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SHORT COMMUNICATION

Increased plasma levels of soluble TNF receptor I in patients with bipolar disorder

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Abstract Bipolar disorder (BD) has been associated with a proinflammatory state in which TNF-α seems to play a relevant role. The aim of the present study was to evaluate the plasma levels of TNF-α and its soluble receptors (sTNFR1 and sTNFR2) in BD patients in mania and euthymia in comparison with control subjects. We evaluated 53 BD patients (34 in mania and 19 in euthymia) and 38 healthy subjects. All subjects were assessed by the Mini-International Neuropsychiatry Interview (MINI-Plus). Patients were also evaluated by the Young Mania Rating Scale (YMRS) and by Hamilton Depression Rating Scale (HDRS). Plasma TNF-α and its soluble receptors were measured by ELISA. The plasma TNF-α and sTNFR2 levels did not differ between groups, but higher sTNFR1 levels were found in BD patients. Of note, BD patients in mania had higher sTNFR1 levels than BD patients in euthymia and controls. The sTNFR1 and sTNFR2 levels correlated with BD duration, and sTNFR2 levels correlated with age of patients. Our data indicate a proinflammatory status in BD patients during mania and further suggest that inflammatory mechanisms may be involved with the physiopathology of BD.

Keywords Bipolar disorder · Cytokines · Inflammation · TNF-alpha · Soluble TNF receptors

Introduction

There is a growing body of evidence indicating altered immune response in bipolar disorder (BD). A proinflammatory state has been associated with the physiopathology

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of BD, and TNF-α may play a pivotal role in this process [1–3]. Furthermore, this proinflammatory state could be partially responsible for higher mortality and clinical morbidity observed in BD patients, including an increased risk for cardiovascular disorders [4].

TNF-α is a cytokine produced in response to injury or infection stimuli by macrophages, lymphocytes, neutrophils and other immune and structural cells including astrocytes and neurons [5]. Two specific cell surface receptors, TNFR1 (p55) and TNFR2 (p75), binding to TNF-α with high affinity and functioning as transducing elements, provide the intracellular signal response to TNF-α. The TNF-α binding to TNFR1 may result in apoptosis or neuronal survival, revealing the dual role of TNF-α in cell cycle. TNFR2 does not directly engage the apoptotic pathway, but the stimulation of these receptors induces endogenous membrane-bound TNF-α that subsequently activates TNFR1 [6]. Investigators, looking for natural TNF inhibitors, have identified agents structurally identical to extracellular cytokine-binding domain of two membrane-associated receptors that were named as soluble TNF receptors (sTNFR). These sTNFRs have been used as clinical markers of disease activity in auto-immune disorders, HIV and cancer. Their role has been studied in follow-up studies of these disorders and associated with disease prognosis [7].

In BD patients, four studies have described increased TNF-α circulating levels during mania and depression [8–11]. One study did not detect any difference in TNF-α levels of BD patients in euthymia [12] and other failed to demonstrate relevant differences in mania, depression and euthymia [13]. These inconsistencies could be related to sample heterogeneity regarding mood symptoms, length of illness and effect of medications [13]. Until now, no study investigated the sTNFRs in BD patients, despite the fact that TNF-α is a less stable molecule, and its soluble receptors sTNFR1 and sTNFR2 thus appear to be more reliable markers of TNF-α activity [14]. Moreover, increased plasma sTNFR1 and sTNFR2 levels have been described in major depressive disorder [15, 16], schizophrenia [17] and Parkinson disease [18] in the absence of significant changes in TNF-α levels and further suggesting a low-grade proinflammatory state.

Here, we investigated plasma TNF-α, sTNFR1 and sTNFR2 levels among euthymic and manic BD patients and healthy controls.

Methods

Participants

We recruited 53 patients with the diagnosis of BD type 1 from the Psychiatric Unit and the Bipolar Disorders Unit of the Governador Israel Pinheiro Hospital, Belo Horizonte, Brazil. The diagnosis was independently confirmed by two psychiatrists. Thirty-four patients were in manic state and 19 in euthymia. Patients in mania were recruited in a public hospital and admitted as inpatients or outpatients. Euthymic individuals were all outpatients. The control group ($N = 38$) had no clinical and psychiatry comorbidities or family history of psychiatry disease. Subjects with any infectious disease in the last 4 weeks, using anti-inflammatory medication, corticosteroids or antibiotics, with an autoimmune disease or with dementia were also excluded.

