Research Article (Araştırma Makalesi)

Voxel Based Morphometric Analysis of Regional Gray Matter Alterations Related with Duration of Illness, Number of Psychotic Episodes, Lifetime Antipsychotics Use in Patient with Schizophrenia

Şizofreni Tanılı Hastalarda Hastalık Süresi, Psikotik Atak Sayısı, Yaşam Boyu Antipsikotik Kullanımıyla İlişkili Bölgesel Gri Madde Değişikliklerinin Voksel Tabanlı Morfometrik Analizi

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Özet

Amaç: Etiyolojik etmenler, klinik görünümler ve tedavi yanıtı açısından şizofreninin oldukça ayrışık bir bozukluk olduğu bilinmektedir. Yapısal görüntüleme çalışmalarında gri madde değişikliği olan alanlar, bu çeşitliliğin bir yansıması olarak görünmektedir. Hastalık süresi, antipsikotik tedavisi ve aktif psikoz dönemlerinin, beyindeki yapısal değişikliklerle ilişkisi henüz netlik kazanmamıştır. Çalışmamızın amacı hastalığın ve hastalıkla ilgili süreçlerin (hastalık süresi, ilaç kullanımı, psikotik atak sayısı) beyin yapısına etkisini araştırmaktır.

Gereç ve Yöntemler: Çalışmamıza 33 şizofrenili hasta ile yaş, cinsiyet ve eğitim süreleri açısından eşleştirilmiş 35 sağlıklı gönüllü katıldı. Hastaların yaşam boyu antipsikotik maruziyeti belirlendi ve klorpromazin eşdeğer dozları üzerinden doz-yıl birimine çevrildi. Olguların manyetik rezonans görüntüleri (MRG) 3 Tesla gücündeki cihaz ile elde edildi. Görüntüler İstatistiksel Parametrik Haritalama 8 programı kullanılarak voksel tabanlı morfometri (VTM) yöntemiyle karşılaştırıldı. İstatistiksel değerlendirmelerde veri özelliklerine göre bağımsız gruplar t testi kullanıldı. İstatistiksel anlamlılık düzeyi çift yönlü $p \leq 0.05$ olarak kabul edildi. VTM'de genel lineer model (GLM) kullanılarak yaş, cinsiyet ve toplam beyin hacmi karıştırıcı etkenler olarak analiz matriksinde yer aldı. GLM'de iki grup karşılaştırmasında t-testi ve hastalık süreciyle ilişkili GM değişikliklerini araştırmada çoklu regresyon çözümlemesi yapıldı. VTM'de p değerinin 0.001'in altında ve küme oluşturan alanların 50 voksel üstünde olması koşulu arandı.

Bulgular: Sağlıklı kontrollerle karşılaştırıldığında hastalarda GM yoğunluğunda sağ orta temporal ve inferior temporal girus, bilateral orta frontal girus, sol singulat girus, sol prosentral girus ve sol supramarginal girus'ta azalma saptandı. Kontrollerle karşılaştırıldığında hastalarda GM yoğunluğunda sağ uncus, sol kaudat ve sol posterior singulat korteks'te artış saptandı. Hasta grubunda hastalık süresiyle sol presentral girus ve sol postsentral girus GM yoğunluğu arasında negatif ilişkili bulundu. Yaşam boyu APİ (Antipsikotik ilaç) kullanımıyla pozitif ve negatif ilişkili alanlar sırasıyla; sol inferior frontal girus ve sağ precuneus'tu. Psikotik atak sayısıyla sol medial frontal girus, sağ presentral girus ve sol parasentral lobül GM yoğunluğu arasında pozitif ilişki saptanırken uvula (serebellum) GM yoğunluğu arasında negatif ilişki saptandı.

Sonuç: Şizofrenili hastalarda GM eksikliğinin frontal ve temporal alanlarda ön planda olduğu söylenebilir. Ayrıca hastalık süresi, antipsikotik tedavisi, psikotik atak sayısı beyindeki GM değişiklikleriyle ilişkili görünmektedir. Limbik lobta GM yoğunluğundaki artışı açıklamak için ileri araştırmalara ihtiyaç vardır.

Anahtar kelimeler: Gri madde (GM), Manyetik rezonans görüntüleme (MRG), Şizofreni, Voksel tabanlı morfometri (VTM)

Abstract

Objective: Schizophrenia is known to be quite a heterogeneous disorder in terms of etiological factors, clinical features and, treatment response. Changes in gray matter areas with structural imaging studies seem to be a reflection of this diversity. The relationship of duration of illness, active psychosis periods, and antipsychotic treatment with structural changes in the brain has not been clarified yet. The aim of our study is to investigate the effects of the disease and disease-related processes (duration of illness, antipsychotic treatment, number of the psychotic episodes) on the brain structures.

Material and Methods: Thirty three schizophrenic patients and 35 age, gender and education matched healthy volunteers participated in our study. Life-time antipsychotic exposure determined for the patients and inverted dose/year unit over equivalent chlorpromazine doses. Magnetic resonance images were acquired with a 3 Tesla-powered imaging unit. By using Statistical Parametric Mapping 8, images were compared with voxel-based morphometry (VBM) analysis. Independent samples t-test for statistical evaluation based on the data characteristics were used. By using the general linear model (GLM) age, gender, and total brain volume were included as confounding factors in the analyze matrix in VBM. In GLM, t-test was used to compare two groups and to investigate disease process-related GM changes, multiple regression analysis were applied. In VBM, p values of less than 0.001 and areas with a minimum expected number of voxels per cluster of 50 are required.

