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Effect of Clozapine on Proton Magnetic Resonance Spectroscopy Findings in Hippocampus

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SUMMARY

Objective: The purpose of this study was to investigate the effect of clozapine on proton magnetic resonance spectroscopy (^{1}H-MRS) findings in hippocampus in patients with schizophrenia. In addition, the relationship between the change in ^{1}H-MRS findings and the change in psychopathology and neurocognitive functions was evaluated.

Method: Patients with schizophrenia (n=16) were assessed with the Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression Scale (CGI), a neurocognitive test battery and ^{1}H-MRS at baseline, and twelve weeks after the initiation of clozapine. Healthy controls (n=8) were assessed once with a neurocognitive test battery and ^{1}H-MRS. Bilateral multivoxel and left single voxel NAA/Cr, Cho/Cr, MI/Cr was calculated in the hippocampi.

Results: After 12 weeks of clozapine treatment, PANSS and CGI scores decreased; immediate recall, cumulative learning subtests of the Rey Auditory Verbal Learning Test, Category Verbal Fluency Test and Wechsler Memory Scale's visual reproduction delayed subtest scores increased significantly. Compared with healthy controls and patients after clozapine, hippocampi multivoxel and single voxel NAA/Cr, Cho/Cr, MI/Cr ratios were not different in patients before clozapine. No significant correlations between change in ^{1}H-MRS metabolite ratios and change in psychopathology, neurocognitive functions were detected.

Conclusion: This study is the first longitudinal study to investigate the effect of clozapine in hippocampus with ^{1}H-MRS. There were no significant changes in ^{1}H-MRS findings in hippocampi after twelve weeks of clozapine treatment. Clozapine's effect in hippocampus should be investigated further in longer follow up studies with larger samples to reach a final conclusion.

Keywords: Schizophrenia, hippocampus, clozapine, magnetic resonance spectroscopy

INTRODUCTION

Schizophrenia is a chronic disease with positive, negative, and cognitive symptoms (Kesby et al. 2018). Although not yet fully elucidated, there are many biochemical, genetic and imaging studies to determine the mechanisms underlying the pathophysiology of schizophrenia (Wong et al. 2003, Khandaker et al. 2015). These studies have demonstrated rather than a dysfunction limited to certain regions of the brain, several interrelated brain regions are affected, activity of numerous neurotransmitters change, and impairments in neural network occur in schizophrenia (Schmitt et al. 2011).

The hippocampus, with an important role in the regulation of cognitive and emotional functions, is a region of the brain that is involved in the pathophysiology of schizophrenia (Steinke et al. 2017). Postmortem studies on schizophrenia patients show morphological changes in the size, shape, and arrangement of neurons and synaptic abnormalities such as decreases in presynaptic markers and dendritic spine density in the hippocampus compared with healthy controls (Harrison 2004). Reduction of the hippocampal volume in patients with schizophrenia as compared to healthy controls is a common finding in structural imaging studies (Heckers 2001). Functional imaging studies have shown a decrease in hippocampal glucose

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metabolism at resting state and an increase in hippocampal blood flow during auditory hallucinations (Heckers 2001, Kühn and Gallinat 2011). Morphological changes detected in the hippocampus and the results of structural and functional imaging studies indicate that the hippocampus is involved in the pathophysiology of schizophrenia (Asami et al. 2012, Kraguljac et al. 2013, Nenadic et al. 2015).

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a commonly used neuroimaging technique for examining the brain in schizophrenia. It enables the in vivo measurement of metabolites such as N-acetyl aspartate (NAA), creatinine (Cr), choline (Cho), myo-inositol (MI), and glutamate (Zhu and Barker 2011). The NAA level is considered to provide information about neural integrity; choline levels are related to formation or destruction of cell membrane and myelin; myoinositol levels indicate astroglial proliferation and the creatinine level, which is considered to provide information about energy metabolism, is used as a reference value while calculating metabolite levels (Zhu and Barker 2011).

