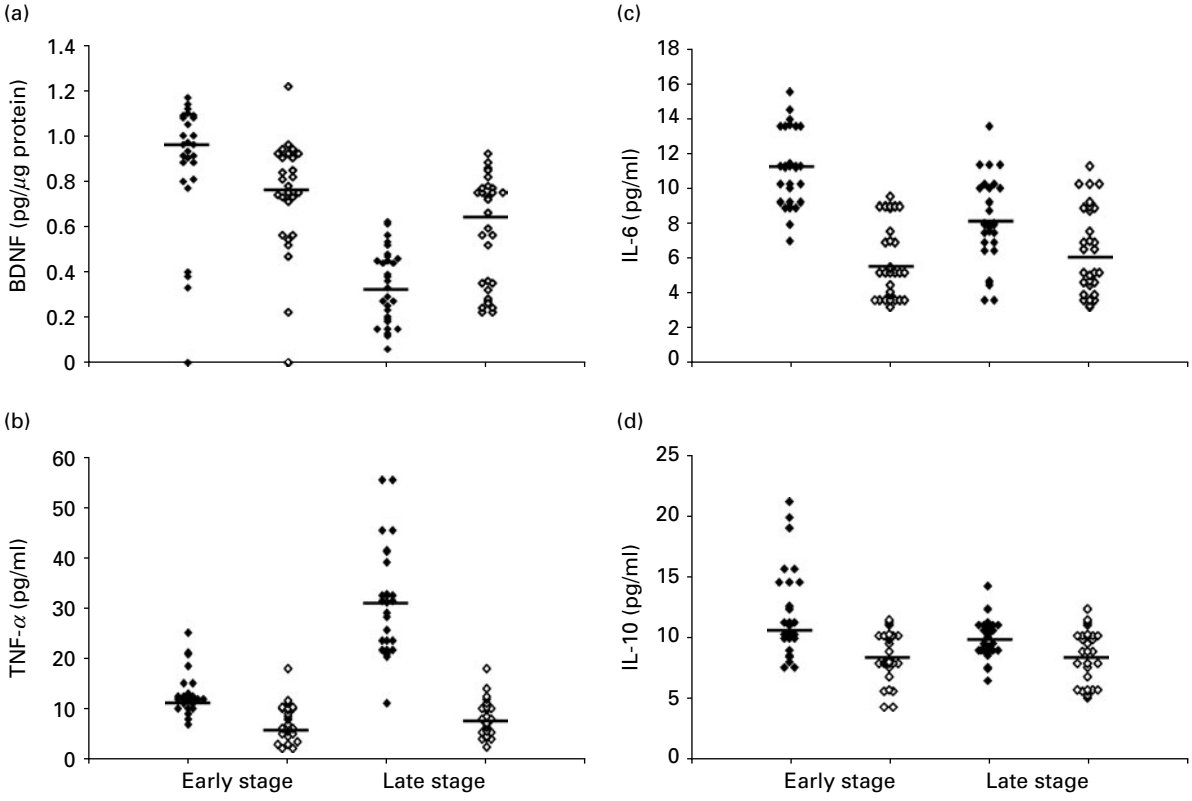

Figure 1. Statistically significant differences: (a) BDNF: early-stage (ES) patients vs. late-stage (LS) patients ($p<0.001^a$). LS patients vs. LS controls ($p<0.001^b$). (b) TNF-α: ES patients vs. LS patients ($p<0.01^a$). ES patients vs. controls ($p<0.01^b$). LS patients vs. controls ($p<0.001^b$). (c) IL-6: ES patients vs. LS patients ($p<0.001^a$). ES patients vs. controls ($p<0.001^b$). LS patients vs. controls ($p<0.01^b$). (d) IL-10: ES patients vs. controls ($p<0.001^b$). [a] Comparisons between patients ES vs. LS were controlled for age, gender, education (HAMD and YMRS). [b] Comparisons with respective control group were controlled for age, gender and education. ◆, Patients; ◇, controls.

late stages of BD without controlling for confounders, but did not hold statistical significance after the above covariates were added to the model ($F=1.0$, d.f. $=7$, 52, $p=0.307$). These findings were confirmed by a positive correlation of BDNF levels with IL-6 levels ($r=0.56$, $p<0.001$) and a negative correlation with TNF-α levels ($r=-0.56$, $p<0.001$), but no significant correlation with IL-10 levels.