Results: Compared to controls, patients showed decrements in gray matter density in the right middle and inferior temporal gyrus, bilateral middle frontal gyrus, left cingulate gyrus, left precentral gyrus, left supramarginal gyrus. Nevertheless, patients showed increased GM density in the right uncus, left caudate, and left posterior cingulate cortex as compared to controls. In the patient group, duration of illness was negatively associated with GM density in the left precentral gyrus and left postcentral gyrus. The lifetime exposure to antipsychotics correlated negatively and positively with gray matter density in, respectively; left inferior frontal gyrus and right precuneus. The number of psychotic episodes was positively associated with GM density in the left medial frontal gyrus, right precentral gyrus and left paracentral lobule whereas negatively in the uvula (cerebellum).

Conclusion: It can be said that GM deficits in schizophrenic patients are prominent in frontal and temporal areas. Besides illness duration, antipsychotic treatment, and number of psychotic episodes seem to be associated with changes in brain GM. Further studies are needed to clarify the increase in the limbic lobe GM density.

Keywords: Gray matter (GM), Magnetic resonance imaging (MRI), Schizophrenia Voxel-based morphometry (VBM)

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INTRODUCTION

Serious behavioral findings related to schizophrenia have accelerated efforts to understand the neurobiology of this complex illness. While understanding schizophrenia and developing new approaches to it, brain imaging methods not only combine regional, morphological and physiological alterations with clinical and neuro-behavioral researches but also help to demonstrate abnormalities in the entire brain (1-4).

Studies with structural magnetic resonance imaging (MRI) on schizophrenic patients have accelerated as MRI allows distinguishing gray and white matter and measuring the volumes of cortical and subcortical structures (1,5). The most consistent results obtained from these studies suggest the reduction in the whole-brain gray matter (GM) and increasing in the lateral and 3rd ventricular volumes (1-3). Besides the whole brain volume changes in the brains of schizophrenic patients, regional volume changes have also been reported (4,6).

Researchers have also seemed to take into account the possible factors that may influence the brain volume changes of schizophrenic patients. One of these possible factors is the duration of illness (2,5,6). The results obtained from first-episode psychotic patients and chronic psychotic patients and the brain volume changes observed over time in longitudinal follow-up studies emphasize the importance of the duration of illness (2,6). In a 5-year follow-up study conducted among schizophrenic patients, it has been reported that brain volume alterations, especially progressively increase in lateral ventricular volume over time, are not at the same grade throughout the illness. These alterations are evident especially during the first 20 years of the illness or before the age of 45 (2). The number of psychotic episodes also seems to be an important factor as it has been shown that recurrent psychotic episodes, as well as the duration of illness, are associated with neurodegenerative progression in some brain regions in schizophrenic patients (7).

Another possible factor is the use of the antipsychotic drugs (APs) and the type of APs (8-10). In a study conducted by Dazzan et al. increased basal ganglion volume and decreased cortical volume were found in patients under typical APs treatment, while increased thalamus volume was found among patients under atypical APs treatment (8). In a randomized-controlled follow-up study conducted by Lieberman et al., while the significant decrease in GM volume was observed in patients treated with haloperidol, it was not observed in patients treated with olanzapine (9). There are also studies reporting the increased cortical GM volume following the use of atypical APs in the relevant literature (11,12). Due to their different mechanisms of action, typical and atypical APs have been reported to have different effects on brain structures. It has been reported that atypical APs increase cellular resistance, have an agonistic effect on NMDA receptors, increase expression of trophic factors and stimulate neurogenesis. Besides it has been reported that typical APs have potential toxic effects, leading to oxidative stress and neurotoxicity (9,11,12).

Since Kraepelin, schizophrenia has been considered a progressive disorder. Although long-term follow-up studies about schizophrenia have revealed clinical impairment in the early stages of the disease, the pathophysiology of this clinical phenomenon has not been fully determined. This aspect of the disease may have a critical prescription to recognize the pathogenesis of schizophrenia and determine preventive treatment strategies. Changes in brain morphology of schizophrenic patients have long been the subject of many studies. We have limited information about when these changes occur during the onset of the disease and whether they are progressive. So, the purpose of this study is to investigate the regional GM changes in schizophrenic patients comparing with the healthy controls and to examine the relationship between duration of illness, the number of psychotic episodes, lifetime APs use, and regional GM alterations in the brain in schizophrenic patients.

MATERIALS AND METHODS

This study was performed with the patients who were followed up with the diagnosis of schizophrenia at Kocaeli University Medical Faculty Hospital Department of Psychiatry outpatient clinics. The control group consisted of healthy volunteers. Patients and healthy controls were matched according to age, gender, and education. Thirty-three patients and thirty-five healthy volunteers were included in the study. Patients who were diagnosed with schizophrenia according to the DSM IV (Diagnostic and Statistical Manual IV) diagnostic criteria, who had moderate to mild positive symptoms, or patients with clinical remission were included in our study. The control group included healthy volunteers who did not have any psychiatric disorder, who had no psychotic disorder in their first-degree relatives were included to our study. The exclusion criteria for the patient and control groups was determined as meeting the

criteria of alcohol and substance dependence diagnosis (except caffeine and nicotine dependence) according to DSM-IV for the last 6 months, to have other medical (having pace-maker, joint prosthesis, etc.) or mental disorder (claustrophobia) that will prevent the MRI study from being performed, to have any chronic neurological disorder or were any history of cranial trauma with loss of consciousness that lasted longer than 3 minutes, pregnancy, and the risk of suicide at the time of interview and examination. The study was approved by the Kocaeli University Ethics Committee for Clinical Research (Protocol Code: KOU KAEK 2014/169 Date: 06.06.2014). Written informed consent was obtained from each patient. The study was conducted by the principles of the Declaration of Helsinki.