Studies employing ^{1}H-MRS have shown changes in metabolite levels or ratios in various regions of the brain in schizophrenia (Schwerk et al. 2014). Although the hippocampus is a region of the brain that has been frequently investigated by H-MRS, the results of these studies are inconsistent. In addition to the studies reporting reduced NAA levels in the hippocampal gray matter of patients with schizophrenia as compared to healthy controls (Steen et al. 2005, Schwerk et al. 2014), there are also studies reporting the lack of these differences (Hutcheson et al. 2012, Meyer et al. 2016, Singh et al. 2018). Similarly, there are reports of reduced or unaltered levels of hippocampal myoinositol and glutamate as assessed by ^{1}H-MRS (Kegeles et al. 2000, Steinke et al. 2017, Singh et al. 2018).

Proton magnetic resonance spectroscopy studies on the effect of antipsychotic treatment of schizophrenia on brain regions are generally cross-sectional. In a study by Bertolino et al. (2001), it was shown that antipsychotic treatment increased the NAA levels in the dorsolateral prefrontal cortex (DLPFC) but not in the hippocampus. In another study it was found that NAA levels in the anterior cingulate gyrus were higher in patients with schizophrenia using atypical antipsychotics as compared to patients on typical antipsychotics (Braus et al. 2002). There are few follow up studies which investigated effects of antipsychotics on metabolite levels in the brain. The ^{1}H-MRS study by Kraguljac et al. (2019) did not determine any change in hippocampal metabolite levels after 6 weeks of risperidone treatment. In ^{1}H-MRS follow up studies with 12-months of quetiapine (Grošić et al. 2014) and 24-months of haloperidol and quetiapine treatments (Bustillo et al. 2008), there were no change in NAA levels measured in different regions of the brain.

Clozapine is the prototype of the atypical antipsychotic agents and is the most effective antipsychotic known in treatment-resistant schizophrenia (Kane et al. 1988, McEvoy et al. 2006). Preclinical studies have shown that clozapine has effects on neuroplasticity and myelin integrity (Critchlow et al. 2006, Xu et al. 2010). Clozapine effects on the brain were investigated by magnetic resonance imaging, single-photon emission computed tomography (SPECT), positron emission tomography (PET), and ^{1}H-MRS. Structural imaging studies showed that clozapine could reverse the increase in basal ganglia volume caused by typical antipsychotics and that total gray matter brain volume increased and white matter volume decreased in patients on clozapine compared with healthy controls (Garcia et al. 2015). Functional imaging studies showed that clozapine treatment was associated with changed blood flow in various regions of the brain and mainly in the caudate and frontal lobes (Ertuğrul et al. 2009, Garcia et al. 2015).

On the other hand, the number of ^{1}H-MRS studies that investigated the effects of clozapine are limited in the literature. In a cross-sectional study, patients with schizophrenia who used atypical antipsychotics such as clozapine, olanzapine and risperidone were compared with healthy controls, and there was no difference in NAA levels in the thalamus, left frontal and temporal lobes (Szulc et al. 2007). In a follow up study on mice, there was no change between the studied groups in terms of the NAA/Cr ratio in mouse brains after 1-week of administration of haloperidol, olanzapine or clozapine, whereas the NAA/Cr ratio significantly increased compared with the pretreatment value in the olanzapine group (Lindquist et al. 2000). In the first follow up study investigating changes in ^{1}H-MRS induced by clozapine in patients with schizophrenia, left DLPFC was chosen as the region of interest, and it was shown that the NAA/Cr ratio increased as a result of 8 weeks of clozapine treatment (Ertuğrul et al. 2009). This result was attributed to the positive effects of clozapine on the integrity and functions of the neural circuitry, and could be an evidence for the neurotrophic effect of clozapine (Ertuğrul et al. 2009).

In the literature, there was not a follow up study on ^{1}H-MRS imaging of the effect of clozapine treatment in the hippocampus which has an important role in the pathophysiology of schizophrenia. The hippocampus is a region where neuroplastic processes were investigated intensively (Kang et al. 2016). To understand the effects of clozapine on neuroplasticity, it is important to examine this region by ^{1}H-MRS.

The aim of the present study was to assess the effects of 12 weeks of clozapine treatment on ^{1}H-MRS findings in hippocampus, and to investigate the relationship between the changes in metabolite levels and the changes in psychopathology and cognitive functions. The first hypothesis of the study was that before clozapine treatment the metabolite ratios measured by ^{1}H-MRS and cognitive functions would be lower in the patient group than in the control group. The second hypothesis was that after 12 weeks of clozapine treatment, there would be an improvement in cognitive functions and an increase in metabolite ratios measured by ^{1}H-MRS and that there would be a correlation between improvement in cognitive functions and changes in ^{1}H-MRS metabolite ratios.

