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# Study on Dorsolateral Prefrontal Cortex Neurochemical Metabolite Levels of Patients with Major Depression Using $^{1}$H-MRS Technique

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**SUMMARY**

**Objective:**. The aim of the study is to investigate, by using proton magnetic resonance spectroscopy ($^{1}$H-MRS), the effects of major depression on the biochemistry of the brain, the relationship between the parametric changes demonstrated and cognitive functions, and the effects of antidepressant treatment.

**Method:** The study included 30 patients, diagnosed with moderate/severe non-chronic major depression disorder (NC-MDD) according to the DSM-5 diagnostic criteria, and 30 healthy individuals as the control group. The dorsolateral prefrontal cortex (DLPFC) areas of the patients and the control individuals were scanned bilaterally by $^{1}$H-MRS. The participants were also tested on the brief computerized version of the Wisconsin Card Sorting Test (or, Berg's "Wisconsin" Card Sorting Test-WCST). After antidepressant treatment for a minimum of 8 weeks, the patients who scored below 7 on the Hamilton Depression Rating Scale (HAM-D), were assessed with the $^{1}$H-MRS scan and the WCST.

**Results:** The Glx level in the left DLPFC was significantly lower in the patient group. Differences were not determined between the NAA, Cr, Cho levels in the right and the left DLPFC of the patient and the control groups. After the treatment, Glx level in the left DLPFC increased; but the levels of the other metabolites did not change. Before the treatment, the abilities of the patient group in changing strategy and problem solving, as assessed by the WCST, were lower in comparison to the control group. After the treatment the patient group improved clinically and performed significantly better on the WCST.

**Conclusion:** In the present neuroimaging (NI) study, it was determined that the Glx level in the left DLPFC of patients with moderate/severe NC-MDD improved together with the clinical features after treatment. Neurocognitive functions also improved after treatment. However, a correlation between the change in the metabolite levels and the performance on the WCST could not be demonstrated.

**Keywords:** Depression, magnetic resonance spectroscopy, prefrontal cortex, neurochemical metabolite, cognitive function

## INTRODUCTION

Major depression disorder (MDD) is the most common mood disorder with high incidences of recurrence and chronicity that can frequently cause physical and psychosocial disability (Judd 1997, Kessler et al. 1994) On the basis of the World Health Organization data of 2001, depression is foreseen to become the second among the diseases causing death in 2020 and will be the most burdening disorder worldwide by taking the first place among the diseases affecting working life. Given the increases in the chronicity, prevalence and the difficulty of treatment of depression, importance of the research in this field is gradually increasing (Küey 1998).

There are numerous studies showing the impairment of attention, memory and executive functions in depression (Fossatia et al. 1999, Egeland et al. 2003, Hill et al. 2004, Mahurin et al. 2006). Sensitivity of the WCST has been

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accepted especially in measuring the functions of dorsolateral prefrontal cortex (DLPFC) (Rogers et al. 2004)

Some of the imaging investigations in depression have reported hypoactivity, hypometabolism and reduction in the glial cell numbers and gray matter volume in the DLPFC (Galynker et al. 1998, Rajkowska et al. 1999, Narita et al. 2004, Siegle et al. 2007, Vasic et al. 2008). Levels of the neuronal metabolites such as N-acetylaspartate (NAA), glutamate-glutamine (Glx), creatinine (Cr), myoinositol (Myo-I), and choline (Cho) playing a role in neuronal functions, cell membrane and energy metabolism can be detected by means of the proton magnetic resonance spectroscopy ($^1$H-MRS) method, (Kwock et al. 1998). Next to the $^1$H-MRS studies specifying neuronal metabolic level change in the prefrontal cortex in relation to the neurodegenrative processes in depression (Gruber et al. 2003, Portellaet al. 2011, Wang et al. 2012, Michael et al. 2003), there are also studies not showing any changes (Neryet al. 2009, Henigsberget al. 2011, Oğuzhanoğlu et al. 2014).

Portella et al. (2011) determined lower Glx and total NAA levels in recurrent and chronic depression patients in comparison to healthy controls and the first-stage depression patients; and raised Cho levels showing a positive correlation with the disease duration in chronic depression patients.

The NAA/Cr and Cho/Cr ratios in the prefrontal cortex, seen to be lowered in depression patients as comapred to healthy controls, are increased after antidepressant treatment (Gönül et al. 2006, Zhang et al. 2015). Also, the decreased Glx level in the left DLPFC of MDD patients was shown to increase after electroconvulsive therapy (ECT) (Michael et al. 2003). These reported increases in the metabolite levels after treatment of depression may be correlated with repair of neuronal damage or increased neuroplasticity.

