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Plasma cytokine abnormalities in drug-naïve, comorbidity-free obsessive–compulsive disorder

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ABSTRACT

Growing evidence in the last decade suggest significant role of immune alterations in the pathogenesis of obsessive–compulsive disorder (OCD). Cytokines, mediators of inflammation, alter the neurotransmitter concentration and result in a hyposerotonergic and hyperglutamatergic state implicated in pathogenesis of OCD. However, only few studies have examined cytokine abnormalities in OCD with inconsistent results possibly due to confounding effects of medications and comorbid anxiety–depression. We examined 20 comorbidity free, drug free OCD patients and 20 age and sex matched healthy controls. Clinical severity was assessed using Yale Brown Obsessive Compulsive Scale, Hamilton anxiety rating scale, Hamilton depression rating scale and Clinical Global Impression. Levels of different cytokines, Interleukin (IL)-2, IL-4, IL-6, IL-10, Tumor necrosis factor (TNF)-α and Interferon (IFN)-γ were assessed using Cytometric Bead Array. OCD patients had significantly greater plasma levels of IL-2, IL-4, IL-6, IL-10 and TNF-α levels than controls but not IFN-γ. Reanalysis of data with only drug naïve patients (excluding 4 drug free patients) did not alter the results. Presence of these abnormalities in drug-naïve patients suggests the possible role of cytokines in the pathogenesis of OCD. Study findings have potential clinical utility in development of novel therapeutic options targeting cytokine aberrations in OCD.

1. Introduction

Obsessive compulsive disorder (OCD) is a common neuropsychiatric disorder with a prevalence of approximately 2% (Bebbington, 1998). Evidence from different lines of research suggests possible role of immune abnormalities in the pathogenesis of OCD (Murphy et al., 2006); (i) higher prevalence of OCD and tics is seen in patients with Sydenham's chorea and group A Streptococcal infection (Swedo, 1994; Abbas et al., 1996; Swedo et al., 1998) (ii) OCD patients have higher concentration of anti basal ganglia antibodies than healthy controls (Dale et al., 2005; Bhattacharyya et al., 2009) though the evidence is not unequivocal (da Rocha et al., 2008) (iii) basal ganglia volume is decreased in children with streptococcus-associated OCD than in the healthy children (Giedd et al., 2000) and basal ganglia volume correlates with anti basal ganglia antibody titers (Giedd et al., 2000; Peterson et al., 2000) (iv) children with OCD have increased prevalence of B lymphocyte marker D8/17 compared to healthy controls (Murphy et al., 2001).

Cytokines are well-recognized as mediators of inflammation. In addition to their role in peripheral inflammation cytokines also affect central nervous system function through their own receptors (Schobitz et al., 1994) and through stimulation of vagal afferents (Rothwell and Strijbos, 1995). Cytokines alter the neurotransmitter concentration through kyneurenine pathway; proinflamatory cytokines stimulate indoleamine dioxygenase (IDO) and result in a altered serotonin and glutamate neurotransmission which are robustly implicated in pathogenesis of OCD (Chakrabarty et al., 2005). Despite the strong association between OCD and neuroinflammation, only few studies have examined cytokine abnormalities in OCD. However, findings from these studies are inconsistent; while few studies reported altered levels of cytokines (Brambilla et al., 1997; Denys et al., 2004; Fluitman et al., 2010; Konuk et al., 2007; Monteleone et al., 1998) others reported absence of difference compared to controls (Maes et al., 1994; Weizman et al., 1996; Marazziti et al., 1999; Carpenter et al., 2002).

A meta-analysis of proinflammatory cytokines in OCD identified confounding effect of medication and comorbid depression on cytokine levels and recommended future studies examining immune abnormalities in OCD should control for these confounding factors (Gray and Bloch, 2012); elevated cytokine levels are

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reported in depression as well (Leonard and Myint, 2009) and selective serotonin reuptake inhibitors (SSRI) are shown to decrease cytokine levels (Maes et al., 1999; Kenis and Maes, 2002). As SSRIs are the first line of treatment for OCD it is important to examine patients in drug naïve state to control the confounding effect of medication. A recent study also reported a positive correlation between relative antidepressant dose and sTNFr2 levels (Fontenelle et al., 2012). Also, previous studies examined levels of individual cytokines but not a comprehensive analysis of all cytokines. Hence the aim of our study was to conduct a comprehensive analysis of plasma cytokine levels in drug naïve, comorbidity free OCD patients in comparison to matched healthy controls.

2. Methods

2.1. Subjects

Subjects included 20 patients with OCD (10 males; mean age=27.05 ± 7.99 years) and 20 healthy volunteers (10 males; mean age=27.85 ± 6.06 years) matched on sex ($\chi^2=1; p=1.00$) and age ($t=0.35$; $p=0.72$). The study was conducted at National Institute of Mental Health and Neurosciences, Bangalore, India. The study was approved by the Institute ethics committee. After complete description of the study written informed consent was obtained from all subjects. All patients were interviewed using the MINI international neuropsychiatric interview (M.I.N.I) Plus (Sheehan et al., 1998) and DSM-IV diagnosis of OCD was established. We excluded patients with lifetime diagnosis of bipolar disorder, schizophrenia and substance abuse/dependence and any current comorbid Axis I psychiatric disorder including major depressive disorder and generalized anxiety disorder. All patients were independently examined by a qualified psychiatrist and diagnosis was confirmed. Healthy controls were evaluated to rule out Axis-I psychiatric disorder using the MINI Plus. Subjects (patients as well as controls) were excluded if they had evidence of traumatic brain injury, hepatic or renal impairment, seizure, stroke, active infection, allergy, rheumatoid arthritis, cancer or on treatment with steroids or non-steroidal anti-inflammatory drugs.

