MAGNETIC RESONANCE IMAGING STUDY OF THE AMYGDALA-HIPPOCAMPAL COMPLEX AND SUPERIOR TEMPORAL GYRUS IN TREATMENT-RESISTANT SCHIZOPHRENIA AND NON-TREATMENT-RESISTANT SCHIZOPHRENIA - A PILOT STUDY

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

HUSM	Hospital Universiti Sains Malaysia
TRS	Treatment-resistant schizophrenia
MRI	Magnetic Resonance Imaging
AHC	Amygdala-hippocampal complex
STG	Superior temporal gyrus
AHC COR	Amygdala-hippocampal complex in the coronal plane
AHC AX	Amygdala-hippocampal complex in the axial plane
STG COR	Superior temporal gyrus in the coronal plane
STG SAG	Superior temporal gyrus in the sagittal plane
PACS	Picture Archiving and Communication System
SD	Standard Deviation
SPSS	Statistical Product and Service Solution
SI	Signal intensity
cm	centimetre
ms	Millisecond

ABSTRAK

Latar belakang: Struktur yang tidak normal di dalam otak merupakan salah satu faktor penyebab kepada pesakit skizofrenia yang tidak memberi tindak balas positif terhadap rawatan. Kawasan otak utama yang dipercayai terlibat adalah struktur di lobus temporal. Pengimejan resonans magnetik (MRI) konvensional adalah sangat sensitif, tidak mengunakan sinaran ion, dan diguna secara meluas dalam mengenal pasti ketidaknormalan di otak bagi pesakit skizofrenia yang tidak tidak memberi reponse positif terhadap rawatan. Disebabkan oleh orientasi struktur di lobus temporal, imej diambil secara 'coronal oblique'. Tujuan utama kajian ini adalah untuk membandingkan struktur dan isipadu amygdala-hippocampal complex (AHC) dan superior temporal gyrus (STG) bagi pesakit skizofrenia yang tidak bertindakbalas terhadap rawatan (TRS) dan yang bertindakbalas terhadap rawatan (non-TRS).

Kaedah: Seramai 30 pesakit sckizofrenia, termasuk 14 TRS and 16 pesakit non-TRS telah terlibat dalam penyelidikan ini yang dijalankan Hospital Universiti Sains Malaysia (HUSM). Pesakit- pesakit ini akan menjalani MRI otak menggunakan protokol khas untuk penyakit sawan. Pengesanan isipadu dan analisa struktur AHC dan STG menggunakan imej MRI T1, T2, dan FLAIR. Ukuran isipadu struktur tersebut dibuat menggunakan perisian Philips Intellispace Portal Software. Purata bagi jumlah isipadu setiap struktur (SD) akan dikira dan perbezaan yang ketara diambil kira jika nilai P kurang daripada 0.05.

Keputusan: Terdapat perbezaan yang ketara pada isipadu AHC di sebelah kanan dalam imej coronal (AHC COR) dan axial (AHC AX) bagi pesakit TRS berbanding pesakit non-TRS (*P value*<0.05). Purata jumlah isipadu AHC COR di sebelah kanan adalah lebih tinggi untuk pesakit TRS iaitu 25.3 cm^3 (SD=18.8 cm^3) berbanding dengan pesakit non-TRS iaitu18.8 cm^3 (SD=5.4 cm^3). Keputusan kajian ini juga menunjukkan jumlah purata yang tinggi untuk AHC AX di sebelah kanan bagi pesakit TRS iaitu 29.0 cm^3 (SD=11.1 cm^3) berbanding, 21.9 cm^3 (SD=5.5 cm^3) untuk pesakit bukan TRS. Selain daripada itu, tiada perbezaan ketara untuk ukuran isipadu AHC kiri dan STG untuk kedua-dua kumpulan. Jumlah purata isipadu bagi AHC COR kiri bagi pesakit TRS adalah 21.2 cm^3 (SD=7.7 cm^3) dan pesakit bukan TRS adalah 16.6 cm^3 (SD=6.4 cm^3). Manakala jumlah purata isipadu AHC AX kiri bagi pesakit TRS adalah 25.0 cm^3 (SD=10.7 cm^3) dan pesakit bukan TRS adalah 20.4 cm^3 (SD=5.1 cm^3). Jumlah purata isipadu bagi STG COR kanan bagi pesakit TRS adalah 10.9 cm^3 (SD=5.9 cm^3) dan pesakit bukan TRS adalah 12.0 cm^3 (SD=3.0 cm^3). Bagi STG COR kiri untuk pesakit TRS, jumlah purata isipadu adalah 17.3 cm^3 (SD=9.5 cm^3) dan bukan TRS adalah 14.4 cm^3 (SD= 2.4 cm^3). Jumlah purata isipadu bagi STG SAG kanan bagi pesakit TRS pula adalah 14.8 cm^3 (SD=3.6 cm^3) manakala pesakit bukan TRS 14.5 cm^3(SD=7.6 cm^3). Jumlah purata isipadu bagi STG SAG kiri bagi pesakit TRS adalah 24.1 cm^3 (SD=6.1 cm^3) dan pesakit bukan TRS adalah 16.2 cm^3 (SD=5.4 cm^3). Analisa struktur AHC dan STG didapati tiada perbezaan yang ketara (p-value>0.05).

