Comparing Brain Magnetic Resonance Spectroscopy Findings of Pediatric Treatment-Naive Obsessive-Compulsive Disorder Patients with Healthy Controls

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SUMMARY

Objective: It is believed that biochemical alterations in different brain regions are involved in the pathophysiology of obsessive-compulsive disorder (OCD) in children and adolescents. The aim of this study was to explore possible metabolic variations between pediatric OCD cases and healthy controls in brain regions which were implicated in OCD pathophysiology.

Method: Children and adolescents between 8 and 16 years of age with OCD (n:15) and case matched healthy controls (n:15) were recruited for the study. After detailed clinical and neuropsychological evaluations, all subjects underwent the multiregional magnetic resonance spectroscopy (MRS) procedure with a long echo time (TE:135).

Results: Significantly lower n-acetylaspartate (NAA) ratios in the left inferior frontal gyrus, right occipital grey matter, left anterior cingulate cortex and lower choline (Cho) ratios in right and left anterior cingulate cortex and higher Cho ratio in left lenticular nucleus was observed in the OCD group. Also we found a negative correlation between OCD duration and left insular cortex NAA/Cho ratio.

Conclusion: We found significant metabolic alterations in the brain regions which were implicated in OCD pathophysiology. Lower NAA and Cho ratios in anterior cingulate cortex and lower NAA ratios in the left inferior frontal gyrus containing lateral orbitofrontal cortex can be possibly related to higher activation in OCD patients. Also further studies of the occipital lobes and insula should be continued in OCD.

Key Words: Pediatric obsessive-compulsive disorder, magnetic resonance spectroscopy, neuroimaging

INTRODUCTION

The prevalence of Obsessive-Compulsive Disorder in children and adolescent is reported between 1% and 4% (Douglas et al. 1995, Zohar 1999). OCD is usually a chronic condition which may continue in adulthood. The disorder causes social, personal and economic problems (Keeley et al. 2007). Countless research has been conducted to date with aims to elucidate the pathogenesis of the disorder.

Adult OCD neuroimaging studies mostly focus on frontal lobes and nucleus caudatus, where as pediatric studies focus on thalamus and striatum (Friedlander et al. 2006).

Brain metabolite ratios can be measured by Magnetic Resonance Spectroscopy (MRS): N-acetyl Aspartate (NAA) reflects neuronal density, viability, integrity and is produced in mitochondrias of neurons; glutamate is a marker of excitator neurotransmitters; choline (Cho) is related to cell membrane turnover-myelin levels and pathological processes such as inflammation and gliosis; myo-inositol (mI) which reflects phospholipid metabolism and creatine (Cr) the marker of intracellular energy mechanisms and used as a reference point for analyses (Maier 1995).

To date, MRS studies on children and adolescents with OCD indicated the following results: higher caudat nucleus glutamatergic ratios which had been reversed with serotonergic drugs (Rosenberg et al. 2000, Moore et al. 1998, Bolton et
al. 2001), lower NAA (Fitzgerald et al. 2000) and higher Cho (Rosenberg et al. 2001, Smith et al. 2003), Cr ratios in the right and left medial thalamus (Mirza et al. 2006), higher dorsolateral prefrontal cortex NAA levels (Russell et al. 2003) and lower anterior cingulate glutamatergic levels (Rosenberg et al. 2004). Arnold and colleagues determined a correlation between a polymorphism in the “Glutamate receptor, ionotropic, N-metil-d-aspartate 2B” gene and decreased anterior cingulate cortex glutamatergic concentrations with brain MRS in pediatric OCD patients (Arnold et al. 2009).

The aim of this study was to compare the metabolic ratios of the healthy children and adolescents in those diagnosed with OCD and to find metabolic alterations in the brain areas that have been previously mentioned in OCD pathophysiology.