The studies reporting unchanged neurochemical metabolite levels after treatment of depression, also shwed that the metabolite levels of the patient and healthy control groups did not differ significantly before the treatment (Oğuzhanoğlu et al. 2014, Kaymak et al. 2009, Bajs et al. 2014). These studies with negative results in mild depression cases have suggested repeating the investigations in depression patients with more severe clinical symptoms. In the present study, patients receiving antidepressant treatment were excluded to eliminate the possibility of resultant changes in neurochemical metabolite levels. Positive neurotropic effects of antidepressant treatment especially on the NAA/Cr ratios have been reported (Gönül et al. 2006, Wang et al. 2012). There are limited number of investigations, especially in moderate and severe MDD, on the relationship of the neurochemical metabolite levels in the prefrontal cortex to the depression symptom cluster and neurocognitive functions.

The current study was planned to investigate the possible effects of depression on brain biochemistry using the $^1$H-MRS method in moderate and severe major depression patients not receiving any antidepressant treatment and to determine any correlation between the changes observed and the neurocognitive functions. Patients with nonchronic moderate/severe MDD were evaluated in comparison to a healthy control group. The correlation between neurochemical metabolite levels in the prefrontal cortex and he depression symptom cluster as well as the treatment effect on metabolite levels were investigated.

## MATERIALS and METHOD

### Participants

All patients, who consecutively consulted the psychiatry outpatient clinic of Pamukkale University Medical Faculty between July 2015 and May 2016 and were diagnosed with moderate/severe major depression according to the DSM-V criteria, were evaluated with respect to the inclusion criteria of the investigation. The Hamilton Depression Rating Scale (HAMD) was used during the interviews. The study enrolled 30 patients and 30 healthy individuals, who gave written informed consent for participation.

The research project was approved by the Ethics Committee of Pamukkale University Faculty of Medicine with the decision dated 07.07.2015 and numbered 2015/10. Funding was granted by the Scientific Research Projects Commission for $^1$H-MRS scans (Project no: 2015TPF024).

### The Exclusion Criteria of the Study

These were determined as 1) Declining to participate in the study, 2) Being under 18 and over 60 years of age, 3) Presence of schizophrenia, bipolar mood disorder, anxiety disorder, the presence of a psychiatric disorder such as alcohol and substance dependence, 4) Presence of a neurological disease, 5) Presence of a physical or cognitive problem preventing communication, 6) being illiterate.

The healthy control group was formed by the applications rece ived after the announcement made in the hospital on the characteristics of the healthy individuals required for the study. Following interviews conducted by the researcher on the basis of the DSM-5 diagnostic criteria, volunteering individuals without any lifelong mental and medical illness and without a of any mental illness in their first degree relatives were accepted for inclusion in the healthy control group. Payment was not made for participation.in the study.

## Stages of the Study

A questionnaire on the sociodemographic and clinical characteristics applicablewithin the context of the research was completed by 30 moderate/severe major depression patients and 30 healthy individuals, who met the criteria for inclusion in the study. The DLPFC regions of the patients and the healthy individuals were scanned bilaterally by the $^{1}$H-MRS method. Subsequently, the participant groups were tested by the researcher on the abbreviated computerized version of the Wisconsin Card Sorting Test (The Psychology Experiment Building Language (PEBL)-Berg's "Wisconsin" Card Sorting Test).

After the completion of $^{1}$H-MRS scanning and the WCST procedures, antidepressant treatments were started with fluoxetine (20mg/day) on 14 patients, sertraline (100mg/day) on 9 patients, venlafaxine (150-300mg/day) on 3 patients, duloxetine (60-120mg/day) on 3 patients, and citalopram (40mg/day) on 1 patient. All patients were placed under clinical observation every 15 days and the drug dosages were adjusted. After eight weeks of treatment, the control $^{1}$H-MRS scan and WCST could only be repeated in 20 of the patients with HAM-D scores under 7, since 5 patients did not come without giving a reason, 2 patients had moved out of town, and 3patients did not accept the control tests. Since the missing participants could not be interviewed and HAM-D assessment could not be made at the time of leaving, accurate data on thedetails such as treatment resistance, improvement, sequele and others were not acquired on their health status. This introduced a limitation to the evaluation of the total aims of the study.

**The Hamilton Depression Rating Scale (HAM-D):** Published by Max Hamilton in 1960, the HAM-D is a 17-item scale that examines the depressive symptoms of the previous week. The validity and reliability study of the Turkish language version of the HAM-D was conducted by Akdemir et al. (1996). The subscales on difficulty in falling asleep, waking up at midnight, early morning waking, somatic symptoms, genital symptoms, weight loss and insight were graded between 0-2; and the other subscales were rated between 0-4. The degree of severity for each item, such as mild, moderate, major, is determined by the expert on the basis of the answers given. In grading the results, 0-7 points indicate absence of depression, 8-15 points indicate mild depression, 16-28 points indicate moderate depression, and 29 points indicate major depression (Williams 1978).

