Right-left and sex dependent differences of hippocampus and amygdala sizes and their relation to the clinical symptoms of schizophrenia: a comparative brain segmentation study

Amani Elfaki*1, Tahir Osman Ali2, Amira Mohamed Osman3, Meltem Acar Gudek1, Murat Golpinar1, Ibrahim Erkan4, Bunyamin Sahin1

1Department of Anatomy, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey.
2Department of Anatomy, Faculty of Medicine, National Ribat University, Khartoum, Sudan.
3Department of Psychiatry, Faculty of Medicine, International University of Africa, Khartoum, Sudan.
4Department of Biostatistics and Medical Informatics, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey.


Abstract

Objectives: The hippocampus and amygdala are believed to be central to the cognitive deficits associated with the schizophrenia. In the current study, the volumes of the hippocampus and amygdala have been examined depending on the right and left sides and sex in patients with schizophrenia and controls. We also evaluated the relation between the quantitative data and their relation to the clinical symptoms of patients.

Methods: 82 controls (47 male, 35 female) and 54 schizophrenic patients (28 male, 26 female) participated in the study. Structural magnetic resonance images were used to determine hippocampal and amygdala volumes that were automatically obtained using automatic brain segmentation software.

Results: The volume of right hippocampus in patients (3.80cm³) was less than controls (4.21cm³), additionally; right hippocampus was less in female patients (p≤0.05). The volume of left hippocampus in patients (3.65cm³) was less than controls (4.05cm³), while no sex difference was found (p>0.050). The volume of right amygdala shows no difference between patients (1.39cm³) and controls (1.50cm³), although it was significant less in female patients (p≤0.05). The volume of the left amygdala in patients (1.26cm³) was less than controls (1.42cm³), additionally; right amygdala was less in female patients (p≤0.05).

Conclusions: The present study demonstrates that abnormal volumes of the hippocampus and amygdala were evident in patients with schizophrenia in comparison with the controls. Female patients have hippocampal volume reduction on the right side. In addition to bilateral volume reduction of amygdala. In this study, patients with schizophrenia displayed marked thought disorder and social withdrawal.

Keywords: Schizophrenia, positive symptoms, negative symptoms, cognitive deficits, magnetic resonance imaging, automatic brain segmentation.

Introduction

In latest years, using magnetic resonance imaging (MRI), scientists try to establish suggestions about the role of hippocampus and amygdala in schizophrenia. The hippocampus is known to play a critical role in declarative memory and declarative memory is one of the most consistently impaired functions in schizophrenia [1]. So it has been hypothesized that the hippocampal volume and shape changes seen in schizophrenia may lead to the well-documented memory abnormalities seen in this disorder [2]. Direct emotional responses to information, especially to fear and anxiety, occur in the amygdala, in addition, the amygdala is thought to be involved in emotional memory retention [3]. It is believable that symptoms of schizophrenia such as inappropriate or flattened affect might relate to a change in the structure of the amygdala and its connections [4]. Previous studies discovered significant gender-dependent differences in patients with schizophrenia. These differences are believed to be a result from the effects of sex hormones along with neurodevelopmental and psychosocial sex differences [5]. There is evidence that female schizophrenia patients display less hippocampal and amygdala size reduction than male schizophrenia patients [6]. Abnormalities of various morphological characteristics and asymmetries of cortical surfaces in schizophrenia have also been shown to be mediated by sex differences [7].
Although, the hippocampus and amygdala have been studied extensively over the past two decades in patients with schizophrenia [8], to our knowledge this is the first study evaluate these two brain regions among Sudanese patients with schizophrenia, or in general the north-eastern African depending on side and gender differences. We also haven’t observed a study evaluating the side differences of the amygdala and their relation to the clinical symptoms.

In the present study, the volumes of the hippocampus and amygdala of patients with schizophrenia and a normal comparison group were evaluated using MRI and brain segmentation. The clinical symptoms of patients were measured with the positive and negative syndrome scale (PANSS), to define whether relationships would exist between positive and negative symptoms and specific volumetric deficits in the hippocampus and amygdala in patients with schizophrenia. Additionally, the current study aimed to determine whether the volumetric lateralization of the medial temporal lobe structures is specific to schizophrenia.

