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# Abnormal gene expression of proinflammatory cytokines and their membrane-bound receptors in the lymphocytes of depressed patients

Hooriyah S. Rizavi, Xinguo Ren, Hui Zhang, Runa Bhaumik, Ghanshyam N. Pandey *

*University of Illinois at Chicago, Department of Psychiatry, Chicago IL 60612, USA*

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ABSTRACT

Abnormalities of protein levels of proinflammatory cytokines and their soluble receptors have been reported in plasma of depressed patients. In this study, we examined the role of cytokines and their membrane-bound receptors in major depressive disorder (MDD). We determined the protein and mRNA expression of proinflammatory cytokines, interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and mRNA expression of their membrane-bound receptors in the lymphocytes from 31 hospitalized MDD patients and 30 non-hospitalized normal control (NC) subjects. The subjects were diagnosed according to DSM-IV criteria. Protein levels of cytokines were determined by ELISA, and mRNA levels in lymphocytes were determined by the qPCR method. We found that the mean mRNA levels of the proinflammatory cytokines IL-1β, IL-6, TNF-α, their receptors, TNFR1, TNFR2, IL-1R1 and the antagonist IL-1RA were significantly increased in the lymphocytes of MDD patients compared with NC. No significant differences in the lymphocyte mRNA levels of IL-1R2, IL-6R, and Gp130 were observed between MDD patients and NC. These studies suggest abnormal gene expression of these cytokines and their membrane-bound receptors in the lymphocytes of MDD patients, and that their mRNA expression levels in the lymphocytes could be a useful biomarker for depression.



## 1. Introduction

There is some evidence to suggest that neuroimmune abnormalities may be associated with depression Leonard and Myint (2009). There are many interactions of the neuroimmune and neuroendocrine systems and since abnormalities of the neuroendocrine system have been observed in depressive illness this has led to the suggestion that abnormalities of the immune system may also be involved in brain disorders such as depression. Among the major mediators of neuroimmune function are the proinflammatory cytokines which are released from the immune cells. Recently several investigators have proposed a cytokine hypothesis of depression (Miller et al., 2009; Schiepers et al., 2005).

Cytokines, generally known as chemical messengers between immune cells, comprise a heterogeneous group of messenger molecules produced by immunocompetent cells, such as lymphocytes and macrophages. They regulate the immune responses and interact with the central nervous system (CNS). There is some evidence to suggest the involvement of cytokines in depression. Several studies report that the administration of cytokines to animals or humans causes behavior known as sickness behavior which is similar to depression (Capuron et al., 2004; Dantzer, 2001a,b; Dantzer and Kelley, 2007). In humans, it has been reported that the administration of proinflammatory cytokines, such as interferon (IFN)-α or IFN-γ to cancer patients causes symptoms similar to depression (Capuron et al., 2001; Capuron et al., 2004; Dantzer et al., 1999). All these evidences taken together suggest the involvement of proinflammatory cytokines in the etiology of depressive illness.

However, the main direct evidence suggesting the involvement of cytokines in depression is derived from the observation of several studies showing that the levels of proinflammatory cytokines and their soluble receptors are abnormal in the plasma of depressed patients [see reviews by Dowlati et al. (2010), Hiles et al. (2012), Howren et al. (2009), Liu et al. (2012), Schiepers et al. (2005)]. A meta-analyses by Dowlati et al. (2010) and by Hiles et al.

*Abbreviations:* BP, bipolar; BMI, body mass index; CNS, central nervous system; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; ELISA, enzyme-linked immunosorbent assay; GLM, generalized linear model; Gp130, glycoprotein130; HDRS, Hamilton depression rating scale; IL, interleukin; IL-1R, interleukin-1 receptor; IL-1RA, interleukin-1 receptor antagonist; IL-6ST, Interleukin-6 signal transducer; MAP kinase, mitogen activated protein (MAP) kinase; MDD, Major depressive disorder; NC, normal controls; PRP, platelet-rich plasma; qPCR, real-time RT-PCR; RIN, RNA integrity number; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor

* Correspondence to: University of Illinois at Chicago, Department of Psychiatry (MC 912), 1601 W. Taylor Street, Chicago, Illinois 60612, United States.
Tel.: 312 413 4540; fax: 312 413 4547.
*E-mail address:* gpandey@psych.uic.edu (G.N. Pandey).

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(2012) indicated that the levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α, as well as the IL-1 receptor antagonist (IL-1RA) are increased in patients with depressive illness. It has recently been reported by Cattaneo et al. (2013) that the leukocyte mRNA levels of IL-1β, IL-6, and TNF-α were significantly higher in depressed patients compared with normal controls.

Like the neurotransmitter receptors, the biological effects of cytokines are mediated through their membrane-bound receptors (Hohmann et al., 1989). Thus proinflammatory cytokines exert their biological effects on a wide variety of target cells through the specific plasma membrane-bound receptors. Cytokine receptors exist in two forms: (i) soluble cytokine receptors, and (ii) membrane-bound cytokine receptors (Fernandez-Botran, 1991). The soluble cytokine receptors arise from the proteolytic cleavage of the extracellular domains of the membrane-bound receptors or by synthesis from alternatively spliced variants. Thus, whereas the membrane-bound receptors are involved in the signal transduction system mediating the biological effects of cytokines, soluble receptors are devoid of such effects.