Sociodemographic data form, Structured Clinical Interview for DSM IV (SCID-1), Positive and Negative Syndrome Scale (PANSS), Global Assessment of Functioning Scale (GAF), and Clinical Global Impression Scale (CGI-S) were applied to the patients. Sociodemographic data form and Structured Clinical Interview for DSM IV (SCID-1) were applied to the control group.

Sociodemographic Form:

This form includes the information about the age, gender, education, marital status, type of the illness, age of onset of the illness, duration of illness, number and length of hospitalization, number of psychotic episodes and lifetime total APs use of the patients. APs used by the patients have been converted to dose-year units by calculation in milligrams according to equivalent doses of chlorpromazine (mg) (13).

Structured Clinical Interview for DSM IV (SCID-I):

It is a structured clinical interview questionnaire designed to investigate the DSM-IV diagnosis of Axis I disorder (14). In the study reliability of the Turkish form, overall percent agreement was found as 98.1% and the kappa coefficient was found as 0.86 in all diagnoses (15).

Positive and Negative Syndrome Scale (PANSS):

It is a semi-structured interview scale with 30 items. The severity is rated on seven points (16). This scale includes 30 psychiatric variables which consist of 7 positive symptom subscales, 7 negative symptom subscales, and 16 remaining general psychopathology subscales. Turkish reliability and validity study of the scale is available (17).

Global Assessment of Functioning Scale (GAF):

It is used to assess the mental, social and occupational functioning of the patient on-axis V of the DSM-IV. The clinician examining the patient evaluates the overall compliance level of the individual using a scale score ranging from 0-100 using his/her judgment. A higher score indicates better functioning while a lower score indicates worse functioning (18).

Clinical Global Impression Scale (CGI-S):

It is used to measure the general evaluation of mental disorders (19). Each area is scored between 0 and 7. A higher score indicates that clinical symptoms are severe.

Magnetic Resonance Imaging (MRI) Protocol:

All images were performed on a 3T Philips MRI unit, with the patient in a supine position via an 8-channel head coil (Philips Achievea Intera Release Eindhoven, Netherlands, Philips Medical Systems Achieva 1 2008-07-18 Release, Software 22).

Conventional MRI: Cranial MRI was performed in the patient group. Sequences involve Axial T1AG, sagittal T2AG, dual PD images.

Volumetric Imaging: The 3D Fast Field Echo T1 Weighted Image (3D FFE T1AG) sequence on the sagittal plane.

Processing Images:

MATLAB and SPM (Statistical Parametric Mapping) programs were installed to perform voxel-based analysis. The obtained 3D FFE T1-weighted images were converted from DICOM (The Digital Imaging and Communications in Medicine) to NIfTI (Neuroimaging Informatics Technology Initiative) format by SPM version 8 (20). The coordinates of the anterior commissure (nerve fiber bundle connecting the two hemispheres along the midline) were taken as the coordinate center on the sagittal plan images of each case formed by the NIfTI formations. The right-left and anterior-posterior directions on axes of the x, y, and z of all cases were corrected according to the zero coordinate systems on the anterior commissure. Direction and symmetry settings made on the x, y, and z axes were reset at these coordinate values. The data that orientation and coordinate settings were made were transferred to the SPM program using the data segmentation tab. By using these data, GM, white matter (WM), and cerebrospinal fluid (CSF) volumes were determined on brain images. After separating the images by SPM 8 segmentation function new drafts were produced by using SPM 8 DARTEL (diffeo-

morphic anatomical registration through exponentiated lie algebra). Images that were produced on the DARTEL format, and that were formally corrected and modulated were adapted to MNI (Montreal Neurological Institute) domain. These drafts were reattached with the images on the hand and the images obtained after this process was smoothed. Smoothing is the process of filtering mages with an isotropic Gaussian kernel value. Normalized images were smoothed to Full Width at Half Maximum (maximum width at half height) 8 mm kernel value to enable statistical analysis on them.

VBM is a method that reveals differences in the anatomical structure of the brain and allows patient-healthy comparisons to be made statistically and shows these differences on a stereotaxic map. This technique relies on dividing the images into different tissue types, taking into account the density differences in the images. Although the ROI (region of interest) which is the manual drawing method is accepted as the gold standard, the VBM method has advantages over ROI because it is fully automatic, objective, fast and does not require the hypothesis. Consistency is monitored in ROI and VBM measurements (21).

The tissues are separated from each other by density differences with the VBM technique. Because the areas with GM probabilities were detected and the results were taken over the number of voxels with this technique, we used the term "density" instead of "volume" in our study.

Statistical Analysis

SPSS for Windows 20 statistical package program was used. Suitable parametric and nonparametric tests for statistical analysis were used after testing whether they showed statistically normal distribution characteristics. To compare the differences between the groups and the mean of continuous variables student t-test was used. In terms of statistical analysis, $p<0.05$ was considered significant. A general linear model (GLM) was used at Voxel-Based Morphometry. Age, gender, and total brain volume were included in the analysis matrix as confounding factors. In the general linear model, the first analysis was made with the t-test to compare the two groups. In the second analysis, a multipl regression analysis in the GLM was performed to investigate the relationship between duration of illness, lifetime APs use, number of psychotic episodes, and regional grey matter volume. To avoid false-negative results, the p-value was asked to be less than 0.001 in VBM and the cluster forming areas were asked to be above 50 voxels.