**The Wisconsin Card Sorting Test (WCST):** As an effective neuropsychological test for the evaluation of frontal lobe functions, the WCST is one of the leading tests used to measure executive functions (Heaton 1981, Karakaş 2004) by evaluating the problem-solving ability and the ability to change the problem solving strategy in accordance with the varying conditions. Depending on the given feedback regarding the accuracy of the behaviour , the patient's ability to make the classification principle, ability to pay attention selectively, ability to use this principle as long as it is valid, and ability to change the setup when it causes wrong behaviors are measured. It is considered to be sensitive especially for measuring functions of the DLPFC (Rogers et al. 2004).

In the present study, the brief computerised version of the WCST (The Psychology Experiment Building Language (PEBL)-Berg's "Wisconsin" Card Sorting Test) was used (Bergs 1948, Mueller et al. 2014). The instructions in the PEBL battery were entered into the software and translated to the Turkish.language. When this format was compared to the classical version which includes 64 response cards, both applications were determined to yield similar results (Fox et al. 2013).

**$^{1}$H-MRS Application:** Proton magnetic resonance spectroscopy ($^{1}$H-MRS) was preferred in this study by on the grounds of being a non-invasive and easily applicable method not containing ionizing radiation, not having known side effects, providing in vivo quantitative biochemical data, facilitating the search for correlations between brain metabolism and the brain (Kwock 1998).

$^{1}$H-MRS scan was conducted at the Radiology Department of Pamukkale University by a qualified technician using a standard head coil with the 1.5 Tesla Magnetic Resonance Spectroscope (GE Philips Ingenia; Philips Medical System, Best, Netherlands). At first, the guiding whole brain image was taken in the sagittal plane in order to determine the sequential positions and to evaluate the orientation. Then, in coronal plane, magnetic resonance protocol was completed with the T2-weighed "fastspin echo (FSE)" sequence by using a thickness of 5 mm and "Time of Repetition/ Time of Echo; 5200/110, Field of View; 14, FOW 230/260" parameters. $^{1}$H-MRS was performed by the single voxel ($^{1}$H-voxel) technique that was placed on each dorsolateral prefrontal cortex region (Figure 1). The examined volume of interest (VOI) was placed a radiologist experienced in functional neuroanatomy manually and visually in order to ensure that it mainly covers the brain tissue associated with the identified regions. The chemical shift selective pulse (CHESS) method was used to suppress waterborne signals. This was followed by using the point-resolved spectroscopy (PRESS) technique which localiz the spectroscopy volume (TR/TE: 5200- 144). As a result, long TE-period spectra were obtained from the VOI in the right and left dorsolateral prefrontal cortex region