METHOD

Participants

This study was conducted at the Department of Psychiatry in the Hacettepe University School of Medicine. Sixteen outpatients diagnosed with schizophrenia based on the DSM-IV criteria who were decided to be treated with clozapine due to treatment refractoriness to previous antipsychotics, and 8 healthy controls matched to the patients on age, sex, and education level were included in the study. Schizophrenia diagnoses were confirmed in the patient group and Axis I disorders were excluded from the control group with the SCID-I, the Structured Clinical Interview for DSM-IV. Patients with history of alcohol dependency, head trauma, and major neurological or medical disorder were excluded. This study was designed as part of a comprehensive follow up study on the effect of clozapine treatment on brain imaging results. Part of this study was previously published (Ozcelik-Eroglu et al. 2014). Approval coded 'LUT 10/02' of the Hacettepe University Faculty of Medicine Ethics Committee was obtained for this study.

Procedure

Before initiation of clozapine treatment, psychopathology was evaluated by the Positive and Negative Syndrome Scale for Schizophrenia (PANSS) and Clinical Global Impression (CGI) scale, neurocognitive functions were evaluated with Digit Span Test, Verbal Fluency Test, Auditory Consonant Trigram Test, Trail Making Test, Rey Auditory Verbal Learning Test (RAVLT), and Wechsler Memory Scale's (WMS) visual reproduction test, and ^{1}H-MRS was performed. Psychopathology and neurocognitive functions were reevaluated after 12 weeks of clozapine treatment, and ^{1}H-MRS was repeated. Healthy controls were evaluated once by neurocognitive test battery and ^{1}H-MRS.

Assesment of Psychopathology

PANSS is a semi-structured interview containing 30 items that rate the symptom severity of schizophrenia and other mental disorders on a 7-point Likert-type scale. Each item is rated from 1 (no symptoms) to 7 (very severe). The total score of 30 items is recorded as the PANSS total score. The PANSS was developed by Kay et al. (1987) primarily to evaluate positive and negative symptoms in schizophrenia; and its validity and reliability after translation to the Turkish language was carried out by Kostakoğlu et al. (1999).

CGI scale generally assesses the severity of any disease or the improvement in its symptoms. Clinicians rank the severity of the disease or the degree of improvement between 0 (not sick) and 7 (very severely sick) on the basis of their general experience with the disease (Guy 1976).

Assessment of Neurocognitive Functions

Digit Span Test, a subtest of the Wechsler Memory Scale (WMS) comprises of two parts (Wechsler 1987). The "Forward" section evaluates attention and the "Backward" section evaluates working memory. In the latter section, the patient is asked to repeat the mentioned numbers in the reverse order. The validity and reliability of the Turkish language version was performed by Karakaş et al. (1996).

Verbal Fluency Test is performed to measure the flow and speed of verbal production and the ability to develop strategies and organize thoughts and working memory. This test comprises of two parts. In the word fluency test, the patient is expected to recall words beginning with certain letters; and in the category test, the patient is expected to generate the words from a specific category in the prescribed time (Lezak 1995). Standardization of the test in the Turkish language was done by Bingöl et al. 1994. The total number of recalled words was calculated in the word fluency test, and the total number of words in each category was calculated in the category test.

Auditory Consonant Trigram Test aims to measure short-term memory, divided attention, and information processing capacity in adults (Peterson and Peterson 1959). The test evaluates working memory. The validity and reliability of the test after translation to the Turkish language was conducted by Anıl et al. (2003). The total number of correctly recalled letters is calculated.

Trail Making Test evaluates attention speed, mental flexibility, visual scanning, and motor speed (Spreen and Strauss 1998). In part A, points 1–25 are combined with a single continuous line, and in part B, a letter and a number are alternately combined. Time score is calculated for each section. Standardization of the test in the Turkish language was done by Cangöz et al. (2007).