RESULTS

Thirty three patients and 35 healthy volunteers participated in the study. The patient group consisted of 10 (30.3%) female and 23 male individuals (69.7%); control group consisted of 10 (28.6%) female, 25 (71.4%) male individuals. The mean age of the patient group was 36.42±10.42 years and the mean age of the healthy volunteers was 36.42 ±10.04 years. The mean of successfully completed education years for the patient group was 11.45±3.67 years and it was 12.09±6.67 years in the control group. The majority of the control group (60%) were married while the majority of the patient group (66.7%) was not married. While not significant difference encountered between groups in terms of mean age, gender, and mean years of education, there was a significant difference in terms of marital status between the groups.

The mean age of onset of illness was 24.58 ± 8.07 years and the mean duration of illness was 11.88±8.05 years. While the mean number of hospitalizations was 2.18±2.53, the mean of the length of hospitalizations was 64.42±75.96 days. The mean number of a lifetime psychotic episodes was 4.18±3.85 for the patient group. APs used by the patients have been converted to dose-year units by calculation in milligrams according to equivalent doses of chlorpromazine (mg) (1 dose-year is equal to 100 mg chlorpromazine daily per year) (21). The mean total APs use (dose-year) of lifetime was 14.48±12.43.

The mean of Positive and Negative Syndrome Scale (PANSS) was 75.76±24.6, the mean of Positive Syndrome Subscale was 16.33±8.29, the mean of Negative Syndrome Subscale was 21.03±7.27; the mean of general psychopathology subscale was 38.48±11.34. While the mean of the Global Assessment of Functioning Scale (GAF) was 59.82±12.43, the mean of the Clinical Global Impression Scale (CGI-S) was 3.67±1.19.

GM Statistical Parametric Mapping Analysis of the Patient and Control Groups

GLM was performed at the VBM. Age, gender, and whole-brain volume were included in the analysis matrix as confounding factors. In the GM comparisons, the T-test was performed in the SPM program to determine whether the patient group and the 'control' group of individuals differed from each other. Differences were given as the number of voxels per field. According to this, it was accepted that there is a difference between comparisons if the voxel value was 50 and above. 50 voxels and below voxel values were ignored and evaluated in-

correctly. To avoid false-negative results, the p-value was asked to be less than 0.001 in VBM and the cluster forming areas were asked to be above 50 voxels.

As a result of comparisons of patient and control groups, a decrease in GM density in frontal, temporal, limbic, and parietal lobes as shown in the patient group (**Table 1** and **Figure 1**).

The decrease in GM density in frontal lobe was detected in left inferior frontal gyrus (BA 9) (k:410 voxel, T=3.95, p<0.001), left medial frontal gyrus (BA 9) (k:410 voxel, T=3.80, p<0.001), right medial frontal gyrus (BA6) (k=52 voxel, T=3.92, p<0.001), left medial frontal gyrus (BA 6) (k=197, T=3.86, p<0.001 and k=197, T=3.71, p<0.001) and left precentral gyrus (BA 6) (k=197, T=3.39, p<0.001) (**Table 1** and **Figure 1**).

The decrease in GM density in temporal lobe was detected in right medial temporal gyrus (BA 21, k=1029 voksel, T=4.57, p<0.001 and BA 22, k=1209 voxel, T=4.11, p<0.001) and right inferior temporal gyrus (BA 37, k=1209 voxel, T=4.09, p<0.001) (**Table 1**).

The decrease in GM density in the parietal lobe was detected in the left supramarginal gyrus (BA 40, k=282 voxel, T=4.19, p<0.001) (**Table 1**).

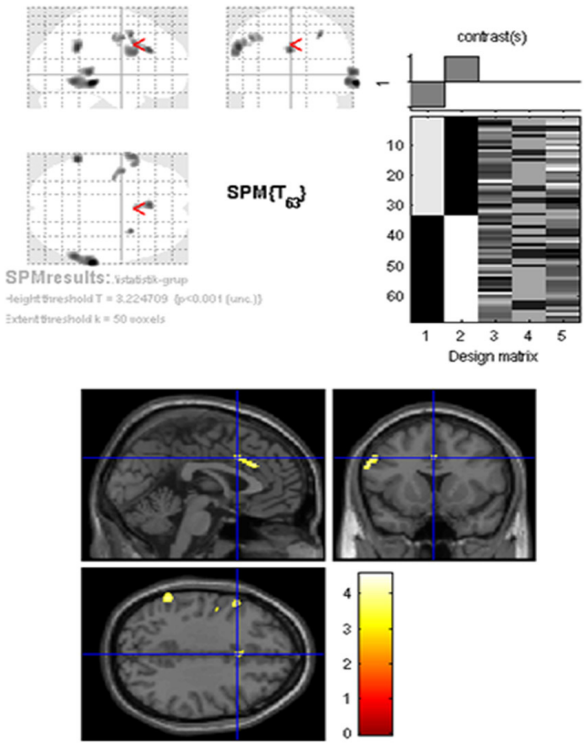

Figure 1. Areas showing the difference in gray matter density between groups.
T=3.22, p<0.001 (uncorrected), k=50 voxels, GM deficiency was shown as colored areas in the sagittal, coronal and axial planes.