Whereas protein and gene expression of these cytokines and their soluble receptors have been studied in depression (Cattaneo et al., 2013; Dowlati et al., 2010; Schiepers et al., 2005), to our knowledge the gene expression of membrane-bound cytokine receptors have not been studied in the blood of patients with depression. The gene expression studies may possibly be more appropriate biomarkers for depressive illness, as has recently been suggested by studies of Padmos et al. (2008) for bipolar (BP) illness, who determined the gene expression of proinflammatory cytokines IL-1β, IL-6, and TNF-α as well as their protein expression in monocytes of BP patients.

In light of these observations, we determined the gene and protein expression of the proinflammatory cytokines IL-1β, IL-6, TNF-α, as well as the gene expression of their receptor subtypes, such as IL-1R1, IL-1R2, TNFR1, TNFR2, IL-6R1 and IL-6 signal transducer (IL-6ST), also known as glycoprotein 130 (Gp130), and the receptor antagonist for IL-1, known as IL-1RA, in the lymphocytes obtained from drug-free, hospitalized depressed patients and normal control subjects.

## 2. Materials and methods

### 2.1. Subjects

The subjects for this study were hospitalized patients with major depressive disorder (MDD) and non-hospitalized NC subjects. The patients were admitted to the research wards of the University of Illinois General Clinical Research Center. This study was approved by the Institutional Review Board of the University of Illinois at Chicago. All subjects gave informed consent for the study. During hospitalization, the patients were kept drug-free for up to two weeks before they started treatment. Blood samples were drawn from the patients (n=31) in the morning under a fasting state. The clinical assessments were performed at the end of the drug-free period before initiation of treatment.

The NC subjects were non-hospitalized subjects who were recruited for the study through advertisements on hospital notice boards or by referral from normal controls or by referral from hospital employees. Control subjects had no history of psychiatric or major medical disorders and they abstained from any medication for at least two weeks before assessment or blood drawing.

### 2.2. Clinical assessment

Patients were diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria, derived by consensus between two trained raters and based on clinical interviews and other available clinical information. Diagnostic and clinical assessments were conducted at admission and at discharge. The BP depressed patients were not included in the study. The discharge diagnosis was considered definitive. Symptom ratings included scores on the Hamilton Depression Rating Scale (HDRS).

### 2.3. Blood processing

For each participant, 30 ml of venous blood was collected in the morning into tubes containing 3.8% (w/v) sodium citrate in DEPC treated water and (1 vol: 9 vol blood) for plasma. The samples were centrifuged immediately at 210 g for 15 min and the platelet-rich plasma (PRP) was removed for platelet isolation. To the red blood cell (RBC) layer, 15 ml of saline was added, mixed gently, and then transferred on Ficoll (2:1 respectively). The samples were then centrifuged at 400 g for 40 min. The upper layer above the interface layer was discarded and the interface layer was processed for lymphocyte isolation. The isolated lymphocytes are stored at −80 °C until assayed. Plasma was obtained by centrifuging the PRP secretion at 6000 g for 10 min.

### 2.4. RNA isolation

Total RNA was extracted, from lymphocytes as previously described (Pandey et al., 2015), using TRIZOL reagent (Invitrogen) and DNase treatment were performed for each sample. The RNA concentration and purity was determined by measuring the optical density at 260-nm wavelengths using NanoDrop®ND-1000 (NanoDrop Technologies, Montchanin, DE, USA) and 260/280 nm ratio with expected values between 1.8 and 2.0. RNA quality was assessed using Agilent Bioanalyzer 2100 (Agilent). All samples used had 28S/18S ratios > 1.2, and RNA integrity number (RIN) above 6.6 with mean RIN values of 8.1 ± 0.7.

cDNA Synthesis was synthesized as previously described (Pandey et al., 2015), briefly, 1 μg of total RNA was reverse transcribed using 50 ng random hexamers, 2 mM dNTP mix, 10 units ribonuclease inhibitor, 50 mM Tris–HCl (pH 8·3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, and 200 units MMLV-reverse transcriptase (Invitrogen) in a final reaction volume of 20 μL. Thermal cycle conditions were 37 °C for 60 min, 70 °C for 15 min and stored at −20 °C immediately. Real-time PCR was performed in duplicates using MX3005p sequence detection system (Agilent). Predesigned Taqman gene expression assays (Applied Biosystems, Foster City, CA) were used for all target and internal control genes, description is given in Table 1.

The geNorm algorithm (Vandesompele et al., 2002) was used to determine stability and optimal numbers of internal controls, the two parameters used are: gene stability: *M* (average expression stability) and *V* (pair wise variation), where a low *M* value signifies a more stable gene and a *V* value of 0.15 is the proposed cut-off below which the inclusion of an addition gene is not necessary, of the 12 genes tested in our sample set, ACTB and GAPDH were identified to be the most stable internal controls. PCR efficiency for all genes, after 5-log dilution series of pooled cDNA, was similar. Each reaction was carried out using 10 μL of cDNA (diluted 1:10) in 1X TaqMan Universal PCR Master Mix (Applied Biosystems) as per manufacturer's instructions. Each qPCR plate included a "no reverse transcriptase" and "no template" control to eliminate non-specific amplification and each sample was assayed in triplicate. Q-RT-PCR data were analyzed using the 2–(ΔΔCt) method, where ΔΔCT=(CT target–CT internal control) Subject – (CT target – CT internal control) Control, and $CT_{\text{internal control}}$ is the geometric mean of ACTB and GAPDH CTs. For further statistical analysis ΔCT values are used.