Rey Auditory Verbal Learning Test (RAVLT) was developed by Rey (1964). The purpose of this test is to evaluate verbal learning and memory. The first list, comprising 15 words, is read to the subject at a rate of 1 word/second and the subject is then asked to say the words that are recalled from the list (free recall). The first stage (RAVLT-1) measures immediate recall to provide information about the subject's attention and working memory. The word list is read to the participant five times, and each time the participant is asked to repeat as many words as she/he can recall. In this way, one's learning skill is evaluated (RAVLT-5). Following this, a different list is read and the participant is again asked to recall the words. In the seventh stage (RAVLT-7) the participant is asked to recall the words in the first list after 20 minutes. This stage evaluates short-term memory. The standardization of the RAVLT in the Turkish language was made by Açıkgöz (1995).

Visual Reproduction Test-1, 2 is included in the most recent revised version of WMS-R. The visual memory subsection evaluates immediate and delayed visual memory. Immediate recalling and recognition of visual stimuli as well as recalling and recognition after 30 minutes are evaluated. The validity and reliability of the Turkish language version was carried out by Karakaş et al (1996).

Proton Magnetic Resonance Spectroscopy

Proton MR spectroscopy (^{1}H-MRS) imaging was performed using a system with 1.5-Tesla magnetic field and 28-mT/m gradient power (Symphony Tim, Siemens, Erlangen, Germany). T2-weighted (A) turbo spin-echo (repetition time (TR)/echo time (TE): 4000/100ms, matrix: 448 × 88) was obtained on three orthogonal planes for guidance. Each of these sequences contains 20 sections with a thickness of 5 mm and a cross-section of 0.5 mm. ^{1}H-MRS was carried out after the evaluation of these images. Single-voxel chemical shift imaging covering the left hippocampus (TR/TE: 1500/35 ms, voxel size: 1.5 × 1.5 × 1.5 cm3) and multivoxel chemical shift imaging covering bilateral medial temporal structures (TR/TE: 1500/135 ms, FOV A-P, R-L:90 mm) were performed. Following the data acquisition, raw data transferred to the Leonardo workstation (Syngo, Siemens) were analyzed with semi-automated spectroscopy software by the radiologist participating in the study. The NAA/Cr, Cho/Cr, and MI/Cr ratios were measured by using single-voxel and bilateral multivoxel imaging in the left hippocampus.

Statistical Analysis

Statistical analyses were made using the SPSS 22.0 package software for Windows. Patients and control groups were compared using the Mann–Whitney U-test for numerical variables and chi-square test for nominal variables.

Mann–Whitney U-test was used to compare the neurocognitive test scores of the patient and control groups before starting clozapine treatment. Normality of the data on PANSS, CGI scale and the ^{1}H-MRS data was assessed using the Shapiro–Wilk test. In the case of normal variance of the measurement parameters before and after treatment, the paired sample t-test was used; otherwise, Wilcoxon-signed rank test was used.

Spearman's correlation analysis was used to evaluate the relationship between the changes in the patient scores on the PANSS, CGI scale and the neurocognitive tests and the changes in ^{1}H-MRS results after 12 weeks of clozapine treatment.

RESULTS

The mean age of the patient and the control groups were, respectively, 34.3±11.25 years (62.5% male and 37.5% female) and 33.88±11.70 years (62.5% male and 37.5% female). Significant intergroup differences were not determined with respect to age (p=1.00), gender (p=1.00) and education level (p=0.88). After 12 weeks of follow up in the patient group, the total and subscale scores on the PANSS (p<0.001) and on the CGI scale (p=0.001) decreased significantly as compared to the scores before clozapine treatment. Demographic and clinical data are shown in Table 1.

The verbal fluency-word fluency (p=0.038); category fluency-animal (p=0.027), category fluency-human (p<0.001),

Table 1. Demographic Characteristics and Clinical Variables

	Patient group (n=16) Mean (SD)	Control group (n=8) Mean (SD)	Statistical analysis p-value
Age	34.4 (11.25)	33.88 (11.70)	1.00[a]
Sex			
Female	6	3	1.00[b]
Male	10	5	
Education (years)	10.94 (3.66)	11.38 (3.20)	0.88[a]
Age of onset	22.25 (7.46)		
Duration of illness (years)	11.62 (9.17)		
Number of hospitalizations	1.81 (1.60)		
Last antipsychotic dose (chlorpromazine equivalent mg/day)	572.54 (268.81)		
Last used drug			
Typical antipsychotics	1		
Atypical antipsychotics	10		
Typical+atypical antipsychotics	3		
No drug	2		
Clozapine dose (mg/g)	317.19 (98.62)		