Table 1. Areas with gray matter deficiency in the patient group in comparison of patient group with control group

	Anatomic Localization	Voxel Number (k)	T	P	X	Y	Z*
1	Right MiddleTemporal Gyrus (BA 21)	1209	4.57	<0.001	64	-34	-5
2	Right MiddleTemporal Gyrus (BA 22)	1209	4.11	<0.001	62	-37	5
3	Right İnferior Temporal Gyrus (BA 37)	1209	4.09	<0.001	58	-53	-4
4	Left SMG, (BA 40)	282	4.19	<0.001	-58	-41	37
5	Left Cingulate Gyrus (BA 32)	182	4.09	<0.001	-2	26	26
6	Left Cingulate Gyrus, (BA 24)	182	3.65	<0.001	0	14	33
7	Left Inferior Frontal Gyrus (BA 9)	410	3.95	<0.001	-58	10	24
8	Left Middle Frontal Gyrus (BA 9)	410	3.80	<0.001	-51	12	34
9	Right Middle Frontal Gyrus (BA 6)	52	3.92	<0.001	30	8	46
10	Left Middle Frontal Gyrus (BA 6)	197	3.86	<0.001	-44	-1	43
11	Left Middle Frontal Gyrus(BA 6)	197	3.71	<0.001	-35	-10	43
12	Left Precentral Gyrus (BA 6)	197	3.39	<0.001	-47	-4	34

T= t test analysis in the SPM (statistical parametric mapping), *x,y,z: Coordinates of the relevant field according to Talairach Atlas
The p-value is less than 0.001 for all of the presented results and voxel values were not taken into account for volume differences below 50.
BA: Brodmann Area, SMG: Supramarginal gyrus

The decrease in GM density in the limbic lobe was detected in left cingulate gyrus (BA 32, k=182 voxel, T=4.09, p<0.001 and BA24, k=182 voxel, T=3.65, p<0.001) (**Table 1**).

As a result of comparisons of patient and control groups, decrease in GM density was detected in right uncus (BA 38, k=249 voksel, T=4.44, p<0.001), left caudate volume (k=783 voksel, T=4.31, p<0.001 and k=783 voksel T=3.98, p<0.001 and k=783 voksel, T=3.98, p<0.001) and left cingulate cortex (k=180 voksel, T=4.30, p<0.001) in the control group (**Table 2**, **Figure 2**).

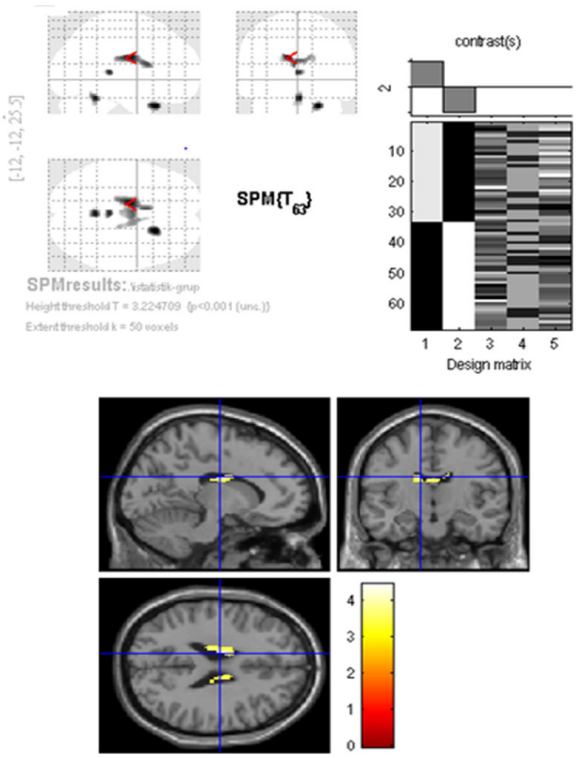

Figure 2. Gray matter density difference in the left caudate body.
Bright colors indicate the smaller area of gray matter in the control group. k=783 voxels, T=4.31
p <0.001 (uncorrected), coordinates according to Talairach Atlas (x, y, z): - 11, -12, 24

No significant volume changes were detected in the right and left hippocampus in the group analysis of the GLM generated by the SPM program [p<0.001(uncorrected) and areas over 50 voxels were considered as significant].

Statistical Parametric Mapping Analysis of Changes of GM Related With The Clinical Data in The Patient Group

Multiple regression analysis was performed in the GLM to investigate the relationship between duration of illness, lifetime APs use, number of psychotic episodes, and regional GM density. Age, gender, and total brain volume were included in the analysis matrix as confounding factors.

Duration of illness was reversely correlated with left precentral gyrus (Brodmann 6, k=166, Z=3.67, p<0.001, x/y/z=-52/-2/21) and left postsentral gyrus (Brodmann 43, k=166, Z=3.28, p<0.001, x/y/z=-57/-10/19) (**Table 3** and **Figure 3**).

There was no regional GM area that is correlated with the duration of the illness.

Number of psychotic episodes was positively correlated with left medial frontal gyrus GM (BA 6, k=188, Z=4.43, p<0.001, x/y/z=-9/-11/65), right precentral gyrus GM (BA 4, k=72, Z=3.58, p<0.001, x/y/z=55/-2/15) and left sol parasentral lobule GM (BAn 6, k=64, Z=3.52, p<0.001, x/y/z=-8/-28/65). Number of psychotic episodes was also found to be reversely correlated with uvula GM (k=181, Z=3.35, p<0.001, x/y/z=12/-84/-25) (**Table 4**, **Figure 4**).

Lifetime APs use was positively correlated with left inferior frontal gyrus GM (BA 9, k=63, Z=3.62, p<0.001, x/y/z=-52/4/24) and reversely correlated with right precuneus GM (k=109, Z=3.47, p<0.001, x/y/z=29/-75/34) **(Table 5, Figure 5)**.