Finally, we examined the correlation between BDNF and cytokines with length of illness. There was a significant negative correlation of length of illness with BDNF ($r=-0.67$, $p<0.001$) and with IL-6 levels ($r=-0.41$, $p=0.001$). There was also a positive correlation between TNF-α levels ($r=0.61$, $p<0.001$) and length of illness. There was no correlation between IL-10 and length of illness. Given that all these variables also showed a significant correlation with age and some with mood symptoms, we investigated the association between serum levels and length of illness in a linear regression model controlling for age and mood symptoms. The association between cytokines and length of illness was no longer statistically significant after controlling for confounders ($p>0.05$). However, the length of illness remained as a significant correlate with BDNF levels ($F=14.9$, d.f. $=4$, 50, $\beta=-0.016$, $p=0.039$), and the model predicted up to 50% ($R^2=0.54$) of the variance in BDNF levels (Figure 2). In the same vein, BDNF levels ($r=-0.34$, $p=0.017$) and IL-6 ($r=-0.37$, $p=0.006$) showed a negative correlation with number of previous depressive episodes, whereas TNF-α showed a positive correlation with number of previous depressive episodes ($r=0.34$, $p=0.012$) and number of previous manic episodes ($r=0.51$, $p<0.001$). Given that the number of mood episodes showed a significant correlation with length of illness ($r=0.57$, $p<0.001$), as expected, we only included length of illness in the regression model, as it is less subject to recall bias.

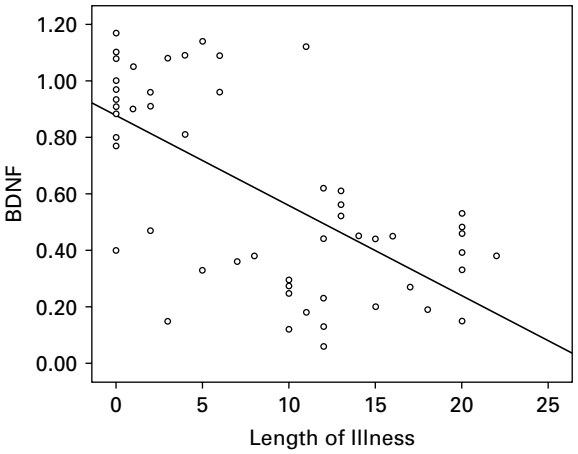

Figure 2. Correlation between BDNF levels and length of illness. Linear regression (age, YMRS, HAMD and length of illness): $\beta = -0.016$, $p = 0.039$; $R^2 = 0.54$.

Discussion

This is the first study to examine BDNF and cytokine levels at different stages of BD. The results suggest that BD is associated with changes in neurotrophins and cytokines that vary from early to late stages of illness. For instance, TNF-α and IL-6 cytokines were increased in early and late stages of BD, while BDNF levels were decreased in the late stage of BD but not in the early stage compared to controls. The anti-inflammatory IL-10 was increased in the early stage of BD, but not in later stages. The decrease in BDNF levels appears to be in proportion with length of illness, regardless of mood symptoms and age. There was also a negative correlation of BDNF levels with number of mood episodes. These data indicate that patients with BD are in a pro-inflammatory state, which is worse in the later stages of illness. Furthermore, these results indicate that there is a decrease in the protective mechanisms in BD from early to later stages as indicated by reductions in BDNF levels and anti-inflammatory IL-10 in the later stages of the disorder.

Previous studies have found decreased BDNF levels in multi-episode patients with BD. BDNF was decreased in those with manic (Cunha et al., 2006; Machado-Vieira et al., 2007) as well as in those with depressive symptoms (Cunha et al., 2006). The two studies that have investigated BDNF levels during euthymia showed decreased (Monteleone et al., 2008) and similar (Cunha et al., 2006) BDNF levels compared to a healthy group. The inconsistency in findings in euthymic patients might be related to the heterogeneity of the samples with regard to the length of illness, which was longer in the former study. Consistent with this, our results showed decreased levels of BDNF in late-stage BD patients who had longer duration of illness but not in those in the early stages of BD. Information about BDNF in the early course of BD is limited to only one recent preliminary study which showed a marked decrease in BDNF levels in BD shortly after the onset of first psychotic episode; the levels progressively increased towards control values during 1-yr follow-up (Palomino et al., 2007). Interestingly, recent data suggest that there was a significant difference in BDNF levels between early and late stages of Alzheimer's disease defined by memory performance scores. Further, BDNF serum levels correlated with mini-mental examination scores in these patients (Laske and Eschweiler, 2006; Laske et al., 2006). Similarly, in multiple sclerosis patients, those with a longer history of disease and with incomplete recovery after relapse showed lower BDNF levels (Caggiula et al., 2005). This is in line with the finding that BDNF levels may vary in proportion with length of illness in BD (Figure 2).