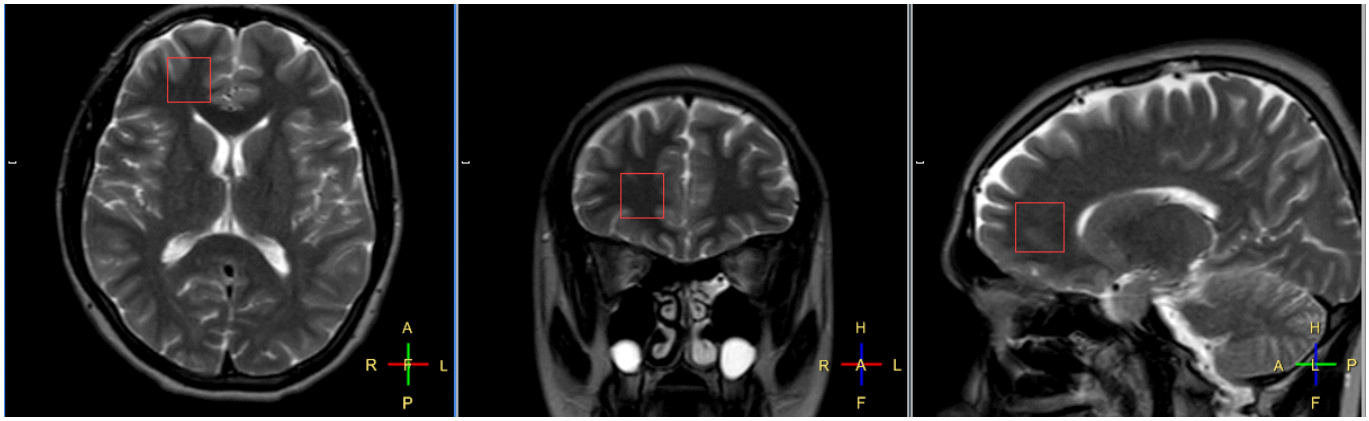


**Figure 1.** 1H-MRS with Single Voxel Placed in Every DLPFC Region

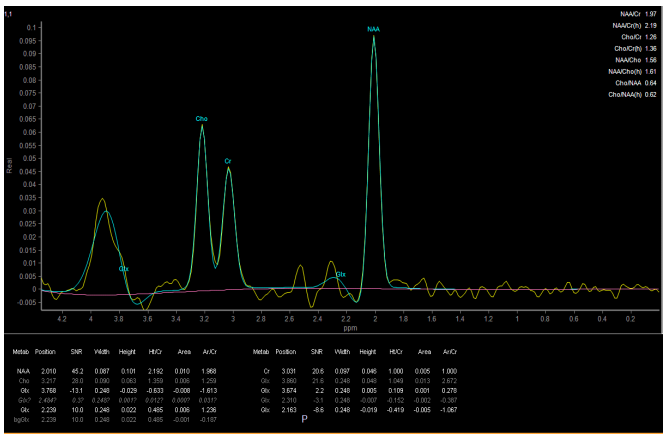


**Figure 2.** Metabolite Values of the DLPFC Region Obtained by 1H-MRS and the Graphical Illustration of the Peak Values

and the metabolite ratios, obtained by 'General Electric software spectral analysis program", were evaluated (Figure 2).

In the $^{1}$H-MRS analyses, levels of the compounds containing N-Acetyl Aspartate (NAA), Cholin (Cho), Glutamate+Glutamine (Glx) and Creatinine (Cr) in DLPFC were investigated and NAA/Cr, Cho/Cr ratios were calculated. Total creatinine was accepted as a reference value based on the assumption of the constancy of its resonance giving information on the energy metabolism and that it was not affected by various pathologies (Monkul et al. 2004).

### Statistical Analyses

SPSS 22.0 package program was used for statistical analysis of the study data. Continuous variables were expressed as ''mean ± standard deviation", and categorical variables were expressed as ''number and percentage". Significance test for the difference between two mean values was used for the comparison of independent group differences when the parametric test assumptions were met. The Mann-Whitney U test was used for the comparison of independent group differences when the parametric test assumptions were not met. In dependent group comparisons; significance test for the difference between two mean values was used when the parametric test assumptions were met and the Wilcoxon Signed Rank Test was used when the parametric test assumptions were not met. Spearman Correlation Analysis was used to examine the correlations between continuous variables and the Chi-square analysis was used to examine the differences between categorical variables.

## RESULTS

The participating group of 30 patients with moderate and severe MDD comprised 23 (76.7%) females and 7 (23.3%) males with a mean group age of 38.8 (±11.1) years and a mean educational period of 9.7 (±3.34 years). The mean age and educational period of the 30 control individuals were, respectively, 33.4 (± 8.7) and 12.1 (± 4.48) years. The two groups did not differ significantly with respect to age, gender and marital status (p: 0.76**, p: 0.26*, p: 1*, respectively; *Chi-Square test, **Mann-Whitney U test). On the other hand, education level in the control group was found significantly higher than in the patient group (p:0.009, Mann-Whitney U test)

Before treatment for depression, the mean total HAM-D score of 30 patients was 23.39 (±4.95); and after treatment, the HAM-D score of the 20 patients, who continued with the test program, was 2.14 (±1.88). Depression level of 5 MDD patients was severe with a mean score of 30.8±1.09; and the mean score of 25 patients was 21.8 (±4) indicating moderate depression. After the treatments with antidepressant agents, severity of depressive symptoms, based on the mean HAM-D score, was significantly decreased (z: 4.631, p<0.001, Wilcoxon test).

**Table 1.** Comparison of the Pre-treatment Neurochemical Metabolite Levels in the Right DLPFC of the Patient and Control Groups

| Metabolites | Patient group S:30 mean±SD | Control group S:30 mean±SD | P |
|---|---|---|---|
| NAA | 0.0848±0.01946 | 0.0860±0.01791 | 0.810* |
| Cho | 0.0559±0.01363 | 0.0556±0.01456 | 0.461* |
| Cr | 0.0418±0.00847 | 0.0445±0.01205 | 0.320* |
| Glx | 0.0158±0.01152 | 0.0134±0.01037 | 0.371** |
| NAA/Cr | 2.0273±0.18092 | 1.9833±0.33692 | 0.203** |
| Cho/Cr | 1.3377±0.18095 | 1.3507±0.24804 | 0.620** |

*T-test, **Mann- Whitney U test

The mean age of depression onset in the patient group was 36.4 (± 11.6) years, corresponding to the middle age period with 70% of the patients being in the the first depressive episode. The group mean disease duration was 9.9 (± 8.26) years. Majority of the patients (n= 24, 80%) did not have a history of depression in the first degree relatives. Statistically significant differences were not found between the patient group and the control group in terms of the right DLPFC NAA, Cho, Cr, Glx, NAA/Cr, Cho/Cr levels when measured before the treatment (*T-test, **Mann- Whitney U test, p>0.05) (Table 1).