[a]: Mann–Whitney U-test, [b]: Chi-square test, SD: Standard deviation, p<0.05

Table 2. Clinical Evaluation Results of the Patient (Before and After Clozapine Treatment) and Control Groups

	Control group Mean (SD)	Patient group (BC) Mean (SD)	Patient group (AC) Mean (SD)	Statistical analysis (Control/BC)[a]	Statistical analysis (BC/AC)[b, c]
PANSS					
Total		83.5 (12.57)	56.31 (12.19)		t = 7.087[a] p < 0.001
Positive		22.44 (4.03)	12.44 (3.58)		t = 7.402[a] p < 0.001
Negative		21.06 (4.15)	16.00 (3.86)		t = 5.589[a] p < 0.001
General psychopathology		39.75 (6.94)	28.19 (6.40)		t = 6.829[a] p < 0.001
CGI Scale		4.62 (0.81)	2.81 (0.91)		z = –3.449[b] p = 0.001
Word fluency	28.62 (9.41)	18.44 (10.87)	21.62 (12.74)	z = –2.085 p = 0.038	z = –1.877[c] p = 0.061
Category fluency-animal	19.88 (4.29)	14.81 (6.53)	14.31 (7.31)	z = –2.179 p = 0.027	z = –0.126[c] p = 0.900
Category fluency-human	24.25 (3.01)	15.38 (6.08)	17.19 (5.89)	z = –3.283 p < 0.001	z = –2.000[c] p = 0.045
Category fluency-alternation	9.12 (1.80)	4.69 (2.60)	6.06 (2.72)	z = –3.289 p < 0.001	z = –2.611[c] p = 0.009
Digit span-forward	6.00 (1.77)	5.44 (2.10)	6.00 (2.42)	z = –0.475 p = 0.653	z = – 1.557[c] p = 0.120
Digit span-backward	5.88 (2.90)	4.31 (1.99)	4.62 (1.96)	z = –1.238 p = 0.238	z = –0.914[c] p = 0.361
Digit span-total	11.88 (4.36)	9.75 (3.47)	10.62 (4.10)	z = –0.954 p = 0.350	z = –1.878[c] p = 0.060
Auditory consonant trigram test	47.50 (8.32)	36.25 (10.45)	38.81 (8.40)	z = –2.361 p = 0.016	z = –1.427[c] p = 0.154
RAVLT-immediate recall	6.50 (1.77)	4.75 (1.81)	6.12 (2.03)	z = –2.018 p = 0.045	t = –2.627[b] p = 0.019
RAVLT-learning	11.00 (2.07)	8.25 (2.89)	8.81 (2.93)	z = –2.285 p = 0.023	t = 1.013[b] p = 0.327
RAVLT-total learning	47.50 (10.10)	33.75 (11.91)	39.2 (13.07)	z = –2.483 p = 0.011	t = –3.40[b] p = 0.004
RAVLT-delayed recall	9.12 (3.27)	7.19 (2.74)	6.4 (2.94)	z = –1.392 p = 0.172	t = –1.441[b] p = 0.170
WMS-immediate	31.38 (4.14)	25.88 (9.08)	27.25 (8.97)	z = –1.472 p = 0.153	z = –0.804[c] p = 0.422
WMS-delayed	28.50 (6.52)	18.94 (10.39)	26.25 (10.12)	z = –2.213 p = 0.027	z = –3.184[c] p = 0.001
Trail making test A-duration	31.00 (7.78)	69.50 (65.55)	63.12 (66.78)	z = –2.696 p = 0.006	z = –1.657 p = 0.098
Trail making test B-duration	100.00 (65.06)	135.54 (78.78)	103.00 (57.62)	z = –0.991 p = 0.351	z = –1.836 p = 0.066

[a]: Mann–Whitney U-test, [b]: Paired sample t test, [c]: Wilcoxon signed-rank test, SD: Standard deviation, BC: Before clozapine, AC: After clozapine, PANSS: Positive and Negative Syndrome Scale, CGI: Clinical Global Impression Scale, RAVLT: Rey Auditory Verbal Learning Test, WMS: Wechsler Memory Scale

