Inflammation, the neopterin–tetrahydrobiopterin pathway, and nitric oxide levels in children and adolescents with obsessive–compulsive disorder

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Abstract

Background: Approximately 80% of obsessive–compulsive disorder (OCD) cases begin in childhood. Various genetic, psychological, sociological factors and biological mechanisms are involved in the etiology of OCD. To the best of our knowledge the relationship between inflammation and OCD is unclear. Chronic inflammation was shown to increase neopterin and decrease tetrahydrobiopterin (BH4) levels by activating the neopterin–BH4 pathway. In addition, studies have shown that it can be an important biomarker in psychiatric disorders. Objective: This study compared serum TGF-1β, TNF-α, IL-1β, IL-2, IL-6, IL-10, IL-17, neopterin, BH4, and nitric oxide (NO) levels between child and adolescent patients diagnosed with OCD and a healthy control group. Methods: The study included 29 patients diagnosed with OCD (comorbidity free, drug free) and 28 healthy children as an aged and sex matched control group. For the measurement of neurobiological markers, venous blood samples were collected, and analyzed by using enzyme-linked immunosorbent assay (ELISA). Results: All cytokine levels were found to be low, but this decrease was statistically significant only for TGF-1β. The neopterin and NO levels were significantly higher and BH4 significantly lower in children with OCD compared to the healthy control group. Conclusion: The results of our study show that the levels of TGF-1β and NO and the activation of the neopterin–BH4 pathway may be implicated in the pathophysiology of OCD. Additionally, anti-oxidant and BH4 adjuvant therapies should be investigated as treatment options for OCD.

1. Introduction

Obsessive–compulsive disorder (OCD) is a common neuropsychiatric disorder characterized by obsessions and/or compulsions that cause significant distress and interfere with normal functioning at work, at home, in social activities, or in personal relationships. Approximately 80% of OCD cases begin in childhood; the prevalence in children and adolescents is 0.1–4% [1]. Psychiatric comorbidity is high in child and adolescent OCD patients, with anxiety, mood, and tic disorders being the most common comorbidities [2].

The biological mechanisms underlying OCD are not sufficiently elucidated [3]. Neuroinflammation-mediated changes in the brain caused by stress and changes in the immune system may contribute to the etiology of OCD. Recent reports have identified immune system alterations in OCD patients. Despite strong recent interest in immunologic abnormalities in OCD, few studies have examined cytokines in this disorder [4]. Cytokines, which increase with chronic inflammation, were shown to increase neopterin and decrease tetrahydrobiopterin (BH4) levels by activating the neopterin–BH4 pathway. Neopterin, a biopterin precursor that is released by macrophages, is accepted as a biochemical marker of cell-mediated immune responses [5].

In addition, studies have shown that it can be an important biomarker in psychiatric disorders, especially major depressive disorder [6]. BH4 is the main cofactor for the speed-limiting steps in the conversion of phenylalanine to tyrosine, hydroxylation of tyrosine and tryptophan, and the formation of serotonin, noradrenaline, and dopamine. Furthermore, BH4 plays an important role in regulating presynaptic release of neurotransmitters from nerve terminals [7].

Nitric oxide (NO) levels are associated with increased oxidative stress cytokines (especially IFN- g), and neopterin elevates NO levels by increasing reactive oxygen species such as NADPH-oxidase (NOX) and superoxide anion (O_2^-) and by activating inducible nitric oxide synthase (iNOS) [8]. The relationship between inflammation, the neopterin – tetrahydrobiopterin pathway, and nitric oxide is shown in Fig. 1. NO participates in the regulation of neurotransmission in the central nervous system. The importance of monoaminergic systems in the functioning of the brain is clearly shown by the number of severe neuropsychiatric diseases caused by impairment of monoaminergic neurotransmission. NO has been implicated in a number of physiological functions such as noradrenaline and dopamine release, memory and learning, and certain pathologies such as schizophrenia, bipolar disorder, and major depression [9–12].

The cytokines in this research were chosen after literature review and the selection criteria was mainly based on; the most evaluated cytokines in adult OCD patients along with the least evaluated cytokines in pediatric OCD patients.

To our knowledge, this is the first study comparing serum TGF-1β, TNF-α, IL-1β, IL-2, IL-6, IL-10, IL-17, neopterin, BH4, and NO levels with child and adolescent patients diagnosed with OCD and a healthy control group.