Also, statistically significant differences were not found between the patient group and the control group in terms of the left DLPFC NAA, Cho, Cr, NAA/Cr, Cho/Cr levels when measured before the treatment (*T-test, **Mann- Whitney U test, p>0.05) (Table 1). The Left DLPFC Glx values of the patient group were lower than those of the control group before the treatment (*T-test, **Mann- Whitney U test, p<0.05) (Table 2).

In the assessment of the WCST results prior to the treatment protocol, statistically significant differences were found between 30 patients and 30 healthy controls in the number of categories completed, the total number of correct responses, the total number of false responses, the number of non-perseveration errors, the number of trials in the first category,

**Table 3.** Comparison of the Pre-treatment WCST Scores of the Patient and the Healthy Control Groups

| WCST | Patient group (S:30) Mean ± SD | Control group (S:30) Mean ± SD | Analysis P |
|---|---|---|---|
| Number of defined categories | 2.3±1.18 | 3.4±0.97 | 0.0001* |
| No. of Total correct answers | 47.9±4.2 | 52.3±3.39 | 0.0001* |
| No. of Total false answers | 16.1±4.2 | 11.6±3.39 | 0.0001* |
| Number of persevering responses | 16.5±7.46 | 19.6±4.89 | 0.088* |
| Number of persevering errors | 7.33±4.05 | 7.66±3.33 | 0.894* |
| Non-persevering errors | 8.76±6.09 | 3.96±2.6 | 0.0001* |
| No. of trials in the first category | 19.1±14.6 | 14.5±8.19 | 0,043* |
| Setup Failure Score | 1.66±1.24 | 1.03±1.12 | 0,031* |
| No. of Conceptual Level Responses | 42.9±6.16 | 49.2±4.1 | 0.0001* |
| Ratio of Conceptual Level Responses | 67.0± 9.6 | 76.8±6.4 | 0.0001* |

*Mann Whitney U test

the failure to maintain the setup score, and the number and percentage of conceptual level responses treatment (Table 3). The pre-treatment WCST results of the patients indicated a worse performanceby this group than the control group.

After the treatments, comparisons of the metabolite levels determined by $^{1}$H-MRS scans and WCST scores were based on the data of the 20 patients, who completed the study, since the post treatment control data could not be obtained from 10 patients with depression of moderate severity .

Statistically significant differences were not found between the patient group and the control group in terms of the right DLPFC NAA, Cho, Cr, Glx, NAA/Cr, Cho/Cr levels measured after the treatment (*T-test, **Mann- Whitney U test, p>0.05) (Table 4).

**Table 2.** Comparison of the Pre-treatment Neurochemical Metabolite Levels in the Left DLPFC of the Patient and Control Groups

| Metabolites | Patient group S:30 mean±SD | Control group S:30 mean±SD | P |
|---|---|---|---|
| NAA | 0.0836±0.01844 | 0.0909±0.2145 | 0.075** |
| Cho | 0.0713±0.09112 | 0.0690±0.05842 | 0.196** |
| Cr | 0.0404±0.00655 | 0.0441±0.00998 | 0.096* |
| Glx | 0.0087±0.00638 | 0.0128±0.00841 | 0.044** |
| NAA/Cr | 2.0627±0.21624 | 2.0593±0.20760 | 0.836** |
| Cho/Cr | 1.3553±0.17876 | 1.3163±0.14447 | 0.357* |

*T-test, **Mann- Whitney U test

**Table 4.** Comparison of the Post-treatment Neurochemical Metabolite Levels in the Right DLPFC of the Patient and the Control Groups

| Metabolites | Patient group S:20 mean±SD | Control group S:30 mean±SD | P |
|---|---|---|---|
| NAA | 0.0880±0.02047 | 0.0860±0.01791 | 0.719* |
| Cho | 0.0571±0.01413 | 0.0556±0.01456 | 0.705* |
| Cr | 0.0439±0.01261 | 0.0445±0.01205 | 0.855* |
| Glx | 0.0101±0.00613 | 0.0134±0.01037 | 0.411** |
| NAA/Cr | 2,047±0.26697 | 1.98±0.33692 | 0.188** |
| Cho/Cr | 1.3125±0.14678 | 1.3507±0.24804 | 0.898** |

