

Original article / Araştırma**Serum IL-1, IL-2, IL-4, IL-6, IL-10, TNF- α , and IFN- γ levels in drug-free, comorbidity-free obsessive-compulsive disorder patients**

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ABSTRACT

Objective: Increasing evidence suggests that certain inflammatory factors, especially cytokines, can lead to neurochemical and behavioural changes via their role in the brain signalling system. Studies investigating cytokine abnormalities in obsessive-compulsive disorder (OCD) have yielded conflicting results thus far. The aim of this study was to investigate the serum IL-1, IL-4, IL-6, IL-10, TNF- α , and IFN- γ levels in drug-free, comorbidity-free patients with OCD. **Methods:** Forty comorbidity-free, drug-free OCD patients and 40 age- and sex-matched healthy volunteers were included in the study. Clinical severity was assessed using the Yale-Brown Obsessive-Compulsive Evaluation Scale (YBOCES). **Results:** IL-1 levels were significantly higher and IL-2, IL-6, and TNF- α levels were significantly lower in patients than in controls. No differences were observed in IL-4, IL-10, and IFN- γ levels. However, a negative relationship was found between IL-1 levels and compulsive symptoms (YBOCES) in OCD patients. **Conclusions:** Our findings suggest the possible role of cytokines in the pathogenesis of OCD. It is important to continue investigating the role of cytokines and other immunological markers in both the etiology and treatment of OCD. (*Anatolian Journal of Psychiatry* 2018; 19(2):157-162)

Keywords: OCD, cytokines, immunological markers

İlaç kullanmayan ve komorbiditesi olmayan obsesif kompulsif bozukluk hastalarında serum IL-1, IL-2, IL-4, IL-6, IL-10, TNF- α ve IFN- γ düzeyleri**Öz**

Amaç: Bazı inflamatuvar etkenlerin beyin sinyalizasyon sistemindeki rolü bilinmektedir. Artan kanıtlar, bu inflamatuvar etkenlerin en önemlileri olan sitokinlerin nörokimyasal ve davranışsal değişikliklere neden olabileceğini düşündürmektedir. Obsesif kompulsif bozuklukta (OKB) sitokin anormalliklerini araştıran çalışmalar şimdiye kadar çelişkili sonuçlar vermiştir. Bu çalışmanın amacı, ilaçsız, komorbiditesi olmayan OKB hastalarında serum IL-1, IL-2, IL-4, IL-6, IL-10, TNF- α ve IFN- γ düzeylerini araştırmaktır. **Yöntem:** Çalışmaya komorbiditesi olmayan ve ilaç kullanmayan 40 OKB hastası ile yaş ve cinsiyet açısından eşleştirilmiş 40 sağlıklı gönüllü alındı. Klinik şiddet Yale Brown Obsesyon Kompulsiyon Ölçeği (YBOKÖ) kullanılarak değerlendirildi. **Bulgular:** IL-1 düzeyleri hastalarda anlamlı olarak yüksekti. IL-2, IL-6 ve TNF- α düzeyleri ise hastalarda kontrollerden daha düşüktü. IL-4, IL-10 ve IFN- γ düzeyleri için herhangi bir fark gözlemlenmedi. Bununla birlikte, OKB hastalarında IL-1 düzeyleri ile kompulsiyon (YBOKÖ) puanları arasında negatif korelasyon saptandı. **Tartışma:** Bulgularımız, OKB patogenezinde sitokinlerin olası rolünü ileri sürmektedir. Hem etyoloji, hem de yeni tedavi hedefleri açısından OKB'de sitokinlerin ve diğer immunolojik belirteçlerin rolünü araştırarak ileri çalışmalara gerek vardır. (*Anadolu Psikiyatri Derg* 2018; 19(2):157-162)

Anahtar sözcükler: OKB, sitokin, immünolojik belirteçler

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INTRODUCTION

Obsessive-compulsive disorder (OCD) is characterized by obsessions (i.e. recurrent and persistent unwanted thoughts) and compulsions (i.e. repetitive ritualised behavior). Although the pathophysiology of this condition is unknown, recent studies have focussed primarily on neurotransmitters, such as serotonin, dopamine,¹ and glutamate.^{2,3} Studies show that certain environmental triggers, such as psychosocial stressors, trauma, and other infectious and inflammatory processes alter gene expression in a way that influences the serotonergic and dopaminergic systems, catecholamine modulation, and glutamate pathways.⁴⁻⁶ It is thought that this alteration occurs via various epigenetic mechanisms and can result in OCD symptoms through changes in the circuits that process corticostriatal information.⁷