**METHOD**

Fifteen children and adolescents with OCD and 15 healthy controls matched by age, gender and education level participated in the study. The Ethical Committee of the Medical School at Gazi University approved the study and written informed consent was obtained from all the subjects and families. The control group was recruited from our volunteer outpatient clinic patients who were referred with adolescent problems, interactional problems in the family but not otherwise diagnosed according to DSM-IV-TR criteria (American Psychiatric Association 1994). In the patient group, OCD diagnoses were confirmed and in the control group, psychiatric diagnoses were excluded by the Turkish version (Gökler et al. 2004) of Schedule for Affective Disorders and Schizophrenia for School Age Children – Present and Lifetime Version (K-SADS-PL) (Kaufman et al. 1997). All the K-SADS-PL sessions with subjects and their parents were interviewed by the same child and adolescent psychiatrist, the first author in this study. Only the subjects with right handedness and whose IQ scores were above 90 from Turkish validated (Savaşır et al. 1995) Wechsler Intelligence Scale for Children – Revised (WISC-R) (Wechsler 1974) and between 8 and 16 years of age were chosen for the study. The mean age for the control group was 12.83 ± 2.49 and 12.86 ± 2.44 for the OCD group. Both of the groups had a mean education period of 7.33 ± 2.55 years. Ten girls and 5 boys participated in each group.

In the OCD group the severity of the disorder was determined using the Turkish version (Yücelen et al. 2000) of the Children’s Yale-Brown Obsessive-Compulsive Scale (CY-BOCS) (Scahill et al. 1997). The OCD group had 13.53 ± 2.47 mean points from obsession subscale and 14.26 ± 2.86 mean points from compulsion subscale, with a total of 27.80 ± 4.78 mean points. In this scale 8-15 points refer to mild, 16-23 points refer to moderate, 24 and higher points refer to severe disease. The mean illness duration for the OCD group was 2.43 ± 1.23 years.

Exclusion criteria for each group were: previous use of psychotropic drugs, an IQ below 90, early or late commencement of school, mismatch between school grade and age, diagnosis with any of the following disorders; attention deficit/hyperactivity disorder, specific learning disorder, affective disorders, pervasive developmental disorders, psychotic disorders and eating disorders in clinical and psychometric assessments and existence of epilepsy or other neurological disorders.

All the subjects underwent the MRS procedure in the Radiology Department of Gazi University with 1,5 Tesla Magnetic Resonance Imaging equipment. T2 weighted sequences were used for MRS images and analyses. An axial section passing through basal ganglions was provided for spectroscopic analysis (Figure 1). Long Echo time was used (TE:135) with TR:1000 relaxation time, and 16 mm slices, 20 x 20 matrices.

MRS analyses were made by the same radiologist (T.O) and NAA/Cho, Cho/Cr, NAA/Cr ratios were analyzed for frontal white matter, inferior frontal gyrus (Brodmann areas [BA] 11,44,45 and 47), lentiform nucleus, caudate nucleus, temporoparietale gyrus, occipitale white matter, occipitale grey matter, insular cortex, medial thalamus and anterior cingulate cortex bilaterally.

SPSS 11.5 was used for statistical analysis. MRS metabolic ratios of the two group was compared with Mann-Whitney U test. Correlational analyses between CY-BOCS scores, OCD illness duration and MRS data were calculated with Spearman’s test. Statistical significance was determined at the 0.05 level.
RESULTS

In the OCD group, most comorbid diagnoses due to K-SADS-PL were generalized anxiety disorder (n:5, 33.3%), chronic motor tic disorder (n:1), tourette syndrome (n:1), social anxiety disorder (n:1), panic disorder with agoraphobia (n:1) and enuresis nocturna (n:1).

The metabolic ratio results of the MRS p and Z values are shown in Table 1 for the two groups.