*T-test, **Mann- Whitney U test

**Table 5.** Comparison the Post-treatment Neurochemical Metabolite Levels in left DLPFC of the Patient and the Control Groups

| Metabolites | Patient group (post-treatment) S:20 mean±SD | Control group S:30 mean±SD | P |
|---|---|---|---|
| NAA | 0.0900±0.02112 | 0.0909±0.02145 | 0.362** |
| Cho | 0.0562±0.01214 | 0.0690±0.05842 | 0.378** |
| Cr | 0.0438±0.01123 | 0.0441±0.00998 | 0.351** |
| Glx | 0.0117±0.00793 | 0.0128±0.00841 | 0.590** |
| NAA/Cr | 2.0605±0.19592 | 2.0593±0.20760 | 0.677** |
| Cho/Cr | 1.3040±0.17313 | 1.3163±0.14447 | 0,736* |

*T-test, **Mann- Whitney U test

After the treatments, the left DLPFC Glx level was increased and the statistical difference observed between the patient and the control group before the treatments was eliminated (Table 5).

When the results of the patients on the WCST before and after treatments were compared, statistically significant differences were determined in the scores and percentages of conceptual level responses, the number of categories completed, the total number of truths, the total number of errors, and the number of non-perseveration errors. It was seen that there was an increase in the WCST performance of the MDD patients after the treatment (Table 6).

**Table 6.** Comparison of the WCST Scores of the Patient Group Before and After the Treatment

| WCST | Patient group Pre-treatment (S: 20) Mean ± SD | Patient group Post treatment (S: 20 Mean ± SD | Analysis P |
|---|---|---|---|
| Number of defined categories | 2.05±1.14 | 2.9±0.85 | 0.004** |
| No. of Total correct answers | 47.15±4.08 | 52.6±3.01 | 0.0001** |
| No. of Total false answers | 16.8±4.08 | 11.4±3.01 | 0.0001** |
| Number of persevering responses | 15.5±8.72 | 19.2±3.48 | 0.419** |
| Number of persevering errors | 7.1±4.67 | 7.05±2.18 | 0.966* |
| No. of non-persevering errors | 9.75±6.5 | 4.35±2.75 | 0.03** |
| No. of trials in the first category | 20.0±17.2 | 13.9±8.33 | 0.76** |
| Setup Failure Score | 1.75±1.33 | 2.15±1.13 | 0.352* |
| No. of Conceptual Level Responses | 41.4±5.68 | 49.25±3.94 | 0.0001* |
| Ratio of Conceptual Level Responses | 64.6±8.89 | 76.9±6.16 | 0.0001* |

*T-test, **Wilcoxon test

### Correlation Analyses

Statistically, there was a moderate negative correlation between disease duration and the right DLPFC NAA/Cr ratio measured before the treatments (r:-0.447, p:0.013). Statistically weak positive correlations were determined between the number of depressive episodes and the right and the left DLPFC Cho and Glx values observed before the treatment (r:0.365, p:0.047; r:0.389, p:0,041; r:0.400, p:0.029; r:0.365, p:0.047, respectively- Spearman correlation analysis). Also a weak positive correlation was observed between the number of depressive episodes and the left DLPFC Cho/Cr ratio (r:0.390, p:0,033, Spearman correlation analysis). Moderate positive correlations were found between the number of depressive episodes and the left DLPFC Cho and Cho/Cr ratio after the treatment (r:0.502, p:0.024; r:0.526, p:0.017, respectively; Spearman correlation analysis).

Correlation analysis between the pre-treatment scores on the WCST and the educational level of the patient group showed a moderate negative correlation between the number of trials in the first category and the educational level (r:-0.423, p:0.02; Spearman correlation analysis). After the treatments a moderate positive correlation was determined between disease duration and the WCST performance on failure to maintain setup score (r:0.456, p:0.043; Spearman correlation analysis). Correlations of the change detected in the left DLPFC Glx level before and after the treatment with the WCST data could not be demonstrated.

## DISCUSSION

In this research the correlations between DLPFC neurochemistry and executive functions and their differences from normal controls were investigated in patients with moderate and severe MDD, aged between 18 and 60 years and consisting mostly of females. Glx levels of the left DLPFC metabolites were found to be significantly lower when compared to healthy subjects before the antidepressant treatments, and an increase was observed after the treatments. Other metabolite levels were not found to differ from those of the healthy controls and changes were not observed after the treatments. The performances of the patients on the WCST with respect to abilities of changing strategy according to the conditions and to solve problems were lower in comparison to the healthy individuals but there was significant improvement with the clinical recovery observed after the treatments. Statistical ly sifnificant correlations were not found between neurochemical changes and the executive functions.

In this study the presence of 23 middle-aged females among the 30 patients was consistent with the data of most epidemiological studies reporting that MDD prevalence is

doubled among middle aged individuals of especially female gender (Angst 1992, Köroğlu 2007, Tot et al. 2001).