Table 2. Areas with gray matter deficiency in the control group in comparison of patient group with control group

	Anatomical Localization	Voxel Number (k)	T	P	X	Y	Z*
1	Right Uncus (BA 38)	249	4.44	<0.001	20	12	-26
2	Left Caudate Truncus	783	4.31	<0.001	-11	-12	24
3	Right Caudate Truncus	783	3.98	<0.001	-5	6	18
4	Left Posterior Cingulate Cortex (BA23)	180	4.30	<0.001	0	-47	24

T= t test analysis in the SPM (statistical parametric mapping), x,y,z: Coordinates of the relevant field according to Talairach Atlas Voxel values were not taken into account for volume differences below 50. p<0.001(uncorrected)
BA: Brodmann Area

Table 3. Areas with a significant relationship between duration of the illness and the density of regional gray matter

	Anatomical Localization	Voxel Number (k)	T	P	X	Y	Z*
Negative Correlation	Left Precentral Gyrus(BA 6)	166	3.67	<0.001	-52	-2	21
	Left Postcentral Gyrus (BA 43)	166	3.28	<0.001	-57	-10	19

* x,y,z: Coordinates of the relevant field according to Talairach Atlas. Voxel values were not taken into account for volume differences below 50. p <0.001 (uncorrected) was considered as significant.
BA: Brodmann Area

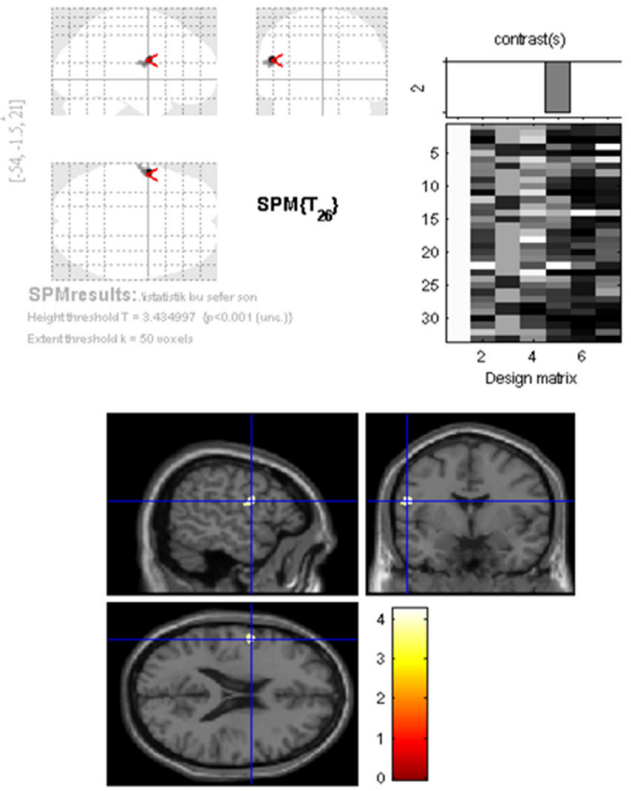

Figure 3. Gray matter area (left precentral gyrus) that is negatively correlated with the duration of illness.
k=166 voxels, Z=3.67, p <0.001 (uncorrected), coordinates according to Talairach Atlas (x, y, z): - 52, -2, 21

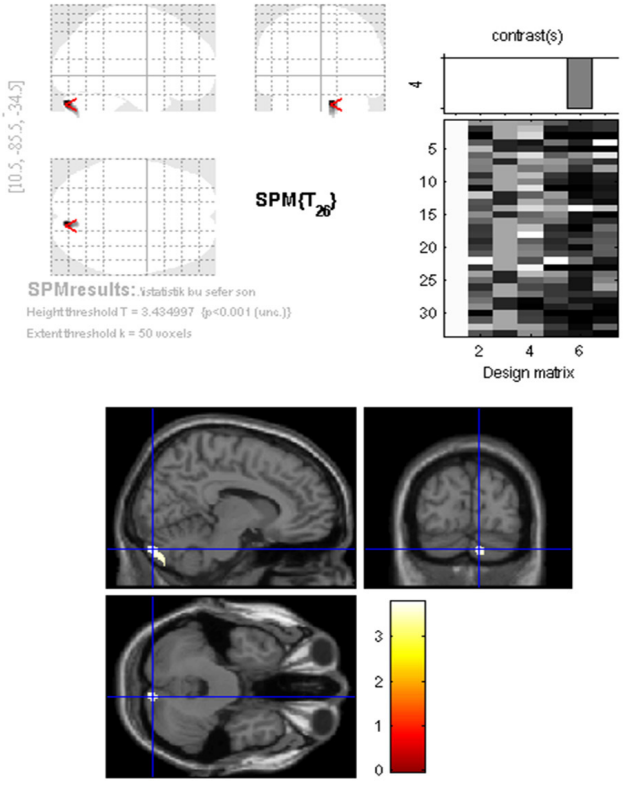

Figure 4. Gray matter area (uvula) that is negatively correlated with the number of psychotic episodes.
k=109 voxels, Z=3.67, p <0.001 (uncorrected), coordinates according to Talairach Atlas (x, y, z): 29, -75, 34

Table 4. Areas with a significant relationship between the number of psychotic episodes and the density of regional gray matter