This is the first time that interleukins have been examined in sample of patients who recently experienced their first manic episode. Our findings are in agreement with previous studies that have reported increased pro-inflammatory cytokines in BD (Brietzke and Kapczinski, 2008; Kupka et al., 2002; O'Brien et al., 2006). A number of recent reports have shown increased levels of the pro-inflammatory cytokine TNF-α in acutely manic or depressed patients with BD (Kim et al., 2007; O'Brien et al., 2006; Ortiz-Dominguez et al., 2007). We found increased TNF-α levels in those with early-stage BD and even a greater increase in those with late-stage BD. Given that TNF-α level is correlated with mood symptoms (Larson and Dunn, 2001), one could argue that higher levels of TNF-α observed in those with late-stage BD might be related to sub-syndromal mood symptoms as sub-syndromal symptoms are more frequent in those with late-stage BD. However, TNF-α levels remained significantly higher in those with late-stage BD even after controlling for increased frequency of sub-syndromal symptoms. It is probable that increased TNF-α levels in late-stage BD might be due to cumulative increase in inflammatory status or failure of protective anti-inflammatory mechanisms or due to a cumulative effect of mood episodes (Kapczinski et al., 2008). Few studies that have examined immune response prospectively in BD showed that IL-6 and TNF-α levels were increased during mania and only IL-6 returned to baseline levels after 6 wk of treatment with mood stabilizers, whereas TNF-α continued to remain high

(Kim et al., 2007). These previous data suggest that TNF-α may be a more enduring change in BD, which is consistent with our findings of a persistent increase in TNF-α levels as well as a decrease in IL-6 levels from earlier to later stages. In addition, IL-6 seems to be more susceptible to the effects of medication (Kim et al., 2007), which would be consistent with decrease in the late-stage group of patients who had been on long-term medications. The differences in medication use and length of illness could also explain inconsistencies in previous studies showing increased IL-6 levels only during depression (Ortiz-Dominguez et al., 2007) or only during mania (Kim et al., 2007). Our finding of no change in IL-10 in late-stage BD is consistent with previous studies which examined IL-10 in multiple-episode patients.

The interaction between neurotrophins and the inflammatory system could occur in a number of ways. BDNF and cytokines seem to cooperate in intracellular signalling (Brietzke and Kapczinski, 2008). When P12 cells are co-stimulated with TNF-α and BDNF, the nuclear translocation of NF-κB – a transcription factor that promotes cell survival – increases greatly. However, BDNF alone does not induce NF-κB translocation (Furuno and Nakanishi, 2006). Similarly, the ability of IL-6 to support the survival of embryonic sensory neurons in vitro depends upon the presence of BDNF and the induction of BDNF in injured adult sensory neurons depends upon the presence of IL-6 (Murphy et al., 2000). Alone, TNF-α can induced dendrite beading, an early feature of neuronal damage, in neuron-rich culture (Suzumura et al., 2006). TNF-α also can act as a trigger to apoptosis through activation of caspase 8 cascade (Mogi et al., 2000; Takeuchi et al., 2006). Thus, under inflammatory conditions, the presence of BDNF might limit the immune injury in the brain (Brietzke and Kapczinski, 2008).

Another possibility is that glutamatergic excitoxicity plays a role in the link between the biochemical changes in BD and cell loss and cognitive decline. Preclinical and clinical studies suggest that the glutamatergic system might be involved in the pathophysiology and treatment of neurodegenerative and mood disorders (Zarate et al., 2002). A recent study showed that TNF-α is the key cytokine that stimulates extensive microglial glutamate release in an autocrine manner by up-regulating glutaminase to cause excitoneurotoxicity (Takeuchi et al., 2006). On the contrary, neurotrophic factors exert a protective effect against excitotoxicity. Neuroprotection by BDNF against glutamate-induced apoptotic cell death appears to be mediated by the phosphatidylinositol pathway (Almeida et al., 2005), which is the same pathway affected by lithium use. These and other studies provide evidence that BDNF may display neuroprotective and anti-apoptotic properties and may counteract the pro-inflammatory cytokines (IL-6, TNF-α).

