Abstract- The Alzheimer’s disease (AD) and mild cognitive impairment (MCI) can affect memory and daily living. Non-invasive diagnostic tools such as MRI can be useful to discriminate the patients from normal group. This study aims to compare the relative volumes of hippocampus and amygdala, to suggest the relative normal volumes, and to evaluate MRI automatic volumetry as a diagnostic tool. The MRI images of 130 subjects were retrospectively studied (Turbo field echo (TFE), acquired with a 3-Tesla Philips scanner). The image data were processed with Free Surfer (automatic segmentation and volumetry). The resultant volumes were corrected for brain size differences with intracranial volumes (ICV), and then analysed with SPSS (v. 17.0). There are differences of hippocampus and amygdala relative volumes between normal, MCI, and AD subjects at p < 0.001. The volume reductions of hippocampus in MCI and AD groups compared to normal group are about 8 % and 28 %, while those of amygdala are about 10 % and 34 %, respectively. The relative volumes of hippocampus (compared to ICV) in normal aging are $0.002617 \pm 0.000278$ (right) and $0.002553 \pm 0.000257$ (left), while those of amygdala are $0.001231 \pm 0.000165$ (right) and $0.001096 \pm 0.000144$ (left). There are no differences of relative volumes affected by gender in normal, MCI, and AD. There is a highly significant difference of relative volume affected by brain side in normal group (p < 0.001) but not in MCI (p = 0.119 and 0.077) and AD (p = 0.713 and 0.250), for hippocampus and amygdala, respectively. These results demonstrate that there are volume losses of hippocampus and amygdala in both diseases. Automatically measured hippocampus and amygdala volumes can be used as a measure indicating MCI and AD. The abnormal disturbance of volume affected by brain side may indicate the progression of both diseases. The hippocampus and amygdala volumes can be used as one of diagnostic tools to confirm the diagnosis of MCI or AD. The volume measurements on high-resolution MRI images can be automatically performed instead of laboriously manual drawings.

Keywords- MRI, hippocampus volume, AD, MCI, automatic brain segmentation

1. INTRODUCTION

Magnetic resonance imaging (MRI) has been increasingly used for clinical diagnosis especially in several brain diseases such as Alzheimer’s disease (AD), mild cognitive impairment (MCI), schizophrenia, and temporal lobe epilepsy [1]. The AD is the most common cause of dementia among elderly with a progressive course, beginning with neuronal dysfunction and irreversible loss of neurons, while MCI can represent a transitional state between normal aging and AD with the density and distribution of the tau-associated neurofibrillary and beta-associated amyloid for long time before the clinical onset of AD [2]. The AD and MCI can affect memory and daily living, thus non-invasive diagnostic tools such as MRI can be useful to discriminate the patients from normal group with early diagnostic and therapeutic interventions [2]. Early pathologic changes of both diseases were also found that related to medial temporal lobe (MTL) structures and initially taken in the hippocampus and amygdala [3,4]. The visual assessment of MRI images is difficult to correctly distinguish between clinical and healthy group because hippocampus and amygdala are subtle structures [4]. Therefore, volume evaluation of both subtle structures requires automatic segmentation and volumetry of MRI images. Manual method has been accepted as gold standard but it required specific anatomical knowledge from experienced radiologist and time consumes [5], thus automatic volumetry is more appropriate for large scale data and clinical used with acceptable consistency and reproducibility [1,6].

The aims of this study are to compare the relative volumes of hippocampus and amygdala in normal aging, MCI and AD subjects, to investigate the affect of brain side (right and left) and gender (male and female) on volumes, to suggest the relative normal volumes of Thai subjects, and to evaluate MRI automatic volumetry as a diagnostic tool. In this study, we used the automatic software tools called FreeSurfer (for segmentation and volumetry), and used intracranial volumes (ICV) to correct the brain size differences.