In the OCD group, left inferior frontal gyrus NAA/Cr (p:0.037), left and right anterior cingulate cortex Cho/Cr (p:0.044; p:0.022), left anterior cingulate cortex NAA/Cr (p:0.019), right occipital grey matter NAA/Cho (p:0.048) ratios were significantly lower than the control group, where as the left lenticular nucleus Cho/Cr (p:0.019) ratio was significantly higher in the OCD group (Table 1).

Also in the OCD group left inferior frontal gyrus NAA/Cho (p:0.077), right anterior cingulate cortex NAA/Cr (p:0.077), right caudate nucleus NAA/Cr (p:0.056), and right frontal white matter NAA/Cr (p:0.085) ratios tended to be lower and the left lenticular nucleus NAA/Cr (p:0.137) ratio tended to be higher than the control group (Table 1).

In the OCD group according to Spearman's test, there was a significantly negative correlation between illness duration and left insular cortex NAA/Cho ratios (p:0.031, r:-0.576).

Significant and positive correlations were obtained between CY-BOCS obsession subscale points and right lenticular nucleus NAA/Cr (r: 0.917, p: 0.000), left tempo-parietale gyrus Cho/Cr (r: 0.797, p: 0.001); CY-BOCS compulsion subscale points and right lenticular nucleus Cho/Cr (r: 0.603, p: 0.022), between CY-BOCS total points and right lenticular nucleus NAA/Cr (r: 0.832, p: 0.000), and left tempo-parietale gyrus Cho/Cr (r: 0.628, p: 0.022) ratios.

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DISCUSSION

We found significant metabolic alterations in the inferior frontal gyrus (including lateral orbitofrontal cortex), anterior cingulate cortex and in the lenticular nucleus which were previously implicated in OCD etiology (Friedlander et al. 2006). Compared to controls, we also unexpectedly found right occipital grey matter metabolic alterations in the OCD group.

Inferior frontal gyrus / lateral orbitofrontal cortex

We found significantly lower NAA/Cr ratios in the OCD group, as compared to controls in the left inferior frontal gyrus. As NAA is a marker of neuronal density and viability (Maier 1995), we suspect neuronal loss-lower neuronal density in this region in the patient group. Apart from the insula and parahippocampal area, the inferior frontal gyrus activity is also closely related with disgust neurocircuitry (Shapira et al. 2003) which is involved in OCD. Also in many previous OCD studies, findings concurrent with our study results were found. In another magnetic resonance imaging (MRI) study, cortical thinning at the left inferior frontal gyrus including left orbitofrontal region was determined in the adult OCD group (Shin et al. 2007). Yoo and colleagues found lower inferior frontal gyrus grey matter volume in adult patients with OCD (Yoo et al. 2008). In a functional MRI study, on pediatric OCD patients, lower left inferior frontal gyrus activation was determined during set shifting task (Britton et al. 2010).

Brodmann areas (BA) 11 and 47 of the inferior frontal gyrus also compose the lateral part of orbitofrontal cortex (Rolls 1996). Consistent with our study, in a voxel based morphometry- (VBM) MRI study, in adult OCD group lower left orbitofrontal cortex (BA 47) and left inferior frontal gyrus (BA 44/45) grey matter volumes were determined (van den Heuvel et al. 2009). There are strong cumulative evidence indicating orbitofrontal cortex dysfunction in OCD pathophysiology (Friedlander et al. 2006, Chamberlain et al. 2005, Rotge et al. 2008). The bilateral volume reduction in the orbitofrontal cortex was revealed in a meta-analysis on studies investigating MRI volumetric measures in adult OCD groups (Rotge et al. 2009). According to Friedlander, higher neuronal activity of the orbitofrontal cortex leads to cellular degeneration and neuronal death in OCD (Friedlander et al. 2006). Several other recent studies supported this view with the findings of higher neuronal activity and smaller volumes of the orbitofrontal cortex (Friedlander et al 2006; Chamberlain et al. 2005; Atmaca et al. 2007, Pujol et al. 2004, Nakao et al. 2005, Nabayema et al. 2008, Rotge et al. 2008). Orbitofrontal cortex and ventromedial areas of frontal lobe activity is related with immediate impulse and gains, behavioural changes due to enviromental variations, decision making due to rewards and related emotional experiences. The orbitofrontal cortex quickly analyses the codes about the reward and is associated with immediate decision and changes. The orbitofrontal cortex is associated with motivational aspect of decision making. Orbitofrontal cortex hyperactivity can cause immediate actions, uncontrollable thoughts and behaviours like in OCD (Aouizerate et al. 2004). In this study, lower left orbitofrontal cortex and associated left inferior frontal gyrus NAA ratios can be due to possible hyperactivity of this region in the OCD group. Also according to Yoo, the hyperactivity of the orbitofrontal cortex observed in the functional imaging studies of OCD may be attributable to residual tissues compensating for decreased grey matter volume (Yoo et al. 2008).