2. Materials and methods

2.1. Study population and sample

This study was planned as a cross-sectional study, and conducted between December 2018 and November 2019 at the Outpatient Clinic of the Department of Child and Adolescent Psychiatry, Manisa Celal Bayar University Faculty of Medicine (MCBUFM). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and its later amendments or comparable ethical standards. Permission for this study was obtained from the MCBUFM Health Sciences Institute Ethics Committee (number 20.478.486, dated 01/08/2018 and 01/10/2019).

For the patient group, children aged between 11–18 years who visited the outpatient clinic and were diagnosed with OCD after the onset of the study were referred to the researcher. A semi-structured psychiatric interview conducted by the researcher was used to identify patients diagnosed with OCD for inclusion in the study based on the inclusion and exclusion criteria, as described below.

For the healthy control group, children aged between 11–18 years who were admitted to the MCBUFM Pediatric Outpatient Clinic, as a prerequisite for swimming course, gym and dormitory admission, who had no chronic or acute disease, and who had not been previously diagnosed with a psychiatric disorder were referred to the researcher. Of these, subjects who did not meet the diagnostic criteria of a psychiatric disorder based on the semi-structured psychiatric interview conducted by the researcher were included in the study as controls if they met the inclusion and exclusion criteria, as described below.

Before the clinical evaluation, informed consent of the children and parents was obtained after the nature of the procedures had been fully explained.

2.2. Inclusion and exclusion criteria

Exclusion criteria for all participants were as follows: use of drugs affecting the immune system in the last 6 months; any immunological, hematological, neurological, metabolical, or significant medical or infectious disease in the last month; substance abuse in the last 3 months; and smoking.

Inclusion criteria for the patient group were as follows: diagnosed with active OCD according to DSM-5; and persistence of an OCD episode for at least 6 weeks.

Patients who had used psychotropic medications in the last 6 weeks and those who were diagnosed with a comorbid psychiatric disorder were excluded from the study.

The exclusion criteria for the healthy control group were a history of psychiatric disorder and psychotropic drug use.

During the research period, 69 children diagnosed with OCD and 41 healthy children were admitted to the study. In the OCD group, 14 subjects were excluded due to psychiatric comorbidities, 13 due to psychotropic medication use and psychiatric comorbidities, 8 because of non-psychotropic medication use, 3 due to smoking, and 2 because of chronic diseases. In the healthy group, 5 children were excluded because of non-psychotropic medication use, 4 due to a history of psychiatric disorders, 2 because of smoking, and 2 due to a newly diagnosed psychiatric disorder. Thus, 29 children with OCD and 28 healthy controls met the inclusion and exclusion criteria and were included in the research.

2.3. Clinical evaluation

Schedule for Affective Disorders and Schizophrenia for School Aged Children Kiddie-SADS-lifetime Version, DSM-5 (K-SADS-PL-DSM-5) and Children Yale-Brown Obsessive Compulsive Scale- (C-Y-BOCS) were applied, and a sociodemographic data form was completed.

2.3.1. Sociodemographic data form

A sociodemographic data form was created by the authors in order to determine sociodemographic characteristics of the children and their parents who participated in the study.

2.3.2. Schedule for Affective Disorders and Schizophrenia for School Aged Children Kiddie-SADS-Lifetime Version (K-SADS-PL DSM-5):

It was adapted by Kaufman et al. (2016) for the evaluation of psychiatric disorders in children and adolescents according to DSM-5 diagnostic criteria. The validity and reliability study of the Turkish version was performed by Ünal et al. in 2019 [13].

2.3.3. Children Yale-Brown Obsessive Compulsive Scale-(C-Y-BOCS):

It is a semi-structured scale developed in 1997 by Scahill L et al. with the scale; obsession and compulsion types are evaluated. After this evaluation, the severity of obsessions and compulsions is measured. There are 19 questions in scale and 5 items (none-mild-moderate-severe-extreme) in each question. In measuring the severity, the first 10 questions of the scale are scored and 0-7 points are subclinical, 8-15 points are mild, 16-23 points are moderate, 24-31 points are severe and 32-40 points are extreme. The validity and reliability study of the Turkish version was performed by Yücelen et al. in 2006 [14].