Data showing an association between Group A β -haemolytic streptococcus infections and OCD onset or exacerbation in some children also indicate that immunological mechanisms play a role in the pathophysiology of OCD.⁸

Increasing evidence suggests that certain inflammatory factors can lead to neurochemical and behavioral changes via their role in the brain signalling system. Cytokines, the most important of these inflammatory factors,^{9,10} are signal proteins with a soluble polypeptide structure that play a role in the initiation and protection of the immune response to infection.¹¹ They are expressed in microglial cells and activated macrophages.^{12,13} Cytokines can be classified into two types: astype-1 (Th1), which consists of pro-inflammatory cytokines (e.g. interleukin (IL)-1 β , IL-2, IL-6, IL-12, IL-18, tumour necrosis factor- α (TNF- α), and interferon (IFN)- γ), and type-2 (Th2) anti-inflammatory (IL-4, IL-10, IL-17) and regulatory (TGF- β , IL-27, and IL-6) cytokines.¹⁴ These are thought to participate in pathophysiological processes by affecting serotonin, dopamine, and glutamate neurotransmission and synaptic plasticity and are also known to play a role in the aetiology of OCD.^{13,15,16}

Despite strong evidence of a connection between OCD and neuroimmunological mechanisms, extant studies of cytokine abnormalities in OCD have yielded conflicting results. These inconsistent findings may be attributable to the effects of small sample sizes and potentially confounding variables, such as drug use and additional psychiatric disorders.^{17,18} Indeed, comorbid psychiatric disorders and psychiatric

drug use are known to potentially affect cytokine levels.¹⁹ We therefore aimed to investigate cytokine levels in a relatively larger sample that was medication-free and had no additional psychiatric disorders. Our basic hypothesis was that proinflammatory and antiinflammatory cytokines may play a role in the pathophysiology of OCD.

METHODS

Subjects

The study protocol was approved by the Ethics Committee of the İnönü University Faculty of Medicine and was conducted in accordance with the principles of the Helsinki Human Rights Declaration (2001 version) and Good Clinical Practice guidelines. The purpose of the study and its implementation were explained to the patients, and their written consent was obtained.

We included 40 patients aged 18-65 years who presented to the İnönü University Faculty of Medicine's Psychiatry Outpatient Department between 01 January 2016 and 01 January 2017. Patients were diagnosed with OCD according to DSM-5 diagnostic criteria. Patient group were who not using any medication for the last six months or had any other psychiatric disorder. Comorbid psychiatric disorder was excluded by a psychiatrist's clinical interview according to DSM-5. In addition 40 healthy age- and gender-matched volunteers as the control group in this study.

The exclusion criteria for the patient group were the presence of a major systemic disease; an endocrinological or neurological disorder known to potentially affect cytokine levels; alcohol, drug, and/or tobacco addiction; previous head trauma or electroconvulsive treatment (ECT); schizophrenia or other psychotic disorders; history of manic or hypomanic episodes; intellectual disability; pregnancy or nursing; and use of oral contraceptives. The control group consisted of 40 age- and gender-matched healthy volunteers who were not pregnant or nursing and who reported no past or current psychiatric, neurological, systemic, or endocrinological disorders; no use of medication; and no current addiction to alcohol or other substances. Considering that the body mass index (BMI) may affect cytokine levels, we were careful to match the patient and control groups in terms of BMI.²⁰ Those with a BMI greater than 30 and those below 20 were not included in the study.

The participants completed the Yale-Brown

Obsessive-Compulsive Evaluation Scale (YBOCES) and the symptom checklist to evaluate disease severity. Blood samples to determine cytokine levels were obtained from all patients and control subjects between 09:00 and 10:00 in the morning, following a 12-hour fast and tobacco abstinence and 30 minutes of rest. The blood samples were centrifuged at 3,500 rpm for 10 minutes. The sera obtained after centrifugation were stored at -80 °C in individual tubes until use.