Anterior cingulate cortex

Another finding of the present study was the significant decrease in Cho/Cr ratios bilaterally at anterior cingulate cortex and lower NAA/Cr ratios at left anterior cingulate cortex, closely related with prefrontal cortex, in the OCD group. Also in OCD subjects, right anterior cingulate cortex NAA/Cr ratios tended to be lower. Cho is related with cell membrane and glia (Maier 1995). Bilateral lower anterior cingulate cortex Cho and NAA ratios in the OCD group can be due to neuronal loss, lower neuronal density at anterior cingulate cortex, particularly on the left. Interestingly, in an adult OCD study, smaller bilateral anterior cingulate cortex volumes were found and were especially obvious on the left side (Atmaca et al. 2007). Again, two VBM-MRI studies reported lower left anterior cingulate cortex grey matter volumes in adult OCD groups (Gilbert et al. 2008, Matsumoto et al, 2010) and another VBM-MRI study also revealed bilateral anterior cingulate cortex grey matter loss in adult OCD group (Yoo et al. 2008). In two meta-analytic studies on VBM-MRI studies in adult OCD patients, lower bilateral anterior cingulate cortex grey matter volumes were determined (Radua et al. 2010, Radua and Mataix-Cols 2009). Again, a meta-analysis of volumetric MRI studies on adult OCD patients reported volume reduction at the left anterior cingulate cortex (Rotge et al. 2009).

There is growing evidence about anterior cingulate cortex hyperactivity in OCD (Rotge et al. 2008). Yücel and colleagues reported lower anterior cingulate cortex NAA levels and the hyperactivity of the same region in their functional-biochemical neuroimaging study on an adult OCD sample (Yücel et al. 2007). Huyser and colleagues reported increased activity at the bilateral anterior cingulate cortex during the Tower of London Test with fMR in a pediatric OCD group (Huyser et al. 2010). Also, lower NAA and Cho levels in the anterior cingulate cortex in our study can be due to hyperactivity of anterior cingulate cortex.

Previous MRS studies investigating the anterior cingulate cortex in OCD are consistent with our results. Ebert and colleagues reported lower anterior cingulate cortex NAA levels correlated with OCD duration on adult sample (Ebert et al. 2005, Nabayema et al. 2008, Rotge et al. 2008). The orbitofrontal cortex and ventromedial areas of frontal lobe activity is related with immediate impulse and gains, behavioural changes due to enviromental variations, decision making due to rewards and related emotional experiences. The orbitofrontal cortex quickly analyses the codes about the reward and is associated with immediate decision and changes. The orbitofrontal cortex is associated with motivational aspect of decision making. Orbitofrontal cortex hyperactivity can cause immediate actions, uncontrollable thoughts and behaviours like in OCD (Aouizerate et al. 2004). In this study, lower left orbitofrontal cortex and associated left inferior frontal gyrus NAA ratios can be due to possible hyperactivity of this region in the OCD group. Also according to Yoo, the hyperactivity of the orbitofrontal cortex observed in the functional imaging studies of OCD may be attributable to residual tissues compensating for decreased grey matter volume (Yoo et al. 2008).
Another adult OCD study indicated an increase of the previous lower anterior cingulate cortex NAA ratios with citalopram treatment (Jang et al. 2006). Sumitani reported lower anterior cingulate cortex NAA ratios, in treatment resistant adult OCD patients who required atypical antipsychotic augmentation (Sumitani et al. 2007).