	Anatomical Localization	Voxel Number (k)	Z	P	X	Y	Z*
Positive Correlation	Left Medial Frontal Gyrus (BA 6)	188	4.43	<0.001	-9	-11	65
	Right Precentral Gyrus (BA 4)	72	3.58	<0.001	55	-2	15
	Left Paracentral Lobule (BA 6)	64	3.52	<0.001	-8	-28	65
Negatifve Correlation	Uvula (Right Cebellum Posterior Lobe)	181	3.35	<0.001	12	-84	-25

* x,y,z: Coordinates of the relevant field according to Talairach Atlas. Voxel values were not taken into account for volume differences below 50. p <0.001 (uncorrected) was considered as significant.
BA: Brodmann Area

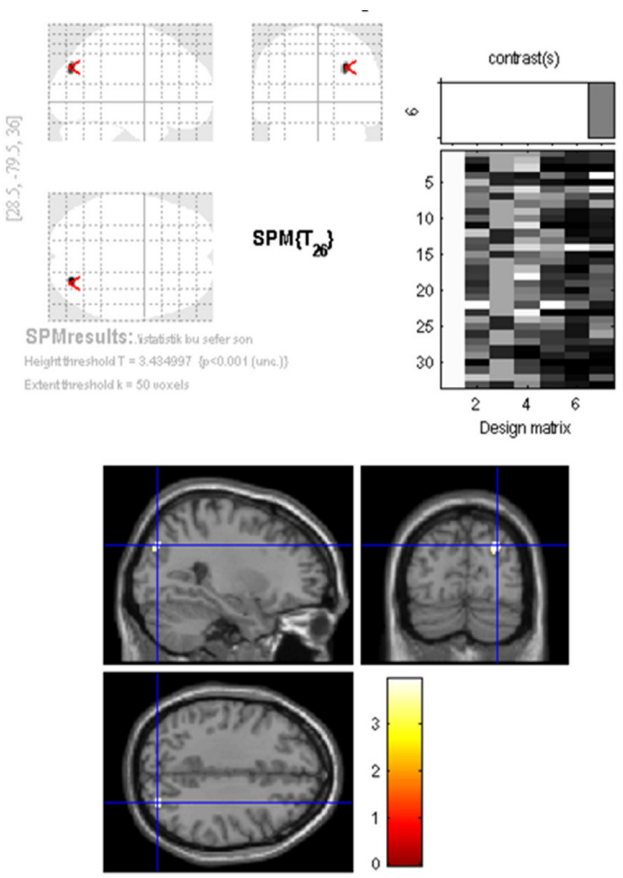

Figure 5. Gray matter area (right precuneus) that is negatively correlated with lifetime antispsychotics use. k=181 voxel, Z=3.35, p<0.001 (uncorrected), coordinates according to Talairach Atlas (x, y, z): 12, -84, -25

DISCUSSION

Our study has demonstrated the GM changes in schizophrenic patients, especially in temporal and frontal lobes (right medial temporal gyrus, right inferior temporal gyrus, left medial frontal gyrus, left precentral gyrus, and right medial frontal gyrus). Furthermore, reduction in the GM volume in the limbic and parietal lobes, left cingulate gyrus and left supramarginal gyrus (SMG) respectively, was also observed. Our findings are consistent with the findings of many previous studies (3,6,22,23). In a meta-analysis study, reduction in the bilateral insular cortex, anterior cingulate cortex (ACC), left parahippocampal gyrus, left medial frontal gyrus, postcentral gyrus, and thalamus volumes were reported in schizophrenic patients (22). In a review conducted by Shenton et al. alterations in GM in the temporal lobe cortical structures, especially the medial temporal lobe structures and in superior temporal gyrus were reported. In the same review, besides the frontal lobe abnormalities especially in the prefrontal GM and orbitofrontal regions, parietal lobe abnormalities especially in the inferior parietal lobe abnormalities including SMG and angular gyrus were also reported consistent with our results (3).

The areas where GM alterations were within the borders of BA 6, 9, 21, 22, 24, 37, 40 in our study. The alterations in the mid temporal gyrus were at BA 21 and 22, while the GM alterations at the inferior temporal gyrus were at BA37. Functional imaging studies have reported that the medial temporal gyrus and inferior temporal gyrus are involved in cognitive processes such as language, semantic memory (medial temporal gyrus) and visual perception (inferior temporal gyrus), and that the left medial temporal gyrus is the region associated with the hallucinations (24). In addition, right medial temporal gyrus that we found alterations in our study, is also found to be associated with hallucinations (25). Finally when schizophrenia is thought to have functional deficits in cognitive domains such as language, semantic memory, and complex visual perception, inferior and medial temporal gyrus seem important in the pathophysiology of schizophrenia (26).

The reduction in the GM densities of the cingulate gyrus and left medial frontal gyrus in our study was also

Table 5. Areas with a significant relationship between the use of lifetime antipsychotics and the density of regional gray matter

	Anatomical Localization	Voxel Number (k)	Z	P	X	Y	Z*
Positive correlation	Left Inferior Frontal Gyrus (BA 9)	63	3.62	<0.001	-52	4	24
Negative correlation	Right Precuneus	109	3.47	<0.001	29	-75	34

* x,y,z: Coordinates of the relevant field according to Talairach Atlas. Voxel values were not taken into account for volume differences below 50. p <0.001 (uncorrected) was considered as significant.
BA: Brodmann Area

consistent with previous studies (22,27). The BAs of these regions are 32 and 9 respectively. It has been suggested that GM reduction in these regions may be related to cognitive control disorders that are considered to be symptoms of schizophrenia (27). ACC is known to play important roles in processing affective-related stimuli, emotion expression, emotion regulation, and high cognitive functions (e.g., planning, problem-solving, decision making) (27).