**Materials and methods**

The study was approved by the ethical committee of the Gezira University/ Sudan, depending on the statement of the ethical principles as developed on the World Medical Association Declaration of Helsinki [9].

**Subjects**

82 control subjects (47 male, 35 female) and 54 patients with schizophrenia (28 male, 26 female) participated in the study.

Patients with schizophrenia met the criteria of international classification of diseases, the tenth revision (ICD/10), and were receiving regular antipsychotic medications as following: 1) Typical antipsychotic (Haloperidol 2–20 mg/day). 2) Atypical antipsychotic medications as following: 1) Typical antipsychotic (ICD/10), and were receiving regular antipsychotic medications as following: 1) Typical antipsychotic (Haloperidol 2–20 mg/day). 2) Atypical antipsychotic (Olanzapine 5–20 mg/day). Table 1 shows the duration of illness, duration of treatment and types of treatment for patients. The patients were selected from Professor Abdelaal Alidresi Psychiatric hospital, Tiganí Almahi Psychiatric hospital and private psychiatry clinics in Sudan.

Controls were Sudanese volunteers with no history of psychiatric disorders and drug medication. Control subjects were matched with patients on the basis of age and body mass index (BMI) (Table 2). Exclusion criteria for both patients and controls include a head trauma, drug abuse, central neurological disorders, and left handed subjects.

Patient’s relatives or patient’s doctor and controls consented to all procedures. Controls and patient’s doctor filled a descriptive questionnaire includes socio-demographic data, physical data and PANSS which is a medical scale used for measuring symptom reduction of patients with schizophrenia.

**MRI acquisition**

Structural neuroimaging was performed on MRI scanner (1.5 Tesla SIEMENS). T1-weighted images were obtained using three-dimensional acquisition by Magnetization Prepared Rapid Gradient Echo (MP-RAGE). Slice distance was 1.0 mm, the field of view was 250 read, 192 mm phase, TR=1657 ms, TE=2.95 ms, bandwidth 180 Hz/pixel, flip angle 15°, ECHO spacing=7.5 ms, phase resolution=100%, slice resolution=50%, and acquisition time = 5 minutes and 18 seconds.

**Automatic segmentation of the MRI**

The DICOM images of participants were analyzed blind to the clinical data using the FreeSurfer software (Fischl, 2012). DICOM images were uploaded directly to FreeSurfer software, which is a collection of tools for the analysis of neuroimaging data that provides a display of algorithms to quantify the functional, connectional and structural properties of the human brain. It has advanced from a package mainly aimed at generating surface representations of the cerebral cortex into one that automatically creates models of most macroscopically visible structures in the human brain given any reasonable T1-weighted input image. Right and left hippocampus and amygdala volumes, as well as intracranial volume (ICV) obtained automatically from the FreeSurfer, and after quality control they are used for the statistics directly.

For calculating the lateralization index (LI) the following formula was used for each subject: 

\[ LI = 2*(R-L)/(R+L) \]

The interpretation of the LI values is as follows: LI > 0 in left hemisphere or LI < 0 in right hemisphere: more leftward asymmetry (left > right), LI < 0 in left hemisphere or LI > 0 in right hemisphere: more rightward asymmetry (right > left) [10].

**Statistical analysis**

Data analysis was performed on a personal computer (specification: Mac OSX 10.9.5, Processor 3.4 GHz, Intel Core i5, Memory 32 GB 1600 MHz DDR 3). SPSS version 21 was employed for all statistical analyses. Different tables and graphs were used to produce the findings. For normality test, Kolmogorov-Smirnov test was used, and all of continuous variables show normal distribution. Cross-sectional demographic differences between the patients and controls were tested using the ANOVA tests for continuous variables (age and body mass index (BMI)) and chi-square test for categorical variable (gender). F tests were used for testing the significant interaction effects between group and gender. The ANCOVA and post-hoc tests were used to compare the mean volume of the intracranial...
al and volume of the hippocampal/amygdala between controls and patients with schizophrenia. Degree of association between volumetric measures and duration of illness, duration of treatment, types of treatment used, and PANSS was tested using standard Spearman correlation test. Chi-square was used to compare the leftward or rightward asymmetry between groups. A p value equal or less than 0.05 was accepted as statistically significant.