Table 3. ^{1}H-MRS Results of the Patient (Before and After Clozapine Treatment) and Control Groups

	Controls Mean (SD)	Patients (BC) Mean (SD)	Patients (AC) Mean (SD)	Statistical analysis (p) Control/BC[a]	Statistical analysis (p) BC/AC[b]
Right hippocampal multivoxel NAA/Cr	1.58 (0.48)	1.47 (0.44)	1.38 (0.5)	0.681	0.730
Right hippocampal multivoxel Cho/Cr	0.79 (0.15)	0.77 (0.22)	0.77 (0.27)	0.875	0.861
Right hippocampal multivoxel MI/Cr	0.43 (0.17)	0.38 (0.12)	0.38 (0.13)	0.428	0.916
Left hippocampal multivoxel NAA/Cr	1.6 (0.51)	1.68 (1)	1.34 (0.64)	0.875	0.064
Left hippocampal multivoxel Cho/Cr	0.75 (0.15)	0.82 (0.19)	0.84 (0.2)	0.428	0.875
Left hippocampal multivoxel MI/Cr	0.49 (0.3)	0.41 (0.24)	0.41 (0.2)	0.238	0.753
Left hippocampal single-voxel NAA/Cr	1.37 (0.32)	1.78 (1.12)	1.79 (0.59)	0.570	1.000
Left hippocampal single-voxel Cho/Cr	0.88 (0.22)	0.88 (0.26)	1.01 (0.25)	0.664	0.649
Left hippocampal single-voxel MI/Cr	0.36 (0.08)	0.4 (0.23)	0.47 (0.34)	0.920	1.000

[a]: Mann–Whitney U-test, [b]: Wilcoxon signed-rank test, SD: Standard deviation, NAA: N-Acetyl aspartate, Cr: Creatinine, Cho: Choline, Mi: Myo-inositol, BC: Before clozapine, AC: After clozapine

and category fluency alternation ($p<0.001$) subtest scores; the Auditory Consonant Trigram test scores ($p=0.016$); the RAVLT- immediate recall ($p=0.045$), learning ($p=0.023$), total learning ($p=0.011$) scores; and the WMS-R delayed visual memory scores ($p=0.027$) of the patients were significantly lower and the Trail Making Test part A duration score was significantly higher ($p=0.006$) in comparison to the control group before starting clozapine treatment. In the patient group, the RAVLT-immediate recall ($p=0.019$) and total learning scores ($p=0.004$); verbal fluency category test-human subtest ($p=0.045$); verbal fluency category test-alternation subtest scores ($p=0.009$); and the WMS-R delayed visual memory scores ($p=0.027$) increased significantly after 12 weeks of clozapine treatment compared with before clozapine treatment (Table 2).

In the right and left hippocampi multivoxel measurements, there was no difference in NAA/Cr, Cho/Cr, and MI/Cr ratios between patients before clozapine treatment and control groups. In the patient group, there was no significant change in multivoxel NAA/Cr, Cho/Cr, and MI/Cr ratios in the right and left hippocampus after 12 weeks of clozapine treatment (Table 3).

There was no significant difference in single-voxel NAA/Cr, Cho/Cr, and MI/Cr ratios in the left hippocampus between patients before clozapine treatment and control groups, and patients before clozapine treatment and after 12 weeks of clozapine treatment (Table 3).