2.4. Biochemical analyses

For the measurement of neurobiological markers, fasting blood samples were collected in 10 mL anticoagulant tubes between 9 and 10 a.m.. The venous blood samples were centrifuged at 3000 rpm for 15 min to be separated from serum and stored at -80degC until analyzed.

Serum samples were analyzed for TGF-1β, TNF-α, IL-1β, IL-2, IL-6, IL-10, IL-17, neopterin, BH4 and NO levels by using enzyme-linked immunosorbent assay (ELISA).

In serum samples, BH4 (Catalogue Number(CN): E-EL-0110), NO (CN: E-BC-K036), TGF-1β (CN: E-EL-H0110), IL-2 (CN: E-EL-H0099), IL-17 (CN: E-EL-H0105) levels were studied with commercial (Elabscience, Houston, Texas, USA) kits; TNF-α (CN: KAP1751), IL-1β (CN: KAP1211), IL-6 (CN: KAP1261), IL-10 (CN: KAP1321), levels were with studied commercial (Dia Source, Ottignies-Louvain-la-Neuve, Belgium) kits; neopterin (CN: RE59321) levels were studied with commercial (IBL international, Hamburg, Germany) kits.

During the analysis, ELISA washing operations were performed with an automatic washing device (BioTek ELx50 BioTek Instruments Inc. Highland Park, Winooski, VT, USA), absorbance readings were carried out in the ELISA reader (BioTekEpoch, BioTek Instruments Inc. Highland Park, Winooski, VT, USA).

2.5. Statistical evaluation

The data obtained from the study were evaluated using the Statistical Package for Social Sciences (SPSS) 21.0 program. Continuous variables obtained by measurement were expressed as mean ± standard deviation, and categorical variables were expressed as percentage and number. The normality of distribution was analyzed with the Kolmogorov–Smirnov test. Student's t-test was used to compare means between two independent groups with normal distribution, and the non-parametric Mann–Whitney U test was used for groups that did not show normal distribution. Chi-square and Fisher's exact test were used to compare the categorical data. In order to determine the direction and level of the relationship between numerical variables, Pearson's test was used for those with normal distribution, and Spearman's correlation test was used for those that did not show normal distribution. In all analyses, $p<0.05$ was considered statistically significant.

3. Results

3.1. Demographic and Clinical Features: The study included 29 (50.9%) adolescent patients with OCD and 28 (49.1%) adolescent healthy controls. No significant difference was found between the groups in terms of age *(p = 0.179)* or sex *(p = 0.198)* . At least one psychiatric disorder was found in a 1st or 2nd degree relative of 17 (58.6%) children in the OCD group and 2 (3.1%) children in the control group ***(p = 0.000)*** . The clinical and demographic features of all cases are shown in Table 1.

3.2. C-YOCBS Features and Scores of the Patient Group: All of the patients had both obsessions and compulsions. Washing/cleaning (82.8%) and controlling (79.3%) were the most common compulsions, and contamination (89.7%) and magical thinking/superstition (44.8%) were the most common obsessions. The least frequent compulsions were hoarding/picking compulsions (17.2%), and the least frequent obsessions were sexual (3.4%) and religious (20.7%) obsessions. Based on the Children's Yale–Brown Obsessive Compulsive Scale (C-YBOCS) total scores, 6 patients (20.7%) were evaluated as moderate, 17 (58.6%) as severe, and 6 (20.7%) as extreme cases.

3.3. Cytokines Levels: No significant difference was found between the groups in the levels of TNF-α *(p = 0.983)* , IL-1β*(p = 0.357)* , IL-2 *(p = 0.135)* , IL-6 *(p = 0.458)* , IL-10 *(p = 0.877)* , and IL-17 *(p = 0.391)* . A significant difference was found between the patient and control groups for TGF-1β***(p = 0.002)*** levels. The data are shown in Table 2. High standard deviation was found for IL-6 in OCD group and the median (minimum-maximum) value was 15.5 (6.27-298.39). In addition, high standard deviations were found for TGF-1β, TNF-α and IL-6 in healthy control group and the median 1-2 (minimum-maximum) values were 136-147 (57-825), 6.18-6.43 (2.64-18.39), 20.98-21.77 (6.01-315.5) respectively. In OCD group, the median (minimum-maximum) values of TGF-1β and TNF-α were 147 (68-441), 5.83 (2.43-13.93) respectively. The Spearman correlation test revealed a weak negative correlation between TGF-1β levels and C-YOCBS total scores in the patient group *(r =–0.124* ; ***p = 0.032)*** . The data are shown in Table 3.