All patients were submitted to the Mini-International Neuropsychiatry Interview (MINI-Plus) [19], the Young Mania Rating Scale (YMRS) [20] and the Hamilton Depression Rating Scale (HDRS) [21]. YMRS was applied to assess severity of mania-symptoms, and HDRS was applied to investigate severity of depressive symptoms. Euthymia was defined by YMRS score <12 and HDRS score <7 points [22]. The study was approved by the local ethics committees. All participants signed the written informed consent.

Procedure

Five milliliters of blood was drawn at 8–10 a.m. from each subject by venipuncture into a vacuum tube with heparin at the moment of clinical interview. The blood was immediately centrifuged twice at 3,000 g for 10 min, and plasma was kept frozen at −70°C until assayed. Plasma TNF-α, sTNFR1 and sTNFR2 were measured according to the procedures supplied by the manufacturer and using ELISA kits for sTNFR1 and sTNFR2 (DuoSet, R&D Systems, Minneapolis, MN, USA). The detection limits were 10 pg/ml for TNF-α and 12 pg/ml for both soluble receptors. Values below the detection limits were assumed to be zero. Concentration is expressed as pg/ml.

Statistical analysis

All variables were tested for normal distribution by means of the Kolmogorov–Smirnov test. Descriptive statistics were used to report sociodemographic and clinical characteristics of the sample. Relationships between dichotomous variables were assessed with χ^2 test or Fisher's exact test when appropriate. Differences between two groups (patients vs. controls) were compared with *t* test or Mann–Whitney test. Differences between three groups (patients in mania vs. patients in euthymia vs. controls) were compared with one-way ANOVA test or Kruskal–Wallis test. Multiple comparisons among levels were checked with Dunn's post hoc test. Spearman correlation analysis was performed to examine relationships between TNF-α, sTNFR1 and sTNFR2 levels, age, length of illness, YMRS and HDRS

scores. All statistical tests were two tailed and performed using a significance level of $P < 0.05$. Data were presented as mean ± standard deviation (SD), median, or percentage, as indicated. Statistical analyses were performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Thirty-four patients in mania and 19 patients in euthymia were included in this study. All patients were medicated. There were no significant differences in proportion of gender or age between control and BD groups. Among BD patients, there were no significant differences for age of first mood episode, length of disease, number of hospitalizations or medication in use. The demographic and clinical features of all groups are shown in Table 1.

Plasma TNF-α levels are shown in Fig. 1a. TNF-α levels did not differ between BD patients and controls (even when comparing BD patient in mania × BD patient in euthymia × healthy controls). TNF-α levels were not correlated with age, length of illness, number of hospitalizations and severity of mania or depressive symptoms.

Plasma sTNFR1 and sTNFR2 levels are shown in Fig. 1b, c, respectively. BD patients had higher sTNFR1 ($P < 0.001$) levels than controls. sTNFR2 did not differ between BD patients and controls ($P = 0.48$).

BD patients in mania and euthymia had higher plasma levels of sTNFR1 than controls (mean ± SD pg/ml for mania: 1,837.9 ± 1,363.1, euthymia: 1,347.6 ± 561.5 and control: 791.3 ± 440.3; $P < 0.001$, $P < 0.01$). Plasma levels of sTNFR1 did not differ in BD patients in mania and euthymia ($P > 0.05$). BD patients in mania also presented higher plasma levels of sTNFR2 in comparison with BD patients in euthymia and controls, but this difference did not reach statistical significance (mean ± SD pg/ml for mania: 1,872.6 ± 800.3, euthymia: 1,616.0 ± 363.6, control: 1,667.4 ± 483.6; $P = 0.41$).

Considering bipolar patients, sTNFR1 and sTNFR2 plasma levels were positively correlated with the length of disease (respectively, $\rho = 0.36$, $P = 0.01$; $\rho = 0.42$, $P = 0.05$). The sTNFR2 plasma levels were also positively correlated with age ($\rho = 0.50$, $P < 0.001$). There was no correlation between sTNFR1 and age. Plasma sTNFR1 and sTNFR2 levels did not correlate with the severity of mania (respectively, $\rho = 0.24$, $P = 0.89$; $\rho = 0.07$, $P = 0.62$), depressive symptoms (respectively, $\rho = -0.18$, $P = 0.21$; $\rho = 0.61$, $P = 0.07$) and the number of hospitalizations (respectively, $\rho = 0.07$, $P = 0.68$; $\rho = 0.06$, $P = 0.65$). Curiously, sTNFR1 and sTNFR2 did not correlate with age in healthy controls (respectively, $\rho = 23$, $P = 0.16$; $\rho = -0.02$, $P = 0.89$).