The balance between cytokine levels and neurotrophin levels is thought to be associated with programmed cell death (apoptosis) (Mogi et al., 2000; Nagatsu et al., 2000). Notably, there is evidence from post-mortem studies that BD pathophysiology may involve apoptotic cell death and neuronal and glial cell loss (Benes et al., 1998; Brietzke and Kapczinski, 2008). Studies have described clear reduction of glial cell numbers in prefrontal cortex of bipolar patients (Rajkowska et al., 2001), as well as signs of necrosis and apoptosis (Uranova et al., 2004). Recent post-mortem studies in BD provide direct evidence for reductions in number and density, as well as changes in cell body size and shape, of neurons and glia. In addition, separation of astroglial cells from cortical neurons in culture leads to neuronal death but cell death can be suppressed by neurotrophic factors such as BDNF (Ohgoh et al., 1998). These alterations at the microscopic level in mood disorders might give rise to the volume reductions as well as metabolic and biochemical abnormalities reported by neuroimaging studies (Rajkowska, 2002). This could also potentially be associated with the persistent cognitive impairment observed in patients with BD, which tends to worsen over time (Torres et al., 2007).

It is conceivable that the increase in cytokines and a decrease in BDNF may synergistically function in favour of neuronal degeneration in BD. One could hypothesize that BDNF levels decrease during mood episodes, as shown in previous studies (Cunha et al., 2006; Shimizu et al., 2003), but the restoration of the levels may be less likely with multiple episodes. The initial increase in pro-inflammatory cytokines could be a part of the disease process itself or represent an adaptative response to insult, i.e. BD onset, which could reach deleterious levels with chronicity. The anti-inflammatory response may be effective in the early course of the disease as indicated by elevation in IL-10 in the early stage of the disorder. However, with multiple episodes, as the protective anti-inflammatory response becomes less effective with continued elevations in pro-inflammatory cytokines, the deleterious effects of these become more apparent. Further, a reduction in BDNF levels seen at the late stage of BD may contribute to a vulnerability to the deleterious impact of cytokines. This hypothesis would be in keeping with recent theories suggesting that longer duration of illness and multiple mood episodes may

have a cumulative effect (Kapczinski et al., 2008; Post, 2007a). This is also in accord with recent ideas of medical staging of BD (Berk et al., 2007).

Some limitations should be considered when interpreting the results of this study. First, the present study has a cross-sectional design and therefore could only examine associations between the serum levels of cytokines and BDNF, but not direct causative mechanisms or the effects of progression of illness. Second, the BDNF was measured in serum. There may be other sources for serum BDNF, although they are not clearly known. However, it has been demonstrated that BDNF can cross the blood–brain barrier, and there is a high positive correlation ($r = 0.81$) between serum and cortical BDNF levels (Karege et al., 2002). Notably, a recent study indicated that serum levels of BDNF might reflect some aspects of neuronal integrity given the association with *N*-acetylaspartate levels (Lang et al., 2007). Third, the cohort included patients from different regions of the world. However, the potential confounding effect was reduced by matching patients with controls in the same region. Nevertheless, this can not exclude the non-specific environmental bias on the direct comparisons between patient groups. Fourth, despite use of mood symptoms as covariates in the analysis, we do not have the information about the exact time since last episode, which could have influenced the results. Finally, these data were obtained in medicated bipolar patients, which could be a potential source of bias. The results presented here could reflect not only the impact of BD, but also effects of chronic medication use. However, previous data indicate that medications may affect BDNF levels in a contrary direction to our findings. Previous data suggest that lithium and divalproex, as well as antidepressants and some antipsychotics increased BDNF levels (Frey et al., 2006). This suggests that BDNF levels could be even lower if patients were medication-free. Regarding the effect of mood stabilizers on cytokines, Th2 cytokine production was found to increase after lithium treatment (Rapaport and Manji, 2001). However, some researchers did not find any significant change of cytokines following treatment in bipolar patients (Su et al., 2002). Chronic lithium treatment, but not acute, induced a decrease in IL-6 and IL-10 in another BD study (Boufidou et al., 2004). Thus, it is not possible to exclude that the decrease in interleukins observed from early to late stage reflect in part an effect of long-term treatment. However, the fact that the levels of IL-6 are still higher than controls suggests that IL-6 may be involved in the pathophysiology of BD and its levels are not solely a result of the effects of medication.

In conclusion, our findings suggest that the changes in neurotrophin and cytokine levels from early to late stages of BD may play a role in the pathophysiology of BD. BDNF and TNF-α showed the most prominent changes and warrant further investigation as potential markers of illness progression. Prospective studies are needed to confirm these findings. Future studies with neuroimaging techniques and in at-risk populations are needed to elucidate to what extent these findings reflect neurodevelopmental abnormalities, progression of the illness involving loss/atrophy of glia and neurons, cognitive impairment, and clinical outcomes.

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