Clinical symptoms severity was assessed using the Yale Brown Obsessive Compulsive Scale (YBOCS) severity and symptom check list (Goodman et al., 1989), Clinical Global Impression – Severity (CGI-S), Clinical global impression-improvement (CGI-I) (Guy and Bonato, 1970), Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) and Hamilton Anxiety Rating Scale (HARS) (Hamilton, 1959). All 20 patients had mixed OCD. Aggressive obsessions were the commonest ($n=15$) followed by contamination ($n=14$), pathological doubts ($n=13$), miscellaneous ($n=9$), religious ($n=8$), symmetry ($n=8$), sexual ($n=5$) and hoarding ($n=2$). Among compulsions washing ($n=16$) was the commonest followed by repeating ($n=15$), checking ($n=12$), ordering ($n=8$), hoarding ($n=5$) and miscellaneous ($n=19$).

16 patients were drug naïve (never received anti-OCD medication) and 4 were drug free with a mean drug free duration of 38.25 months. The age at onset of OCD was 19.20 ± 5.94 years with a mean duration of 7.25 years of illness. Four patients had early onset of illness (age at onset less than 14 years). At baseline YBOCS was 27.95 ± 3.83, CGI – severity scale 4.35 ± 0.74. As none of the individuals had clinical depression or anxiety HDRS (2.30 ± 1.52) and HARS (3.20 ± 1.28) scores were in lower range.

2.2. Cytokine assessments

Blood samples were collected from all subjects between 08:00 and 09:00 h after overnight fast. Samples were collected using K_2 EDTA vacutainer tubes (Becton & Dickinson, U.S.A), mixed well by inversion and centrifuged for 15 min at 1000 × *g* within 30 min of collection. Plasma was separated, aliquoted and stored at −80 °C. All samples were coded and analyzed by investigator blind to the clinical status (SK). Cytokines were assessed using commercial "cytometric bead array (CBA)" kit (BD Biosciences, SanJose, USA) as recommended by the manufacturer. This procedure used multiplex bead array technology to simultaneously detect multiple cytokine proteins. Bead populations with distinct fluorescence intensities coated with capture antibodies specific for each cytokine were used [Limit of detection for IL-2, IL-4, IL-6, IL-10, TNF, IFN-g were 2.6, 4.9, 2.4, 4.5, 3.8, 3.7 pg/mL respectively]. Samples were acquired on a FACSCalibur (BD Biosciences, San Jose, USA) flow cytometer using BD CellQuest™ Pro software. For generation of standard curves, serial dilutions of the standards spanning the concentration range from 0 to 5000 pg/mL were analyzed. The results were tabulated using the "cytometric bead array" software as recommended by the manufacturer.

2.3. Statistical analysis

Data was examined for normative distribution using Shapiro–Wilk's test. As the data was not normatively distributed we used nonparametric test-Mann–Whitney *U* test, to examine differences in cytokine levels between patients and controls. We analyzed categorical variables using Chi square test. Spearman's correlation analysis was employed to examine the relationship between cytokine levels and OCD related clinical variables. As we analyzed 6 cytokines we applied Bonferroni correction for multiple comparisons and adjusted the '*p*' value at 0.008.

3. Results

Cytokine levels for OCD patients and controls are given in Table 1. OCD patients had significantly greater plasma levels of IL-2, IL-4, IL 6, IL 10 and TNF-α levels than controls (< 0.008) but not IFN-γ (Table 1). Reanalysis of data with only drug naïve patients (excluding 4 drug free patients) did not alter the results; Drug-naïve OCD patients had significantly elevated levels of IL-2 ($U=56.0$, $p=0.006$), IL-4 ($U=49.5$; $p=0.002$), IL-6 ($U=42.5$; $p=0.001$), IL-10 ($U=50.5$, $p=0.003$), TNF-α ($U=57.5$, $p=0.007$) but not IFN-γ ($U=65.00$, $p=0.017$). There was no significant correlation between cytokine levels and clinical variables like HARS, HDRS, YBOCS score, duration of illness ($p > 0.05$).

4. Discussion

We examined cytokine abnormalities in drug-naïve, comorbidity-free OCD patients. To the best of our knowledge, this is

Table 1
Plasma cytokine levels between patients and healthy volunteers.

Cytokine	Plasma cytokine levels [pg/ml] Mean (SD)		U^+	p^*
	Control ($n=20$)	Patients ($n=20$)		
IL2	0.33 (0.69)	1.19 (1.07)	108.5	**0.005**
IL4	0.81 (1.04)	1.90 (1.31)	105.0	**0.007**
IL6	2.06 (0.65)	3.23 (1.50)	83.0	**0.002**
IL10	0.78 (1.04)	2.02 (1.54)	101.5	**0.006**
IFN-γ	0.52 (0.95)	1.62 (1.53)	118.5	0.014
TNF-α	0.47 (0.76)	1.31 (0.82)	102.5	**0.005**

*- '*p*' values significant at < 0.008 after correction for multiple comparison.
+ Mann–Whitney *U* test.