Correlations between the changes in the psychopathology and neurocognitive test scores and the change in the ^{1}H-MRS assessed metabolite ratios after clozapine treatment were analysed. A significant correlation was determined between the change in the multivoxel NAA/Cr ratio in the left hippocampus and the change in the CGI score ($r=0.540$, $p=0.038$). There was also a significant correlation between the change in the multivoxel Cho/Cr ratio in the right hippocampus and the change in the Trail Making Test part A score ($r=0.659$, $p=0.010$). However, the changes in the multivoxel NAA/Cr, Cho/Cr, and MI/Cr ratios in the right hippocampus and the multivoxel Cho/Cr and MI/Cr ratios in the left hippocampus did not correlate with the changes in the PANSS and CGI scores. Significant correlations were not demonstrated between the changes in the multivoxel NAA/Cr and MI/Cr ratios in the right hippocampus and the multivoxel NAA/Cr, Cho/Cr, and MI/Cr ratios in the left hippocampus and the changes in the scores on the Digit Span Test, Verbal Fluency Test, Auditory Consonant Trigram Test, Trail Making Test, the RAVLT and the WMS-R visual reproduction subtest. Similarly, there were no significant correlations between the changes in the single-voxel NAA/Cr, Cho/Cr, and MI/Cr ratios in the left hippocampus and changes in the scores on the PANSS, CGI, Digit Span Test, Verbal Fluency Test, Auditory Consonant Trigram Test, Trail Making Test, the RAVLT and the WMS-R visual reproduction subtest. After the statistical correction for multiple comparisons, the correlations between the changes in the ^{1}H-MRS metabolite ratios and the changes in the psychopathology and neurocognitive tests lost their statistical significance.

DISCUSSION

To the best of our knowledge, this is the first longitudinal follow up study investigating the metabolic effects of clozapine therapy on the hippocampus. After 12 weeks of clozapine treatment, the PANSS positive, negative, general psychopathology and total scores and CGI scale scores decreased significantly, whereas the RAVLT-immediate recall and total learning scores, Verbal Fluency Test category scores and WMS-delayed memory test scores increased significantly compared with the baseline scores. On the other hand, there were no significant differences in the ^{1}H-MRS results between the patients before clozapine treatment and the control groups, or in the ^{1}H-MRS results of the patient group before and after clozapine treatment. Also, statistically significant correlations were not found between the ^{1}H-MRS results and the clinical and cognitive test parameters.

Previous studies comparing the NAA levels in the hippocampi of schizophrenia patients with those of healthy controls reported inconsistent results (Steen et al. 2005, Schwerk et al. 2014, Meyer et al. 2016, Singh et al. 2018). The absence of change in the NAA/Cr ratio was proposed to be associated with the sample size (Hutcheson et al. 2012); and the disagreements between the results were attributed to methodological differences or clinical diversities arising from duration of illness, symptom distribution, and the differences in drug treatments (Meyer et al. 2016). In this study, which included 16 schizophrenia patients, the absence of a difference in the NAA/Cr ratio between the patient and control groups may be associated with the sample size, duration of illness or the effects of previous treatments.

It is being claimed that different neurodegenerative and neurodevelopmental processes play a role in the pathophysiology of treatment-resistant schizophrenia (Elkis and Buckley 2016). There are not any reports in the literature on the NAA levels in the hippocampus in treatment-resistant patients. In this study, conducted on patients with treatment-resistant schizophrenia, absence of differences in the hippocampal NAA levels of the patients and the healthy controls suggests that treatment resistance is not associated with metabolic changes in the hippocampus, and that other regions of the brain may have a greater role. Another point to consider is the previous use of various antipsychotic agents by the patient group prior to clozapine treatment. Hence, the potential effects of antipsychotic drugs used before clozapine on the NAA levels in the hippocampus may be another reason for the absence of difference in the NAA levels between the patient and control groups.

In this study, NAA/Cr, Cho/Cr, and MI/Cr ratios in the hippocampus of the patients before clozapine treatment were compared with the ratios after 12 weeks of clozapine treatment, and no significant change was found. There are a limited number of studies in the literature reporting the absence of any effect of different antipsychotics in different brain regions after follow up using ^{1}H-MRS (Kraguljac et al. 2019, Grosic et al. 2014, Bustillo et al. 2008). Failing to demonstrate changed NAA levels in the hippocampus after a 6-week risperidone treatment of schizophrenia followed up with ^{1}H-MRS was attributed to the short duration of treatment, and studies of longer duration were recommended for detection of possible differences after treatment (Kraguljac et al. 2019). In another study, 25 first episode drug naive patients with schizophrenia were started on quetiapine at the onset of disease and followed up for 12 months with a switch to another atypical antipsychotic in 15 patients not responding to quetiapine. The lack of any difference in the ^{1}H-MRS data on the DLPFC NAA/Cr ratios before and after treatment of the entire patient group was attributed to the small size of the patient group, with the suggestion that the change in antipsychotic administration could have affected the results, since it was found in the same study that the DLPFC NAA/Cr ratio increased in the group given the same antipsychotic during the treatment period as compared to the patients whose antipsychotic medication was switched. This was thought to be associated with possible diversity in the action mechanism of different atypical antipsychotic agents (Grosic et al. 2014).