MRI Volumetry of Hippocampus and Amygdala in Normal Aging, Mild Cognitive Impairment and Alzheimer’s Disease Subjects

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II. MATERIALS AND METHODS

A. Subjects

The MRI images of 130 subjects were retrospectively studied and provided by the Thai Brain Mapping (TBM) project of Ramathibodi Hospital, Faculty of Medicine, Mahidol University, Thailand. In this study, all subjects were above 50 years old (range 50-88 years) and have been approved by the Ethical Committee, Mahidol University. They were clinically classified into three groups based on health history, clinical and neurological examinations by clinicians. The first group is healthy controls (HC, n = 100, 31 males and 69 females, mean age = 64.4 ± 7.0 yrs), the second is MCI subjects (n = 12, 5 males and 7 females, mean age = 72.5 ± 8.2 yrs), and the last is AD subjects (n = 18, 9 males and 9 females, mean age = 76.6 ± 9.1 yrs). The mean age, gender, and intracranial volumes (ICV) were shown on Table 1. From the p-value, the samples of three groups are similar based on mean age, gender, and intracranial volumes.

Table 1 Demographic characteristics of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>HC (n = 100)</th>
<th>MCI (n = 12)</th>
<th>AD (n = 18)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs ± SD)</td>
<td>64.4 ± 7.0</td>
<td>72.5 ± 8.2</td>
<td>76.6 ± 9.1</td>
<td>0.157</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>69</td>
<td>58.3</td>
<td>50</td>
<td>0.261</td>
</tr>
<tr>
<td>Intracranial volumes (ICV, mm³ ± SD)</td>
<td>1415283.67 ± 146052.13</td>
<td>1404307.89 ± 130624.82</td>
<td>1405095.34 ± 208616.53</td>
<td>0.948</td>
</tr>
</tbody>
</table>

B. MRI image acquisition

The MRI images were acquired with a 3-Tesla Philips scanner. The Axial T1-weighted images were obtained with the following protocol: Turbo field echo (TFE) technique, matrix size = 512 x 512, slice thickness = 2 mm and then were reconstructed to 3D images with slice thickness 0.94 mm.

C. Hippocampus and amygdala anatomical definition

The hippocampus and amygdala are part of middle arc of the limbic system and located in medial temporal lobe. The hippocampal formation consists of the hippocampus itself, the dentate gyrus and parts of the parahippocampal gyrus [7]. The amygdala is located near to the temporal pole and anterior to hippocampus [7].

D. Automated volumetry using FreeSurfer

The image data were processed using FreeSurfer software package (v 4.0.5) installed on Linux workstation. FreeSurfer is a set of software tool for the study of cortical and subcortical anatomy developed by members of Athinoula A. Martinos Center for Biomedical Imaging and freely available at http://surfer.nmr.mgh.harvard.edu [2,3]. In the preprocessing step, we performed image orientation and image format conversion. FreeSurfer’s processing includes image registration, intensity normalization, skull stripping, labelling, and volumetry [1,4]. FreeSurfer calculates brain subvolumes by assigning a neuroanatomical label to each voxel from a manually labeled training set [6]. The hippocampus and amygdala were automatically delineated using an algorithm that examines variations in voxel intensities and spatial relationships to classify non-neocortical regions on MRI scans [2].

E. Intracranial volumes

Intracranial volumes (ICV) defined as the sum of whole brain and CSF volumes. It represents the size of brain at maximal maturity unaffected by age or disease-related atrophy [6,8,9]. In order to work with average volumes, it is necessary to use relative volumes to normalize the regional brain volumes affected by individual head size [3]. In this study, we divided the normal volumes by ICV that obtained from FreeSurfer to correct the head size differences.

F. Data analysis

SPSS Statistics 17.0 software was used for statistical analysis. We used independent sample t-test for comparison the relative volumes affected by gender, Paired samples t-test for comparison the relative volumes affected by brain side, and ANOVA for comparison among clinical group. The data were tested for homogeneity of variances before apply with ANOVA. In addition, post-hoc analysis (LSD method) was
III. RESULTS

The resultant volumes from FreeSurfer were recorded according to clinical group, gender, and brain side. The comparisons of relative volumes affected by gender were tested with independent sample t-test. The statistical results were listed on table 2.

Table 2 The statistical analysis of comparisons the volumes affected by gender.

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>0.141</td>
<td>0.206</td>
<td>0.280</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.201</td>
<td>0.927</td>
<td>0.546</td>
</tr>
</tbody>
</table>

The comparisons of relative volumes affected by brain side were tested with paired samples t-test. The statistical results were listed on table 3.