RESULTS

Comparison of demographic data between controls and patients with schizophrenia

One-way ANOVA test, showed no significant difference between male/ female controls and patients regarding age and BMI (p=0.050) and chi-square test result also showed no significance for gender (p>0.050).

Correlation between volumetric measurements and duration of illness, duration of treatment and types of treatment used

Spearman’s rho correlation test, showed no correlation between volumes of the right and left hippocampus and amygdala with the duration of illness, duration of treatment and types of treatment used (p>0.050).

Evaluation of the intracranial volume (ICV)

The ICV was larger in males than females both for the patients (p<0.001) and controls (p<0.001). The ICV was less in patients with schizophrenia when it is compared with the controls (p<0.001), the mean differences between control and patient groups was 88.78 cm³ (Table 3).

Evaluation of the hippocampus volume

Right hippocampus: we made ANCOVA with our groups regarding cases and sex as between-group variables, with intracranial volume, age and type of treat-
ment use as covariates. And we found intracranial volume as a significant covariate for right hippocampus volume, then we repeat the analysis with a correction for intracranial volume and we found that the right hippocampus was less in patients than controls (F (1;131)=15.478 p<0.001).

Additionally, we found sex is significantly different between groups (F (1; 131) =4.355 p=0.035). Because of sex significance we produced pairwise comparisons (Bonferroni correction method) and we found that the volume of the right hippocampus was less in female patients with schizophrenia when it is compared with the female controls (p=0.001)(Table 3).

**Left hippocampus:** we made ANCOVA with our groups regarding cases and sex as between-group variables, with intracranial volume, age and type of treatment use as covariates. And we found intracranial volume as a significant covariate for left hippocampus volume, then we repeat the analysis with a correction for intracranial volume and we found that the left hippocampus was less in patients than controls (F (1;131)=5.745 p=0.018).

Although, we found no sex different between groups (p>0.050) (Table 3).

**Right amygdala:** we made ANCOVA with our groups regarding cases and sex as between-group variables, with intracranial volume, age and type of treatment use as covariates. And we found intracranial volume as a significant covariate for right amygdala volume, then we repeat the analysis with a correction for intracranial volume and we found that the left amygdala was less in female patients with schizophrenia when it is compared with the female controls (p=0.011)(Table 3).

**Evaluation of the amygdala volume**

**Right amygdala:** we made ANCOVA with our groups regarding cases and sex as between-group variables, with intracranial volume, age and type of treatment use as covariates. And we found intracranial volume as a significant covariate for right amygdala volume, then we repeat the analysis with a correction for intracranial volume and we found that the left amygdala was less in patients than controls (F (1;131)=3.577 p=0.061).

On the other hand, we found sex is significantly different between groups (F (1; 131) =22.096 p<0.001). Because of sex significance we produced pairwise comparisons (Bonferroni correction method) and we found that the volume of the right amygdala was less in female patients with schizophrenia when it is compared with the female controls (p=0.003).

**Left amygdala:** we made ANCOVA with our groups regarding cases and sex as between-group variables, with intracranial volume, age and type of treatment use as covariates. And we found intracranial volume as a significant covariate for left amygdala volume, then we repeat the analysis with a correction for intracranial volume and we found that the left amygdala was less in patients than controls (F (1;131)=8.870 p=0.003).

Additionally, we found sex is significantly different between groups (F (1; 131) =6.939 p=0.009). Because of sex significance we produced pairwise comparisons (Bonferroni correction method) and we found that the volume of the left amygdala was less in female patients with schizophrenia when it is compared with the female controls (p=0.011)(Table 3).