Among the brain imaging method used in research on the pathophysiology of MDD, the $^{1}$H-MRS is considered as a valuable method, despite the controversial results obtained, in not having ionising radiation and enabling non-invasive analysis of brain neurochemistry (Kwock 1998).

Quite different results are reported in the studies using $^{1}$H-MRS on depressive patients. Next to the studies correlating neurodegenerative processes with decreases in the levels of neurometabolites such as NAA, Cho and Cr in the prefrontal (Gruber et al. 2003, Portellaet al. 2011, Wang et al. 2012), there are also studies that do not report any changes in the metabolite levels (Neryet al. 2009, Henigsberget al. 2011, Oğuzhanoğluet al. 2014). NAA is a compound produced in neuronal mitochondria that shows neuronal integrity and a healthy neuronal function and decreased levels have been associated with hypofrontality (Cecil et al. 2002, Sadocket al. 2007). Decreased neuronal density or neuronal dysfunction and lower than normal NAA/Cr ratios were reported in the prefrontal cortex of patients with MDD (Wang et al. 2012, Gönül et al. 2006, Jiaet al. 2015).

In the present study, the NAA levels and NAA/Cr ratios investigated in the DLPFC areas of nonchronic moderate and severe MDD patients did not sifnificantly differ from those of the healthy group. Onset of depression was in the middle ages of the patients, 70% of the patients were in the first depressive episode and, duration of the disease was less than one year, hence eliminating any chronicity. These data and the observed NAA metabolite levels suggested that there was not any impairment in the neuronal functions and that neurodegenerative change might not have yet begun. It has been reported that variables such as age, severity of disease, number of periods, and duration may affect the research results (Neryet al. 2009, Oğuzhanoğluet al. 2014). Increased age, recurrent and persistent depression are considered to be an indicator of the decrease in neuroplastic capacity because they cause a decrease in the NAA levels (Portellaet al. 2011, Michael et al. 2003, Husarovaet al. 2012). In the present study, negative correlations were found between patient age and the right and left DLPFC NAA/Cr and left DLPFK NAA values before treatment, and between the disease duration and the right DLPFC NAA/Cr ratio. These data suggest that expected cellular abnormalities and neurodegenerative changes in depression may develop in older ages as the disease is prolonged and becomes chronic.Although it has been argued that NAA is an indicator of neuroplasticity and that antidepressant treatment may increase the levels; it was demonstrated in the study by Janovicet al. (2014) that NAA levels did not change in the first depressive episode and in moderate depression cases after antidepressant treatment.

Increased levels of choline, which is the product of the phospholipid metabolism in the cell membrane, giving information about cell density, proliferation and membrane,functions, indicate an increase in membrane anabolism-catabolism cycle and the intensity of cell synthesis (Monkul 2004, Kusumakaret al. 2001). Given the high concentration of choline in oligodendrocytes, it has been suggested that abnormal choline levels may be seen as a reflection of oligodendrocyte changes in the prefrontal cortex of depressive patients that may lead to disorders of myelinization and neuronal networks (Jiaet al 2015). There are works reporting that choline levels are increases with the disease process (Kumar et al. 2002). Portella et al. (2011) reported that prefrontal cortex choline levels in recurrent and chronic depression patients were higher than in the first depressive episode patients. There are also studies reporting that there are not any abnormal changes in prefrontal cortex choline metabolite levels in depression (Kaymak et al 2009, Neryet al. 2009, Oğuzhanoğluet al. 2013). In the present study, the right and left DLPFC choline levels and the Cho/Cr ratios were found to be similar to those in the control group. In the correlation analysis between the number of depressive episodes and the metabolite levels, the weak positive relationship between the number of depressive episodes and the right/left DLPFC choline values and the left DLPFC Cho/Cr ratios and suggest the possibility of increased choline levels with recurrence and chronicity.

The decrease in the Cho/Cr ratio in the same region (Wang et al. 2012, Jiaet al. 2015) shows that the decrease in the choline levels seen in the early stage of the disease may increase with recurrence and chronicity of the disease and this may specify neuronal membrane degeneration, loss of myelin and disturbance in signal conduction system.

Glutamate is the primary stimulatory neurotransmitter of the central nervous system. Since antiglutamatergic agents are beneficial in the treatment of depression, glutamine/glutamate ratio has recently gained attention. The role of glutamate in neuronal cell death in connection with overstimulation of NMDA receptors and that this stimulation can be induced by stress is being debated as one of the possible causes of depression (Ceylan et al. 2001). Glutamatergic system changes resulting from functional abnormalities and reduction of glial cells are important for explaining the pathophysiology of depression (Coyle et al. 2000). In most of the $^{1}$H-MRS research articles, the ''regions of Glx" correspond to glutamate and glutamine presence. The contribution of glutamatergic neurotransmission to the depressive symptoms supports the role of glutamatergic dysfunction in the pathogenesis of mood disorder. In the absence of any change in the glutamate levels, a reduction in absolute Glx values has been reported to indicate the possible modulator role of astrocytes in the pathophysiology of depression (Arnone et al. 2015).