The anterior cingulate cortex has a role in attention, motivation, reward-harm estimation, working memory, problem solving, action planning and conflict monitoring and is associated with the emotional consequences of an action. Hyperactivity of the anterior cingulate cortex leads to obsessive-compulsive, phobic and unroutine ordered actions (Aouizerate et al. 2004).

These metabolic alterations that we found in the anterior cingulate cortex and lateral orbitofrontal cortex could reflect the dysfunctions of these regions and the close connection between these two possible dysfunctional regions in pediatric OCD. Because anterior cingulate cortex is associated with rewards with long term consequences whereas the orbitofrontal cortex is related with immediate rewards (Aouizerate et al. 2004).

**Basal ganglions**

Basal ganglions are widely studied areas in OCD pathophysiology (Chamberlain et al. 2005). We found higher left lenticular nucleus (putamen + globus pallidus) Cho/Cr ratios in the OCD group. This finding can be due to gliosis and myelinization defects or dysfunctions of intracellular signal mechanisms which were previously suggested in pediatric OCD etiology (Mac Master et al. 1999, Rosenberg et al. 2001, Smith et al. 2003). Also in our study the left lenticular nucleus NAA/Cr ratios in OCD group tended to be higher. According to these two findings, a probable pathologic process can be suggested, ending up with gliosis accompanying neuronal hyperplasia and increased volume in the left lenticular nucleus of OCD subjects. Rosenberg suggested that increased brain volumes in OCD may result from delayed or inefficient pruning of neurons (Rosenberg et al 1998), whereas Yoo suggested that increased volumes can be due to compensation of dysfunctional frontal-striatal regions in OCD (Yoo et al. 2008).

Our findings in the left lenticular nucleus is consistent with previous OCD studies. In a fMRI study Lazaro and colleagues reported the decrease of left putamen hyperactivity in a pediatric OCD sample with effective treatment (Lazaro et al. 2008). Again on pediatric OCD samples with VBM-MRI technic, increased left putamen grey matter was recently found (Szeszko et al 2008). Also, two adult OCD studies with VBM-MRI technic reported larger grey matter volume in the putamen (Yoo et al. 2008, Pujol et al. 2004). In two meta-analytic investigations reviewing studies on adult OCD samples using VBM-MRI technic reported increased grey matter volumes bilaterally at the lenticular nucleus (Radua et al. 2010, Radua and Mataix-Cols 2009). On the contrary, in anxiety disorders other than OCD, decreased left lenticular nucleus grey matter volumes were reported (Radua et al. 2010).

Ohara and colleagues did not find any metabolic alterations in the lenticular nucleus with MRS in an adult OCD sample (Ohara et al. 1999), whereas in Tourette syndrome which is closely related to OCD, lower left putamen NAA/Cho ratios were found with MRS (Devito et al. 2005) converse to our results.

We found significant metabolic alterations between the two groups in the left lenticular nucleus; however interestingly in the OCD group we also found a significant positive correlation between the severity of obsessions measured with CY-BOCS and right lenticular nucleus NAA/Cr ratios and between the severity of compulsions measured with CY-BOCS and right lenticular nucleus Cho/Cr ratios. Also, a meta-analytic study reported, increased grey matter volumes in cases with more severe OCD (Radua and Mataix-Cols 2009). Also consistent with our findings, a VBM-MRI study recently revealed that pediatric OCD subjects had significantly higher right putamen grey matter volumes than their siblings (Gilbert et al. 2008).