Kesimpulan: Kajian ini menunjukkan pesakit skizofrenia yang tidak mematuhi rawatan mempunyai isipadu AHC di sebelah kanan yang lebih besar berbanding dengan pesakit skizofrenia yang mematuhi rawatan.

Kata kunci: Treatment-resistant schizophrenia, amygdala-hippocampal complex, superior-temporal gyrus, pengimejan resonans magnetik.

ABSTRACT

Background: Structural brain abnormalities is one of the leading cause of developing treatment-resistant schizophrenia. The main region affected is temporal lobes structures. Conventional magnetic resonance imaging has higher sensitivity, lacks ionising radiation and has been widely used in detecting structural brain abnormality. Because of the anatomic orientation of the temporal lobe, MRI findings are best identified by using a coronal oblique view. The purpose of this study is to compare the structure, signal intensity, and volumes of bilateral amygdala-hippocampal complex and superior temporal gyrus in treatment-resistant schizophrenia (TRS) and non-treatment-resistant schizophrenia (non-TRS) in this view.

Methods: This prospective cohort study was conducted at Hospital Universiti Sains Malaysia (USM) on 30 patients, including 14 TRS patients and 16 non-TRS patients. These patients are subjected to an MRI brain study using epilepsy protocol. MR images were evaluated for loss of volume and abnormal signal intensity of bilateral amygdala-hippocampal complex (AHC) and superior temporal gyrus (STG) on T1- and T2-weighted images, as well as FLAIR images. The volumes of those structures are measured using Philips Intellispace Portal Software. Mean and standard deviations (SD) were obtained for each measurement, and the level of significance was determined (p-value < 0.05). The structure, signal intensity, and volumes of the bilateral amygdala-hippocampal complex and superior temporal gyrus for both groups were studied.

Results: There was a significant mean difference in volumetric values between the groups of right AHC in the coronal plane (AHC COR) and right AHC in the axial plane (AHC AX) (p-value < 0.05). The mean and SD of right AHC COR in TRS patients is 25.3 cm^3 (SD= 9.1 cm^3) were higher than in Non-TRS patients, which is 18.8 cm (SD=5.4 cm^3). The results also showed a high mean right AHC AX value in TRS patients, 29.0 cm^3 (SD=11.1 cm^3) compared to Non-TRS patients, 21.9 cm^3 (SD=5.5 cm^3). There were no significant mean differences of left AHC COR and AX between the patient groups (p-value > 0.05). The mean and SD of left AHC COR in TRS patients is 21.2 cm^3 (SD=7.7 cm^3) and Non-TRS patients is 16.6 cm^3 (SD=6.4 cm^3). The mean of left AHC AX in TRS patients is 25.0 cm^3 (SD = 10.7 cm^3) and Non-TRS patients is 20.4 cm^3 (SD=5.1 cm^3). There was no significant mean difference in volumetric values between the groups of bilateral STG in the coronal plane (STG COR) and sagittal plane (STG SAG) (p-value > 0.05). The mean and SD of right STG COR in TRS patients is 10.9 cm^3 (SD=5.9 cm^3) and Non-TRS patients is 12.0 cm^3 (SD=3.0 cm^3). The mean of left STG COR in TRS patients is 17.3 cm^3 (SD=9.5 cm^3) and Non-TRS patients is 14.4 cm^3 (SD=2.4 cm^3). The mean of right STG SAG in TRS patients is 14.8 cm^3 (SD=3.6 cm^3) and Non-TRS patients is 14.5 cm^3 (SD=7.6 cm^3). The mean of left STG SAG in TRS patients is 24.1 cm^3 (SD=6.1 cm^3) and Non-TRS patients is 16.2 cm^3 (SD=5.4 cm^3). There was no significant association between the brain structures, signal intensity, and group of patients (p-value < 0.05). The finding shows that the proportion of patients in TRS and Non-TRS patients was almost similar in terms of brain structure and diffusion. All patients were detected with no abnormal signal intensity.