Although there are studies on the effects of antipsychotics on brain metabolites assessed by ^{1}H-MRS in patients with schizophrenia, studies investigating the effects of clozapine, which is known to be the most effective antipsychotic for the treatment of schizophrenia, are very few. Among cross-sectional studies on patients with schizophrenia managed with various antipsychotic medications including clozapine, the NAA levels were high in various region of the brain (McLoughlin et al. 2009, Bertolino et al. 2001) or unaltered (Sculz et al. 2007, Bustillo et al. 2001). The disagreement between results were considered to arise from methodological differences involving the voxel size, metabolite ratios or using concentration measurements (Meyer et al. 2016). In the literature, there are also longitudinal animal and human studies on the effects of clozapine. In a study which compared the effects of the administration of haloperidol, olanzapine and clozapine to mice for 1 week, no difference in the NAA/Cr ratios among the animal groups were reported (Lindquist et al. 2000). Similarly, changes in the NAA/Cr ratios in various regions of the brain including the hippocampus were not observed in an animal study after 6 weeks of treatment with clozapine and haloperidol (Bustillo et al. 2004). In a 6-month follow up study in mice, no difference in the NAA/Cr ratio in the striatum was reported following the administration of clozapine and haloperidol (Lindquist et al. 2011). In contrast to these animal studies, the only follow up study in patients with schizophrenia treated with clozapine for 8 weeks demonstrated an increased NAA/Cr ratio in the dorsolateral prefrontal cortex (Ertuğrul et al. 2009). In the present study, on the other hand, there were not any differences in the hippocampal metabolite ratios after 12 weeks of clozapine treatment. The assumption that antipsychotics may have different specific effects in different regions of the brain could be one explanation for this observed lack of clozapine effect in the hippocampus. It should be kept in mind that inconsistencies in results of different studies may arise from methodological differences, potential drug effects or the progressive and heterogeneous nature of schizophrenia. Although a period of 12 weeks is accepted to be sufficient for evaluating the effects of clozapine on clinical symptoms and cognitive functions (Nielsen et al. 2011), considering that there are patients who respond to clozapine within 6–12 months (Meltzer 1989, Rosenheck et al. 1999), longer follow up studies are required for investigating clozapine effect on metabolite ratios.

Several structural and functional imaging studies claim that changes in the hippocampus are associated with psychopathology and various cognitive functions, particularly memory, verbal fluency, and executive functions (Haukvik et al. 2013, Zierhut et al. 2010, Antonova et al. 2004). Different studies using ^{1}H-MRS of the hippocampus have reported contradictory results on the relationship between metabolite ratios and the cognitive functions. Whereas an inverse relationship was reported between verbal memory and the NAA/Cho and Cho/Cr ratios in a cross-sectional study conducted with ^{1}H-MRS (Hasan et al. 2014), a correlation could not be determined between the hippocampal NAA level and executive functions (Rüsch et al. 2008). Also, after a 6-week follow up study on risperidone treatment, a correlation could not be found between the hippocampal NAA and Cho levels and the scores on the positive and negative symptom and on the neuropsychological tests evaluating cognitive functions such as memory and attention (Kraguljac et al. 2019). We studied the relationship between the changes in the scores of neurocognitive tests which assessed different cognitive domains and the changes in ^{1}H-MRS results in the hippocampi after clozapine treatment and no significant relationship was found.

The small number of participants, the relatively short period of observations and not having excluded the previous use of drugs in treatment-resistant patients are among the limitations of this study.

This study is the first longitudinal study to investigate the effects of clozapine on the ^{1}H-MRS findings in the hippocampus, and their relationship with psychopathology and cognitive functions. It was demonstrated that the 12 weeks of clozapine treatment had no significant effect on ^{1}H-MRS findings in the hippocampus. However, clozapine effect on the hippocampus should be investigated further in longer follow up studies with larger numbers of participants in order to be able to reach a definitive conclusion.

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