**Occipitale lobe**

An unexpected finding of lower right occipital grey matter NAA/Cho ratios in the OCD group in our study supports a few previous study results on OCD. Recently in three VBM-MRI researches (one of them on pediatric OCD (Szeszko et al. 2008) and two on adult OCD samples (Koprivova et al. 2009, Togao et al. 2010)), found occipital cortex grey matter volume reductions. Szeszko and colleagues also found a reduction of fractional anisotropy (FA) in occipital white matter with Diffusion Tensor Imaging (DTI) in an adult OCD group, which can be related to myelin loss and axonal destruction (Szeszko et al. 2005). In an another MRI study, adult OCD samples exhibited cortical thinning in the left occipital lingual cortex (Shin et al. 2007). In an electroencephalogram (EEG) coherence study, an OCD sample revealed higher fronto-occipital theta activity which is related with cognitive decline and suggested to be associated with visuospatial dysfunction in OCD (Desarkar et al. 2007). An EEG spectral analysis on adult OCD subjects detected increased alpha wave in occipital lobes and was associated with hypoactivity of this region (Serra et al. 1994). Recently, Starck and colleagues found a negative correlation between occipital glutamate levels and severity of OCD symptoms with MRS (Starck et al. 2008) and Nabayema reported growing occipital-parietale region activity with effective treatment in a fMRI study on adult OCD samples (Nabayema et al. 2008). All of these results and our lower occipital grey matter NAA ratio

finding could be the indicators of occipital lobe hypoactivity in OCD, which would explain the lower NAA ratios that we found in the occipital lobe. Lower NAA ratios in the occipital lobe can directly cause hypoactivity in this brain region, distinct from our findings in different brain regions. There must be further studies researching the occipital lobe in OCD.

**Insula**

Insula has a significant role in disgust neurocircuitry (Shapira et al. 2003, Schienle et al. 2005) and intolerance of uncertainty (Simmons et al. 2008) which are closely associated with OCD symptomatology. There were studies reporting insular grey matter volume reduction (Yoo et al. 2008) and higher FA at subinsular white matter (Nakamae et al. 2008) in OCD. Two fMRI studies conducted in recent years revealed hyperactivity of the left insula in child and adolescent OCD samples (Lazaro et al. 2008, Huyser et al. 2010). Lazaro and colleagues showed decreased hyperactivity of the insula with efficient treatment (Lazaro et al. 2008). Again in a fMRI study on adult OCD samples, increased activity was detected in the left insula. Interestingly, decreased activity was shown during provocation of contamination and symmetry symptoms in a fMRI study on cases diagnosed with pediatric OCD (Gilbert et al. 2009). All these studies and our finding of negative correlation between OCD duration and left insular cortex NAA/Cho ratios indicate that insula deserves more attention in future OCD investigations. There can be a functional hyperactivity of insula which leads to neuronal loss by time in OCD.

**Medial thalamus**

Interestingly, although previous MRS studies on pediatric OCD groups replicated lower NAA (Fitzgerald et al. 2000) and higher Cho (Rosenberg et al. 2001, Smith et al. 2003) ratios in the medial thalamus, we did not find any metabolic alterations between the two groups in this region.

This study had several limitations. First, we studied a relatively small sample size and could not use short echo time (TE). As we used long echo time we could not assess any glutamatergic changes that had been widely investigated in previous OCD studies (Stewart et al. 2007, Arnold et al. 2009). Also, we could not analyze parietal lobe metabolic ratios, which can be associated with visuospatial and nonverbal neuropsychological deficits widely reported in OCD (Irak and Flament 2007).

In this study, we found significant metabolic alterations in brain regions which were shown to be important in OCD pathophysiology, supporting previous other studies. Additionally we detected some variations in insular and occipital regions that may have a role in OCD pathophysiology and deserve to be studied further.

**REFERENCES**