3.4. Neopterin , BH4, and NO Levels: Neopterin***(p = 0.021)*** , BH4 ***(p = 0.001)*** , and NO***(p = 0.013)*** levels were significantly different between the groups. The data are shown in Table 2. High standard deviation was found for neopterin in healthy control group and the median 1-2 (minimum-maximum) values were 7.59-7.59 (3.47-18.45). In OCD group, the median (minimum-maximum) value of neopterin was 8.16 (3.71-34.47). In the patient group, Spearman correlation test results showed a weak negative relationship between BH4 levels and C-YBOCS total scores*(r = –0.218* ; ***p = 0.001)*** , a statistically significant positive correlation between neopterin levels and C-YBOCS total scores *(r = 0.352* ; ***p = 0.002)*** , and a weak positive correlation between NO levels and C-YBOCS total scores *(r = 0.198* ; ***p = 0.005)*** . The data are shown in Table 3.

3.5. Correlation of Illness Duration with C-YBOCS Scores and Biological Data: Spearman correlation analysis revealed statistically significant positive correlations between the illness duration and

the C-YOCBS obsession score *(r = 0.431 ; **p = 0.02**)* and total score *(r = 0.401 ; **p = 0.031**)* . There was no statistically significant correlation between illness duration and serum cytokine, neopterin, BH4, and NO levels. The data are shown in Table 4.

4. Discussion

The relationship between inflammation and OCD is unclear. The fact that children show signs of OCD and tic disorder after a bacterial throat infection suggests that neuroinflammation plays an important role in the etiology of OCD [15]. The most important hypotheses explaining this relationship involve guanosine triphosphate (GTP) cyclohydrolase I and indoleamine 2,3 dioxygenase (IDO) activation.

IDO is activated by many proinflammatory cytokines. As a result of IDO enzyme activation, tryptophan, the primary amino acid precursor of serotonin, is converted to kynurenine, resulting in the potential depletion of serotonin [16] (Fig. 1).

GTP cyclohydrolase I is also activated by many proinflammatory cytokines, especially IFN-g. With the activation of GTP cyclohydrolase I, the production of neopterin increases and that of BH4 decreases in response to GTP. Therefore, neopterin is considered a biochemical marker of the cell-mediated immune response. BH4 in this pathway is the main cofactor for rate-limiting steps in the formation of serotonin, noradrenaline, and dopamine. In addition, BH4 plays an important role in regulating the presynaptic release of neurotransmitters from nerve terminals [5,7]. The pathway of cytokine–neopterin and BH4 affects the formation of NO by increasing reactive oxygen species such as NOX and O_2^- and activating iNOS [8] (Fig. 1).

In this study, all proinflammatory cytokine levels were found to be low, but this decrease was statistically significant only for TGF-1β. There are few previous studies examining the relationship between OCD and cytokine levels in children. In a study in which comorbidities were not excluded but drug use was, TNF-α levels were significantly higher in children with OCD compared with healthy controls; TGF-1β, IL-1β, and IL-17 levels were also low, but not significantly so [17]. In a study of adults with OCD, in which comorbidities and drug use were excluded, the IL-1β level was significantly higher in the OCD group compared to the healthy control group, whereas IL-2, IL-6, and TNF-α levels were significantly lower; no difference was found in IL-10 levels [18]. Considering these findings, the relationship between OCD and proinflammatory cytokines identified in this study is compatible with a recent meta-analysis [19]. Differences among these findings may be explained in terms of the exclusion of psychiatric comorbidities, which might affect cytokine levels, the effect of OCD on stress levels, and the decrease in immune system cell levels and cytokine levels due to increased the hypothalamic–pituitary–adrenal (HPA) axis activity and cortisol levels as a result of this effect [20,21]. In addition, most studies in the literature were conducted in adult patients, and cytokine production in adults may differ from that in children [22]. To our knowledge, this study is the first to find that circulating TGF-1β levels are significantly lower in OCD patients. The relationship between blood concentration of TGF-1β and pediatric OCD must be investigated through additional studies.