Plasma sTNFR1 and sTNFR2 levels did not differ in BD patients categorized according to the presence of other psychiatry comorbidity (i.e. generalized anxiety disorder, panic disorder and obsessive–compulsive disorder), drug addiction (i.e. alcohol, nicotine, cannabis and cocaine) and clinical comorbidities (i.e. arterial hypertension, diabetes mellitus, dyslipidemia and disorders in thyroid). Plasma sTNFR1 and sTNFR2 levels did not differ in BD patients according the medication use, including lithium ($P = 0.99$; $P = 0.94$), anticonvulsants ($P = 0.16$; $P = 0.10$) and antipsychotics ($P = 0.21$; $P = 0.89$).

A secondary analysis was performed in plasma levels of TNF-α, sTNFR1 and sTNFR2 excluding mean outliers of BD patients in mania. Statistical analysis performed after excluding outliers did not show differences in analyzed parameters. The outlier values in TNF-α and its receptors were not found in the same patients.

Table 1 Clinical and demographic features of control and bipolar disorder (BD) subjects

Variables	Control Subjects ($N = 38$)	BD Patients		P-value
		Mania ($N = 34$)	Euthymia ($N = 19$)	
Female gender (%)	52.6	61.8	57.9	0.62
Age in years (mean ± SD)	42.9 (9.7)	49.6 (14.2)	44.5 (10.9)	0.45
HDRS score (mean ± SD)	1.00 (1.5)	3.8 (4.6)	1.94 (1.8)	0.01
YMRS score (mean ± SD)	0	28.5 (6.2)	1.28 (2.5)	<0.001
Age of first mood episode (mean ± SD)	–	30.1 (11.5)	21.3 (7.4)	0.12
Length of disease in years (mean ± SD)	–	19.5 (13.9)	20.2 (10.1)	0.49
Number of hospitalization (mean ± SD)	–	3.6 (3.5)	5.4 (3.2)	0.12
Medication in use (frequency %)				
Lithium	–	68.4	41.2	0.08
Anticonvulsants	–	68.5	61.8	0.76
Antipsychotics	–	63.2	47.1	0.39
Antidepressants	–	2.9	15.8	0.13