**Association between volumetric measurements and PANSS**

Spearman’s rho correlation test showed that: right hippocampus has significant negative correlation with the following positive symptoms (conceptual disorganisation (r=-0.256, p=0.046)) and one negative symptom (passive/ apathetic social withdrawal (r=-0.256, p=0.017)). Left hippocampus has significant negative correlation with the one negative symptom (lack of spontaneity and flow of conversation(r=-0.225, p=0.035)). Right amygdala has significant negative correlation with one positive symptom (conceptual disorganisation (r=0.302, p=0.004)) and the following negative symptoms (emotional withdrawal (r=-0.356, p=0.001) and passive/ apathetic social withdrawal (r= 0.345, p=0.001)). Left amygdala has significant negative correlation with the following positive symptoms (delusions (r= 0.235, p=0.026), conceptual disorganisation (r= 0.237, p=0.025) and suspiciousness/ persecution (r= 0.225, p=0.032)), and the following negative symptoms (emotional withdrawal (r= 0.260, p=0.016), passive/ apathetic social withdrawal (r= 0.296, p=0.006), difficulty in abstract thinking (r= 0.215, p=0.040), and lack of spontaneity and flow of conversation(r= 0.221, p=0.039)).

**Asymmetry of the hippocampus and amygdala**

There is no asymmetry difference in right/left hippocampus and amygdala between male/female controls and patients with schizophrenia (p>0.05). The find-

---

**Table 3. Volume of intracranial, hippocampus and amygdala in controls and patients with schizophrenia**

<table>
<thead>
<tr>
<th></th>
<th>Intracranial volume</th>
<th>Hippocampus volume</th>
<th>Amygdala volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1540.8± 98.45</td>
<td>4.32 ±0.36</td>
<td>4.13 ±0.45</td>
</tr>
<tr>
<td>Patient</td>
<td>1438.9± 110.72</td>
<td>4.00 ±0.47</td>
<td>3.70 ±0.76</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1404.1± 95.56</td>
<td>4.06 ±0.29</td>
<td>3.94 ±0.38</td>
</tr>
<tr>
<td>Patient</td>
<td>1328.4± 99.67</td>
<td>3.59 ±0.38</td>
<td>3.61 ±0.38</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1482.4± 118.19</td>
<td>4.21 ±0.35</td>
<td>4.05 ±0.43</td>
</tr>
<tr>
<td>Patients</td>
<td>1385.7± 118.47</td>
<td>3.86 ±0.47</td>
<td>3.65 ±0.61</td>
</tr>
</tbody>
</table>
ings showed the right hippocampus and amygdala were larger than the left hippocampus and amygdala in male/female healthy control and in patients with schizophrenia (p=0.002).

**DISCUSSION**

Following the recognition that variation in the volume of brain and brain structures may be related to variation in head size, a common approach to the problem of variability has been to employ some correction for overall head size. Normalization by intracranial volume (ICV) reduced variability in the volume measurements. Previously the ICV has been measured by manual delineation of the intracranial portion; recently the ICV can be estimated automatically using brain segmentation software as it was done in the present study.

In the current study the ICV was used to control the hippocampus and amygdala volumes. The ICV was less in patients with schizophrenia when it is compared with the controls. After controlling for the ICV our results for hippocampus and amygdala are independent from it. By accounting for differences in ICV, results will reflect morphological variations in regional areas that are due primarily to the variable of interest rather than differences in physiology.

The patients with schizophrenia had reduced right hippocampus (10.79%), left hippocampus (10.96%) and total hippocampus (10.59%) volumes. These findings are consistent with the post-mortem [11] and MRI [12] findings in schizophrenia.

Cognitive tests involving memory function showed the alteration differences of performance between patients with schizophrenia and healthy controls; such memory deficits appear to be powerfully related to the function of hippocampus [13]. Therefore, hippocampus may be a key structure in the production of schizophrenia [14]. All the patients who participated in the present study, were medicated patients with chronic schizophrenia. Since we did not find relation between volume loss of the right and left hippocampus and treatment used, volume loss in hippocampus do not seem to be a side effect of treatment. On the other hand, hippocampal volumetric changes are present from the earliest stages of the schizophrenia and in untreated patients, as mentioned in the previous studies [15, 16]. Special effects of the antipsychotic medication perform on presynaptic terminals and proteins and mostly restricted to the basal ganglia [17], and not often observed in the hippocampus [18]. This concept suggests that at least part of the disease process occurs early in life.