The inferior parietal lobe [Supramarginal gyrus (SMG) and angular gyrus] are the main parts of the heteromodal association area. SMG and angular gyrus are important parts of the semantic-lexical network in which words are transformed into thoughts after gaining meanings (3). There are studies reporting a reduction in GM in SMG similar to our result (4, 28). Functional imaging studies to be performed on the heteromodal association area may be helpful to understand the importance of SMG in the pathology of schizophrenia.

We found an increase in the GM density of right uncus, left caudate, and left posterior cingulate gyrus in the patient group. While the increase in the caudate volume is consistent with some previous studies (10,29), an increase in the GM in the uncus and posterior cingulate gyrus in the patient group is not consistent with the previous studies (23, 30). Unfortunately, there are limited imaging studies about these regions in schizophrenic patients. To illuminate the role of these regions in schizophrenia, future functional imaging studies are needed.

Although we determined a positive correlation between lifetime APs use and GM density of left inferior frontal gyrus, we determined the reverse correlation between lifetime APs use and GM density of right precuneus. It is known that both schizophrenia and APs treatment modulate brain morphology. But it is not so clear that structural brain abnormalities are because of the illness itself or because of the effects of the APs treatment (12). After reviewing the literature, we have found some studies having similar results with our study suggesting the increase in the GM volume in some regional areas after APs treatment (11, 12,31). In a short follow-up study, researchers suggested that there was an increase in some GM regions especially in the bilateral prefrontal cortex, insula, right thalamus, left superior occipital cortex, and bilateral cerebellum GM volumes after 8 weeks of atypical APs treatment (12). In another study, significant reverse correlations were found between lifetime cumulative APs exposures and whole-brain GM volumes in schizophrenic patients, but no significant association was found between APs use and any specific brain region (5). It can be said that the effect of APs treatment on whole-brain or regional brain volumes of schizophrenic patients is complex than thought. The fact that our study has a cross-sectional design, does not include the drug-naive schizophrenic patients as a comparison group and the typical APs have not been distinguished from atypical APs in our study, thus making it difficult to explain these complex relationships. Both finding frontal GM reduction in the patient group and determining a positive correlation between lifetime APs use and GM density of left inferior frontal gyrus, underline the role of the frontal lobe in the development of schizophrenia and the importance of the frontal lobe in the follow-up process of APs treatment.

In addition to APs use, the type of APs also seems to be important in brain volume changes in schizophrenic patients (8, 31). In 28 days follow up study conducted by Garver et al, researchers have found a significant expansion in the cerebral cortical GM volumes of the patients under Second Generation APs -risperidone and ziprasidone treatment while they have found no significant change in the cortical GM volumes of the patients under haloperidol treatment (11). Also in a 5.5-month follow-up study with quetiapine, researchers have found an increase in the densities of GM in the bilateral inferior frontal cortex / orbitofrontal gyrus and ACC (31). The increase in cortical GM volume observed during atypical APs treatment was thought to be related to neurocognitive improvement and increased quality of life in schizophrenic patients (31). Although we did not distinguish between typical and atypical APs in our study, determining the positive correlation between lifetime APs use and GM density of left inferior frontal gyrus coincides with the previous results from atypical APs (11).

One of the interesting data of our study is that there was a reverse correlation between the density of the left prefrontal gyrus (BA6, frontal lobe), the density of the left postcentral gyrus (BA 43, Parietal lobe), and the duration of the illness. This finding increases the strength of other findings we have found associated with the GM volume in the frontal lobe of our study. Similarly, some studies reveals the reverse correlation between the GM volumes of certain areas of the frontal lobe in schizophrenic patients throughout the illness (5, 32). Structural and functional impairments associated with

schizophrenia have been observed before the onset of the illness, vary according to the levels of the illness, and most of the studies have concentrated on the disease for a limited period, so it is unclear how these abnormalities occurred during the illness (2,32). Besides age of onset and duration of illness without treatment may also contribute to these findings (7).

While we determined a positive correlation between the number of psychotic episodes and left medial frontal gyrus GM (BA 6), right precentral gyrus GM (BA 4), and left sol paracentral lobule GM (BA 6), we determined the reverse correlation between the number of psychotic episodes and right uvula (right cerebellum posterior lobe). Our findings support the literature (7) that recurrent psychotic episodes are associated with the neurodegenerative courses in some brain regions in schizophrenic patients.

There are some limitations of our study. Although the last six months have been taken into acocunt, similar to previous studies, it is a limitation that lifetime alcohol and/or substance use disorder has not been ruled out. The small sample size is another limitation of our study. We did not distinguish between typical and atypical APs in our study. The severity of structural brain abnormalities at the onset of psychosis may contribute to individual differences in the response to APs treatment. It is also a limitation that our study was conducted with chronic or patients who have previously used APs. Another limitation is that we did not examine the patient group as with or without deficit syndrome and with or without a mood disorder.

CONCLUSION

It can be concluded that GM deficiency in schizophrenic patients is predominantly determined in the frontal and temporal areas. Further research is needed to explain the GM increase in the limbic lobe. Also, the number of psychotic episodes, duration of illness, and use of antipsychotic medications are thought to affect the intensity of brain grey matter. There is a need for structural and functional imaging studies to be performed longitudinally with the first episode schizophrenic patients with a more homogeneous and larger number of samples, taking into consideration the duration of psychosis without treatment and the type of antipsychotics used by the patient.

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