In the current study, the level of left DLPFC Glx was found to be lower in the patient group before the treatment as compared to the control group and may indicate the loss of glutamatergic system activity in the affected brain area. Changes in the glutamate metabolism suggest that there may be a defect in glial cell functions.

In MDD, abnormally low Glx values were reported in the anterior cingulate cortex (Chen et al. 2014, Auer et al. 2000), the DLPFC (Michael et al. 2003), the ventromedial prefrontal cortex (Portellaet al. 2011) and in the hippocampal regions (Block et al. 2009). The low level of Glx detected in depressive patients suggests hypometabolism in the affected brain region. It is considered that low Glx may be a metabolic marker for functional changes.

Decreased Glx metabolite levels in the left DLPFC observed in patients with severe and chronic depression suggested that psychomotor retardation and depressive mood symptoms are particularly associated with DLPFC dysfunction and that low Glx in this region may be a biomarker (Michael et al. 2003).

In the present study, the difference seen in the Glx levels that was not detected in the levels of the other metabolites, suggests that the clinics of moderate and severe MDD may indicate early metabolic changes. After antidepressant treatment, the Glx metabolite level in the left DLPFC was observed to increase together with clinical recovery. This increase, that can be accepted as the neurochemical reflection of clinical recovery, indicates the correlation of the functions of Glx metabolites in the left DLPFC and the depression symptom cluster. However, this change could not bet statistically correlated with the HAM-D score, the WCST results, course characteristics of the disease before and after the treatments and the sociodemographic data. Therefore, there are not any data to evince that glutamate change may be related to the any of the characteristics of depression clinics. Previous studies have shown that low Glx levels detected in DLPFCs of depressive patients reach normal levels with treatment and that neuronal damage can be repaired by treatment (Michael et al. 2003, Chen et al. 2014). Long-term antidepressant use was implicated in the structural and functional changes in the glutamatergic synapses of the frontal brain (Mahurin et al. 2006, Chen et al. 2014). Our findings suggest that the glutamatergic system has an important role in the neurobiology and treatment of moderate and severe MDD.

Possibile correlation of cognitive dysfunction with hypometabolism, neuronal loss, and atrophy in DLPFC in depressive patients has been reported (Drevetset al. 1997, Rajkowska et al. 1999). The factors of age, educational level, basic cognitive capacity of the patient, age of onset of the disease, subtype of MDD, presence of psychiatric and medical comorbidities, duration and the frequency of the disease have effects on the extent of impairment in neurocognitive functions (McIntyre et al. 2013).

Failure in the WCST increases with increasing disease duration. As the exposure to the disease process is prolonged, more cognitive functions may be affected. The cognitive impairment observed in this study in the active phase of the disease showed improvement with the reduction in the depressive symptoms after the antidepressant treatment.

Glutamateis an excitatory neurotransmitter known to have a role in learning, (Ceylan et al. 1999), memory, and other cognitive functions The increase observed in this study in glutamate level after antidepressant treatment of the patient group, may have indicated the neurochemical basis of cognitive function change. However, correlation of the change detected in the Glx level with the WCST data before and after the treatment could not be shown in the current study.

The primary limitations of the study stem from the significantly higher educational level of the healthy controls as compared to the patient group because of the effect of educational level on the WCST performance; and also by using only the WCST, out of the available neuropsychological tests, to evaluate the cognitive functions. which are not considered to be a single structure and evaluation by a single test is not sufficient. Another limitation of the study may be due to using $^{1}$H-MRS. Despite being a non-invasive scan method, it is subject of debate on grounds of its controversial results; and the use of the 1.5 Tesla Magnetic Resonance Device involves limitations in technical respects. Despite the demonstration of its inefficiency, having to use the PRESS method under the conditions of equipmentation in the research laboratory in the measurement of glutamate and glutamine is another limitation of the study (Henry et al. 2011).

In conclusion, the study shows that the neurocognitive functions are impaired in cases of non-chronic moderate and severe MDD, and that they improve after tretment with antidepressants. The lowered Glx level in the left DLPFC region before antidepressant treatment, is found to be increased together with clinical recovery after treatment.

For more reliable results, long-term follow-up studies including MDD patients with different clinical features and using the $^{1}$H-MRS scan technique with high field strength such as provided by the 3-4 Tesla equipment may lead to the identification of specific markers for the neurobiology and treatment of depression.

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