Table 3 The statistical analysis of comparisons the volumes affected by brain side.

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>&lt; 0.001</td>
<td>0.119</td>
<td>0.713</td>
</tr>
<tr>
<td>Amygdala</td>
<td>&lt; 0.001</td>
<td>0.077</td>
<td>0.250</td>
</tr>
</tbody>
</table>

The comparisons of relative volumes affected by clinical group were tested with Analysis of variance (ANOVA). The mean, standard deviation of relative volumes, and statistical results were listed on table 4.

Table 4 The mean, standard deviation, and the statistical analysis of hippocampus and amygdala relative volumes affected by clinical group.

<table>
<thead>
<tr>
<th>Relative volumes</th>
<th>HC (mean±SD)</th>
<th>MCI (mean±SD)</th>
<th>AD (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right hippocampus</td>
<td>0.002617 ± 0.000278</td>
<td>0.002417 ± 0.000298</td>
<td>0.001868 ± 0.000409</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Left hippocampus</td>
<td>0.0002553 ± 0.0000257</td>
<td>0.0023334 ± 0.0000344</td>
<td>0.001850 ± 0.0000420</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Right amygdala</td>
<td>0.001231 ± 0.0001165</td>
<td>0.0011074 ± 0.0001254</td>
<td>0.00793 ± 0.0001764</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>0.001096 ± 0.0000988</td>
<td>0.000898 ± 0.0000042</td>
<td>0.00742 ± 0.0002354</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

From the table 2, there are no differences of hippocampus and amygdala relative volumes affected by gender (male and female) in normal group at p = 0.141 and 0.201, respectively. These findings are consistent with the previous study by Li et al [10]. In contrast to raw volumes, there are differences at p < 0.001. The results indicate that ICV can be used for correcting inter-subject variation in head size differences by gender. There are no differences in MCI (p = 0.206 and 0.927) and AD (p = 0.280 and 0.546), for hippocampus and amygdala, respectively. The results in MCI and AD group indicate that there are no the disturbance of volume changes affected by gender. Besides, the study of Shen et al showed that volumes which corrected by ICV were closed to manual volumes more than non-corrected volumes [1].

From the table 3, there are differences of hippocampus and amygdala relative volumes affected by brain side (right and left) in normal group with both p < 0.001. The results show left-less-than-right in normal group that consistent with the previous study by Shi et al [8], and Szabo et al [9]. Meanwhile, there are no differences in MCI (p = 0.119 and 0.077) and AD (p = 0.713 and 0.250), for hippocampus and amygdala, respectively. The abnormal disturbance of volume affected by brain side in MCI and AD may indicate the progression and pathogenesis of both diseases [8]. In our opinion, these findings should be confirmed by larger scale data.

From the table 4, there are differences of hippocampus and amygdala relative volumes between normal, MCI and AD subjects at p < 0.001. The volume reductions of hippocampus in MCI and AD groups compared to normal group are about 8 % and 28 %, while those of amygdala are about 10 % and 34 %, respectively. These findings demonstrate that there are volume loss of hippocampus and amygdala in both diseases. The results support the fact that MCI is the transitional stage of AD and consist with the previous postmortem study [8]. The results also demonstrate that automatically measured hippocampus and amygdala volumes can be used as a measure indicating MCI and AD. Moreover, this study also highlights the importance of amygdala volumes for diagnostic.

The relative volumes of hippocampus (compared to ICV) in normal aging are 0.002617 ± 0.000278 (right) and 0.002553 ± 0.000257 (left), while those of amygdala are 0.001231 ± 0.000165 (right) and 0.001096 ± 0.000144 (left). Providing normal database of hippocampus and amygdala volumes is an essential step in distinguishing both diseases [10].

The limitations of this study should be noted that the sample size of MCI and AD are relatively small. Larger
The hippocampus and amygdala volumes should be proposed as one of diagnostic tools to confirm the diagnosis of MCI or AD. The volume measurements on high-resolution MRI images can be automatically performed instead of laboriously manual drawings.

REFERENCES