HDRS Hamilton depression rating scale; *YMRS* Young mania rating scale

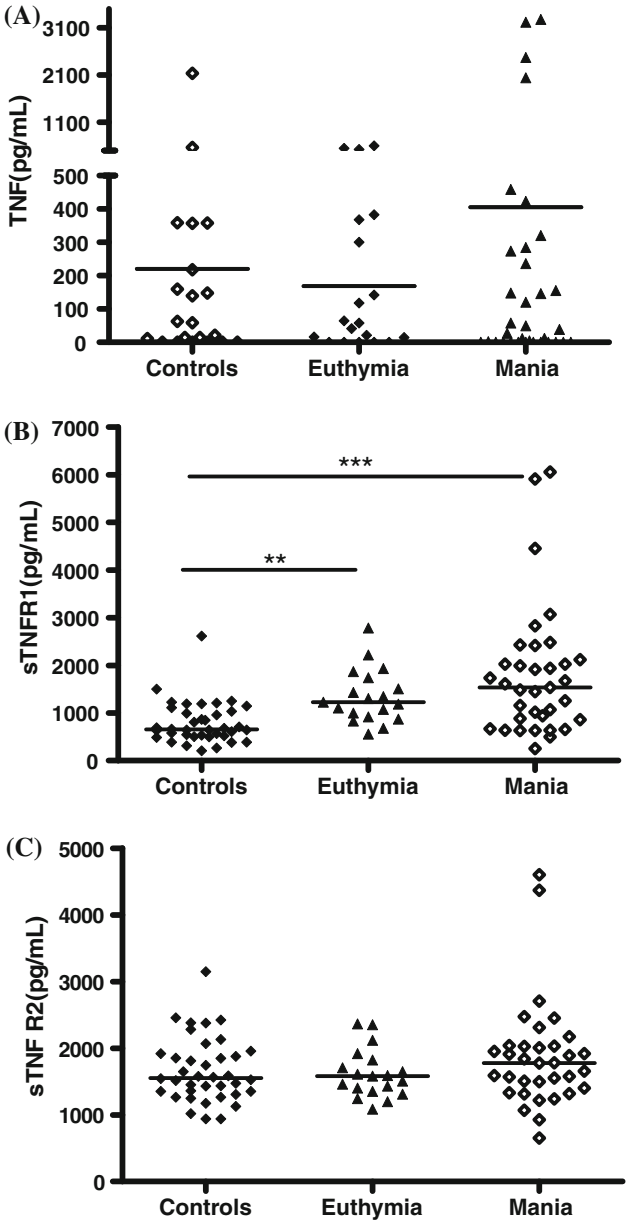

Fig. 1 Plasma levels of TNF α (**a**), sTNFR1 (**b**) and sTNFR2 (**c**) in control subjects and bipolar patients in euthymia and in mania. ** $P < 0.01$, Kruskal–Wallis with Dunn's post test. *** $P < 0.001$, Kruskal–Wallis with Dunn's post test

Discussion

To the best of our knowledge, this is the first study that evaluated TNF-α and its soluble receptors in BD patients. The plasma sTNFR1 levels were significantly increased in BD patients in mania, despite unchanged TNF-α and sTNFR2 levels. Moreover, plasma sTNFRs were correlated with age and length of disease, but not with severity of mood symptoms. Two previous studies have assessed TNF-α and soluble receptors in BD patients [23, 24]. However, the authors did not perform a specific analysis with BD subgroups and have included them into affective disorder group [23] or other psychiatric disorders [24].

Although the present result is in contrast with previous studies showing higher TNF-α levels in BD patients [8–11], it corroborated by the concept of a proinflammatory state in BD, especially in mania. It is worth to mention that TNF-α seems to be less stable than sTNFRs [14]. One possible explanation would be that TNF-α is produced at lower levels in peripheral tissues and degrades soon after release. As sTNFRs are induced by TNF-α, their increased concentrations in plasma may reflect the activity of TNF-α, even when TNF-α itself is not detected. Indeed, there is considerable evidence suggesting that sTNFR1 and sTNFR2 are more reliable markers of TNF-α activity than TNF-α itself. As a consequence, TNF receptors seem to be reliable markers of inflammatory activity in patients with psychiatric disorders [16–18].

TNFR1 has a ubiquitous distribution and mediates most actions of TNF-α. By contrast, TNFR2 seems to be confined mainly in hematopoietic cells. TNF-α is involved in activation of nuclear factor-kappa B (NF-κB) which promotes neuronal survival by inducing the expression of anti-apoptotic proteins (e.g. cIAP) and the overexpression of the proto-oncogenes members of Bcl-2 family (Bcl-2 and Bcl-xL). TNF-α also attenuates the elevation of reactive oxygen species and regulates the expression of various neurotrophic factors, including brain-derived neurotrophic factor [5]. Otherwise, TNF-α is involved in the activation of caspases and apoptotic machinery. Taken together, TNF-α seems to be an essential factor integrating signaling between glial cells and neurons involved in the regulation of synaptic plasticity [6]. In this sense, one *post mortem* study has showed that BD patients presented enhanced expression of the transmembrane form of TNF in the dorsolateral prefrontal cortex and anterior cingulate cortex, areas supposed to be associated with BD [25].

In the present study, plasma sTNFR2 levels were found elevated in older BD patients with longer disease. sTNFR1 levels were also correlated with the length of disease. These results may suggest a cumulative effect of the successive mood episodes and length of disease, enhancing a proinflammatory state in BD [8].

There are some limitations in our study. A pro-inflammatory state has been associated with BD patients in depression and mania. Until now, however, few studies have evaluated bipolar depression patients [2]. BD patients in depression were not included in this study. The majority of our patients had a long period of disease, and the present results do not reflect patients BD onset. All patients were medicated (mood stabilizing agents and/or atypical antipsychotics), but it is still uncertain the role of these medications in proinflammatory parameters [23]. It is important to emphasize that there was no significant difference in the

TNF-α or sTNFRs levels when comparing patients using distinct mood stabilizing agents and/or antipsychotics. It is already known that obesity may be associated with higher sTNFR1 and sTNFR2 plasma levels [26]. Since body mass index (BMI) was not assessed in this study, a higher frequency of obesity in BD could have been a potential confounding factor. However, it is important to note that Himmerich and colleagues [15] did not find significant relationships between obesity and TNF-α levels in major depression. A proinflammatory state, associated with higher sTNFR1 levels [16], has been associated with post-traumatic stress disorder symptoms [16] and could be considered as another potential confounding factor, as this variable was not assessed here.

In conclusion, our results are in line with previous studies and suggest that an inflammatory/immune process may contribute to BD physiopathology. It will also give us the possibility to understand this mental illness as a systemic disease.

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