In the current study, the right and left hippocampus were significantly smaller in patients with schizophrenia. In addition, right hippocampal volume, not left one, in the female patients was significantly smaller than in female controls, and no differences were recorded between male patients and male controls. Female patients with schizophrenia and bipolar disorder exhibited decreased myelination in the hippocampal formation [19], which may lead to decrease the volume of hippocampus in the female patients of the current study. On the other hand, female patients with schizophrenia performed relatively poorer on right hemisphere tasks, and it suggests that female patients with schizophrenia may have right hemispheric dysfunction [20]; these may explain the clear occurrence of right hippocampus volume reduction in our female patients with chronic schizophrenia.

Together with previous study we identifying that right hippocampal volume is associated with chronic schizophrenic patients [21]. Other data revealed that left hippocampal volumes are altered during the early phases of schizophrenia [16]. Longitudinal MRI ultra high-risk (UHR) study confirmed progressive left medial temporal volume reduction during the transition from the UHR phase to first-episode psychosis in patients [15], so that the current study supports the findings of Velakoulis and Pantelis, that reduction of right hippocampal volumes reflects illness duration.

The patients with schizophrenia had reduced right amygdala (7.91%), left amygdala (12.70%) and total amygdala (10.19%) volumes. There is divergence between previous extensively MRI studies and a minor number of post-mortem studies related to the volume of amygdala. A consensus from MRI studies suggests a 5-10% decrease of the amygdala in schizophrenia, but, results from post-mortem planimetry studies are unclear [22]. The main problem may be related to the difficulties of delineating the amygdala and separating it from the hippocampus and from the temporal horn of the lateral ventricle. Several authors decided that the delineation of the amygdala was unreliable and omitted it from their studies. Some of them included it with the hippocampus. Specifically, one of the recent MRI study performed semi-automated segmentation of amygdala found that amygdala was smaller in patients with schizophrenia [23].

Our findings showed that the patient group had smaller amygdala volume than the control group, left amygdala was significantly smaller than right amygdala, also significantly smaller in patients with schizophrenia. In addition, there is no difference between male patient and male control related to the volume of right and left amygdala. However, volume reduction in female patients being particular with both right and left amygdala. This appears to be consistent with neuropsychological observations to some extent, in that the amygdala is thought to be involved in emotional memory retention [3]. Outcome of females forming strong emotional memories is an increased vulnerability to depression [24]. Depression is a frequently occurring...
symptom in schizophrenia [25], these may explain the clear occurrence of amygdala volume reduction in our female patients with chronic schizophrenia. Abnormalities of the hippocampus and amygdala can produce symptoms similar to those of schizophrenia. The proof of the reliability of the current results was approved by the Spearman correlation test between the volumetric data and the clinical symptoms of the patients. Right hippocampus has significant negative correlation with the following positive symptoms (Conceptual disorganisation and Excitement), and one negative symptom (Passive/apathetic social withdrawal). Left hippocampus has significant negative correlation with the one negative symptom (Lack of spontaneity and flow of conversation).

Right amygdala has significant negative correlation with one positive symptom (Conceptual disorganisation) and the following negative symptoms (Emotional withdrawal and Passive/apathetic social withdrawal). Left Amygdala has significant negative correlation with the following positive symptoms (Delusions, Conceptual disorganisation and Suspiciousness/persecution), and the following negative symptoms (Emotional withdrawal, Passive/apathetic social withdrawal, Difficulty in abstract thinking, and Lack of spontaneity and flow of conversation).

The inverse correlation between right hippocampus, right amygdala and left amygdala volumes and severity of conceptual disorganization observed in our study may indicate a possible involvement of this structure in the pathophysiology of formal thought disorder in chronic schizophrenia. In addition, we found that our patient sample displayed predominant negative symptoms mainly passive/apathetic social withdrawal, besides, lack of spontaneity and flow of conversation. Those symptoms lead to cognitive deficit and indicate impaired hippocampal and amygdala functions. The findings are consistent with previous MRI studies and further establish the medial temporal lobe structures as a significant region in the study of schizophrenia [26, 27].