Cytokine assessments

Serum IL-1, IL-4, IL-6, IL-10, TNF- α , and IFN- γ levels were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, Affymetrix Inc., Santa Clara, CA, USA).

Statistical analysis

The Statistical Program for Social Sciences (SPSS) for Windows software, version 17.0, was used for statistical analyses. Descriptive statistics for numerical variables are presented as means and standard deviations (SDs), and the distribution of the data was evaluated with the Shapiro-Wilk test. As the data were not normally distributed, the nonparametric Mann-Whitney U

test was used to evaluate differences in the cytokine levels between patients and control subjects. Categorical variables were analysed using the chi-square test. Associations of cytokine levels and disease severity (YBOCS scores) with disease duration were evaluated with Spearman's correlation analysis because the data did not show a normal distribution. A p value of <0.05 was considered significant.

RESULTS

No significant demographic differences were observed between the patient and control groups with regard to age, gender, or BMI (Table 1) ($p=0.990$, $p=1.00$, and $p=0.643$, respectively). The YBOCS scores and disease duration of patients with OCD are presented in Table 1. We found aggressive obsessions in 22 patients, obsessions about dirt in 35 patients, sexual obsessions in five patients, and religious obsessions in 26 patients. There was no patient with a collecting obsession.

The IL-1 levels were significantly higher in patients than in controls, but the IL-2, IL-6, and TNF- α levels were significantly lower in patients than in controls. No differences were observed

Table 1. Comparison of patient and control sociodemographic characteristics

Demographic data	Patient group (n=40) Mean $\pm$ SD	Control group (n=40) Mean $\pm$ SD	p
Age	32.82 $\pm$ 9.00	32.80 $\pm$ 9.00	0.990
Gender (male/female)	18/22	18/22	1.000
Body Mass Index	24.31 $\pm$ 2.93	24.63 $\pm$ 3.25	0.643
Disease duration (years)	9.50 $\pm$ 7.50		
Yale-Brown Obsessive-Compulsive Scale	18.07 $\pm$ 7.88		
Yale-Brown Obsession	9.55 $\pm$ 4.04		
Yale-Brown Compulsion	8.77 $\pm$ 3.94		

Table 2. Comparison of patient and control cytokine levels

Cytokine	Patient group (n=40) Mean $\pm$ SD	Control group (n=40) Mean $\pm$ SD	Test	p
IL-1	18.56 $\pm$ 9.32	11.50 $\pm$ 10.61	$z=-5.23$	<0.001
IL-2	64.51 $\pm$ 50.64	78.12 $\pm$ 46.38	$z=-3.11$	0.002
IL-4	39.00 $\pm$ 18.77	50.03 $\pm$ 37.25	$z=-0.81$	0.416
IL-6	4.44 $\pm$ 5.29	13.65 $\pm$ 10.33	$z=-5.18$	<0.001
IL-10	5.14 $\pm$ 7.66	5.95 $\pm$ 6.43	$z=1.01$	0.314
IFN- γ	3.83 $\pm$ 2.09	3.98 $\pm$ 2.56	$z=-0.38$	0.707
TNF- α	26.57 $\pm$ 4.25	42.88 $\pm$ 13.61	$z=-5.91$	<0.001

in the IL-4, IL-10, and IFN- γ levels (Table 2).

No statistically significant associations of disease severity (YBOCS total scores) and disease duration with cytokine levels were observed.

DISCUSSION

The main aim of this study was to investigate cytokine levels in OCD patients who did not use medication and had no additional psychiatric diagnosis. The main findings of our study were higher levels of IL-1 and lower levels of pro-inflammatory cytokines, such as IL-2, IL-6, and TNF- α , in OCD patients. No significant differences were observed between the patient and control groups in the levels of anti-inflammatory cytokine levels, such as IL-4, IL-10, and IFN- γ . The higher levels of IL-1 was the only finding that supported our hypothesis.