In this study, the neopterin level was significantly higher and BH4 significantly lower in children with OCD compared to the healthy control group. A prior study conducted in adult patients with OCD found that the neopterin level in the OCD group was not significantly different from that in the control group, but there was a significant decrease in the level of neopterin following the dexamethasone suppression test performed to suppress the immune system [23]. Only a few studies in the literature have investigated the relationship between neopterin levels and psychiatric disorders. In recent years, as a result of studies conducted in adult patients diagnosed with major depressive disorder, neopterin levels have been among the candidates for relevant immune markers in major depressive disorder [6]. A study of children diagnosed with autism spectrum disorder (ASD) found that neopterin levels were significantly higher in children with ASD compared to the control group [24]. In a study of adult patients diagnosed with bipolar disorder, neopterin levels were significantly higher in the bipolar group compared to the control group [25]. We found no study investigating the relationship between OCD and BH4 levels. When psychiatric diseases other than OCD were examined, BH4 levels were found to be significantly lower (e.g., in patients with adult schizophrenia)

compared to healthy controls [26]. Although the proinflammatory cytokine levels did not increase in this study, activation of the neopterin– BH4 pathway could have been due to elevated IFN-g levels and decreased 6-pyruvyl-tetrahydropterinsynthase levels [5,27].

In this study, NO levels were found to be significantly higher in children with OCD compared to the healthy control group. A review of the literature identified no studies examining the relationship between OCD and NO levels in children. A review of studies and meta-analyses conducted in patients with adult OCD revealed that NO levels were significantly increased, in keeping with this study [28–30]. It was thought that increased neopterin levels, and possibly increased IFN- g levels and iNOS activation, may induce an increase in reactive oxygen species and thus in NO levels [8].

The results of this study indicate that the activity of the neopterin–BH4 pathway and changes in inflammatory and oxidative parameters may underlie OCD. In addition, the correlations of TGF-1β ($r<0.3$, weak), neopterin, BH4 ($r<0.3$, weak), and NO ($r<0.3$, weak) levels with C-YBOCS total scores suggest that this pathway may be involved in the etiology of OCD. Furthermore, the positive correlation between illness duration and C-YOCBS obsession and total scores indicates that illness severity increases over time. Consequently, the results of this study show that the importance of early diagnosis and treatment of OCD, and the levels of TGF-1β and NO and the activation of the neopterin–BH4 pathway may be implicated in the pathophysiology of OCD. Additionally, anti-oxidant and BH4 adjuvant therapies should be investigated as treatment options for OCD.

In this study, TGF-1β, IL-2, IL-17, and NO levels were evaluated, which have been examined in few previous studies in children with OCD, and the levels of neopterin and BH4, which have not been previously investigated in children with OCD. In addition, we excluded psychiatric and physical comorbidities and the use of psychotropic and non-psychotropic drugs that might have influenced the biological results.

The present study has some limitations. The cross-sectional study design allowed the measurement of cytokine levels at only a single time point. The small sample size was inadequate to be representative of the general population. Also, TGF-1β, TNF-α, IL-6 and neopterin levels resulted in high standard deviations due to extreme values and the small sample size, and this may have affected the statistical results. However, these extreme values were not excluded from this study because they were within the measuring range of the kits used. In addition, we did not assess the serum levels of neurotransmitters (e.g., serotonin, dopamine, and noradrenaline), cortisol, IFN- g, and 6-pyruvyl-tetrahydropterinsynthase. Further studies involving a larger population and addressing the limitations of this study are needed, and the causal link between TGF-1β, neopterin, BH4 and NO and pediatric OCD requires further investigation.

5. Conclusions

Taken together, in this study, we gained sight into the biochemical mechanisms of OCD from the activity of the neopterin–BH4 pathway and changes in inflammatory and oxidative parameters. Circulating TGF-1β and BH4 levels were significantly lower in the OCD group compared to the healthy control group, whereas neopterin and NO levels were significantly higher. Based on these findings, we postulate that the levels of TGF-1β and NO and the activation of the neopterin–BH4 pathway may be involved in the pathophysiology of OCD. This study not only provides insight into the mechanisms underlying OCD but also presents novel opportunities for the design and development of more effective therapeutic strategies.

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Informed consent: Informed consent of the children and parents was obtained after the nature of the procedures had been fully explained.

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Figure Legends:

Figure 1 The relationship between inflammation, the neopterin – tetrahydrobiopterin pathway, and nitric oxide

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