The asymmetry of brain structures has been studied in schizophrenia to better understand its underlying neurobiology. Brain lateralization is considered highly related to human psychological and behavioral characteristics. According to the present data, the hippocampus and amygdala asymmetry in schizophrenia was similar to the comparison subjects, showed right > left asymmetry present in patients with schizophrenia and controls, also among sex across the groups, which is consistent with previous studies [28].

On the other hand, our findings are replicated the works of the Okada et al. [29] They found rightward asymmetry for hippocampal and amygdala volumes in both patients with schizophrenia and controls in a large number of subjects (3208). We used the same software for images analysis (FreeSurfer) and the same laterality index (LI), defined as the ratio [(left – right)/ (left + right)], even though, our study population are from two different ethnic groups (our study population are Sudanese and Okada’s study population are Japanese). In addition, in our study we consider gender differences.

While our findings were inconsistent with some finding has been reported in the literature, for example; the study of Shenton et al. [30], showed that the patient group has increased asymmetry. This inconsistently can be explained as we used a completely different methodology that abstracts and parameterized individuals; as they studied the amygdala–hippocampal complex rather than the hippocampus and amygdala separately. They also used delineating approach, we used automated segmentation techniques and evaluated a more number of brain regions in a more population of patients.

Our findings indicate that the hippocampus and amygdala are larger on the right side. There were evidence that a more pronounced right > left asymmetry of the hippocampus is at the medial aspect of the hippocampus (subiculum, dentate, and CA3), which is connected with the entorhinal cortex [28], particularly, these subregions of the hippocampus are closely related to cortical regions that support memory and language.

In the review of the Shenton et al. [31], 49 MRI studies that evaluated the medial temporal lobe structures, with 74% reporting positive findings and 26% reporting negative findings. Methodological differences probably contribute to the contradictory findings. For example: 1) some studies depend on thicker MRI sections to estimate temporal lobe structures volume [32]; 2) other studies depend on thin MRI sections to estimate temporal lobe structures volume [33]; 3) almost all MRI studies combine the amygdala and hippocampus into the amygdala-hippocampal complex because to separate them is difficult on coronal slices [34]; 4) Meanwhile, a small number of recent studies have measured these two brain regions separately [12].

In the present study, we analysed 1mm MR images using the automatic segmentation software to obtain reliable quantitative data which has little user defendant variations. We measured the volume of hippocampus and amygdala automatically and separately as the differentiation between those two structures so difficult and the case of inclusion of the amygdala significantly increased the size of the reduction.

There are several potential limitations of our study. First, our analysis only examined cross-sectional studies of individuals with schizophrenia and healthy controls and only examined hippocampus and amygdala. Second, we haven’t examined the hippocampal subfields;
studies including patients with chronic schizophrenia prove differences between hippocampal subfields in sensitivity to illness, and in associations with the clinical symptoms of schizophrenia [35].

**Conclusion**

In conclusion, the findings of present study demonstrate that abnormal volumes of the hippocampus and amygdala were evident in the patients with schizophrenia in comparison with the healthy controls. Female patients have hippocampal volume reduction on the right side, in addition to bilateral volume reduction of amygdala, indicating that the disorder were severe in female patients. Our findings specify that the sizes of hippocampus and amygdala show side and sex differences in the patients with chronic schizophrenia. This might motivate further researchers linking to gender differences on morphometric and cognitive variables in schizophrenia. In this study, chronic patients with schizophrenia displayed marked thought disorder and social withdrawal.

**Acknowledgements**

The authors are highly indebted to the technicians of the department of radiology in the National Ribat University/ Sudan, for their great contribution to this study and to the subjects who participated in collection of data.

This study was supported by the Organization for Women in Science for the Developing World (OWS-DW)/ Italy and the Ministry of Higher Education/ Sudan.

**Conflict of interest**

The authors declare no conflict of interest.

**REFERENCES**


[16] Velakouls D, Wood SJ, Wong MT, McGorry PD, Yung A, Phillips L, et al. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. Arch Gen Psychi-
ELFAKI ET AL: RIGHT-LEFT AND SEX DEPENDENT DIFFERENCES OF HIPPOCAMPUS AND AMYGDALA IN SCHIZOPHRENIA