The results of studies investigating cytokine levels in OCD are contradictory. TNF- α , IL-6, and IL-1 β have been the most extensively investigated cytokines in relationship to this disorder. A meta-analysis of 12 studies on this subject demonstrated a significant reduction in IL-1 β levels in OCD patients relative to controls, whereas there were no overall differences in TNF- α and IL-6 plasma levels.²¹ Stratified subgroup analysis has shown that pediatric OCD patients receiving psychotropic medication have lower plasma IL-6 levels.²⁰ Comorbid depression has also been reported to alter TNF- α levels, with relevant studies reporting increased TNF- α values.²⁰

TNF- α plays a key role in central nervous system functions.²² It is produced by macrophages and circulating monocytes and also has an important role in infectious, inflammatory, and autoimmune conditions.²³ The plasma levels are affected by age, BMI, gender, time of day, drug intake, and other factors.²⁴ The lack of additional diagnoses in our patient group and the fact that they were not on medication eliminated the confusing effects of other psychiatric disorders and drugs, respectively. The control group was also matched to the patients in terms of age, gender, and BMI, increasing the value of our findings by eliminating the effect of other confusing factors.

We found lower TNF- α levels in patients than in control subjects in our study. Decreased TNF- α plasma levels in patients with OCD have also been reported by Denys et al., Brambilla et al., and Monteleone et al.²⁵⁻²⁷ Additionally, one study found markedly increased TNF- α levels in patients with early-onset OCD accompanied by

anxiety or depressive disorder.²² As demonstrated by the meta-analysis results noted above, depression seems to play a role in the higher TNF- α levels.

Another important finding of our study was the high IL-1 levels in OCD patients. Blood monocytes, such as microglia and tissue macrophages, are the major production sources of IL-1 β , which is a pro-inflammatory cytokine.²⁸ Several studies have investigated circulating IL-1 β levels in OCD patients and controls, but their results have varied. This particular cytokine can play a role in central nervous system repair and, particularly, in remyelination, and has been associated with many structural changes in OCD, including those in the corpus callosum; thus, more comprehensive studies are needed.²⁹

We found no relationship between the severity and duration of the disease and cytokine levels. IL-2 has been reported to have a positive correlation with YBOCS scores in children with OCD and a concurrent diagnosis of tic disorder.³⁰ Denys et al. observed a negative correlation between the severity of OCD symptoms and the IL-6 levels.²⁵ The only study evaluating OCD patients without medication found the plasma IL-2, IL-4, IL-6, IL-10, and TNF- α levels of OCD patients to be significantly higher than those of the controls, whereas the same difference was not seen with IFN- γ . This study reported no significant correlations between cytokine levels and clinical variables, such as YBOCS scores and disease duration.¹⁸

The findings of our study also reflect decreased levels of IL-2 and IL-6, which, like TNF- α , are pro-inflammatory cytokines. However, the results reported in the literature vary, and the findings of our study contradict some relevant studies. More information is required to determine the possible role of cytokines in OCD.

The results of this study should be interpreted in consideration of its limitations. First, the relationship between the production of cytokines and the nervous system constantly varies. There is evidence that cytokines increase their own production as well as that of other cytokines through direct or indirect stimulation of vagal afferents,³¹ and this should be taken into account when evaluating our findings. Another limitation is that the symptom subtypes of the patients were not taken into account, and the sample was small. Recently, there has been an increase in the number of studies suggesting that subtypes of OCD, such as washers, controllers, and other symptom clusters, may be associated with

different aetiologies, different functional deficiencies, and different neural pathways.³² Studies that evaluate symptom subtypes separately should elucidate this issue.

Although data provided by both previous research and the current study are important, we have not yet determined how to use biological markers related to the immune system to evaluate the risks for, diagnostic markers of, and

likely progression of disease. Thus, additional studies are needed to investigate acute and chronic immunological responses. Additionally, blood and other samples, such as saliva, should be examined. It is necessary to continue investigating the roles of cytokines and other immunological markers in both the aetiology and the treatment of OCD.

Yazarların katkıları: L.G.E.: Konuyu bulma, makaleyi yazma; N.A.K.: Hasta ve kontrol grubu görüşmeleri, kanları toplama; L.U.İ.: Hasta ve kontrol grubu görüşmeleri, ölçeklerin uygulanması, kanları toplama; S.Y.: İstatistik; Ç.T.: Çalışılacak kitlerin belirlenmesi, kitlerin istemi ve alımı; H.G.T.: Biyokimyasal analiz; S.Ü.: Planlama, araştırmanın yürütülmesi, literatür tarama.

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