Conclusion: In conclusion, our findings show that TRS was associated with an increase in the mean volume of right AHC in both axial and coronal planes relative to non-TRS patients.

Keywords: Treatment-resistant schizophrenia, amygdala-hippocampal complex, superior-temporal gyrus, Magnetic resonance imaging.

CHAPTER 1: BACKGROUND

1.1 Introduction

Schizophrenia is a chronic neuropsychiatric disorder that affects approximately 0.5–1% of the population (Sun *et al.*, 2015; Vita *et al.*, 2019). The symptoms associated with schizophrenia have been divided into three groups: positive symptoms (i.e. hallucinations, delusions, racing thoughts), negative symptoms (i.e. apathy, lack of emotion, poor social functioning), and cognitive symptoms (i.e. impairment in attention, memory and executive function) (Liu *et al.*, 2014). Classic symptoms of schizophrenia are typically seen in adolescence and early adulthood (Shenton *et al.*, 2010; Sun *et al.*, 2015).

Schizophrenic patients are usually treated with antipsychotic medication, but the response to antipsychotic therapy is highly variable (Vita *et al.*, 2019). Some patients with schizophrenia do not respond to antipsychotic drugs and are classified as treatment-resistant schizophrenia (TRS). It was defined in 1988 by Kane *et al.* as a condition characterised by persistent positive symptoms despite two drug trials of different chemical classes with doses equivalent to 1000mg/day of chlorpromazine for six weeks (Kane *et al.*, 2014).

There are many factors that are associated with TRS, including the abnormal neurobiology process, such as structural brain abnormalities (Rajarethinam *et al.*, 2000; Buckley, 2005; Guilherme, Rocha and Elkis, 2010). Over the last three decades, neuroimaging has been used as a powerful research tool in detecting brain abnormalities

at different stages of the disease. In modern times, magnetic resonance imaging (magnetic resonance imaging; MRI) is used to evaluate the structural brain changes in schizophrenia patients (Parkar, Seethalakshmi and Shah, 2006; Yoshida et al., 2009; Liu et al., 2014).

The temporal lobe has been a region of interest in studies on schizophrenia development (Quarantelli *et al.*, 2014; Dazzan *et al.*, 2015; Vita *et al.*, 2019). This lobe is where sound, emotions, auditory, language, certain aspects of visual perception, and speech comprehension systems are processed (Arnold, 1997). Damage to this region leads to thought disorders and hallucinations of dementia praecox (Arnold, 1997). However, neuroimaging has shown that the presence of brain volume reductions leads to schizophrenia. These volume reductions are not restricted to a single brain area but rather involve several different regions, including the prefrontal cortex, temporal cortex (amygdala, hippocampus, parahippocampal gyri, superior temporal gyri, basal ganglia, thalamus and cerebellum (Guilherme, Rocha and Elkis, 2010). Rajarethinam *et al.* reported that left amygdala-hippocampal volume loss was found in TRS patients (Rajarethinam *et al.*, 2000).

There are very limited studies available that look into the signal intensity changes on MRI scanning in schizophrenia patients. There is one preliminary study published in 2012 by Kong et al. found that the right temporal neocortex and the right amygdala appear hypointense on T1W and hyperintense on T2W in schizophrenia patients compared to healthy controls (Kong *et al*., 2012). A previous publication by Jorgensen et al. addressed the issue of signal intensity changes at the lateral temporal gyrus among schizophrenia and bipolar patients by using a contrasted MRI to measure the grey/white matter contrast ratio on T1-weighted images (Jogensen *et al.*, 2016). However, no literatures specifically

mentioned signal intensity changes in MRI brain in TRS patients in our search. Thus, this study aims to contribute a useful information on the signal intensity of AHC and STG in TRS patients which not found in the previous. study.

The advancement of magnetic resonance imaging has allowed for image acquisition with excellent soft-tissue spatial resolution. It can be used for better detection and characterisation of amygdala-hippocampal complex (AHC) and superior temporal gyrus (STG) structural changes, as well as for measurement of brain volumetry (Khashper, Chankowsky and Del Carpio-O'Donovan, 2014). There are many techniques used in obtaining brain volume. It was divided into manual, semi-automated, and automated. In our study, we used manual tracing, which is considered a gold standard for the segmentation of AHC and STG (Buckley, 2005; Daly *et al.*, 2007).

This study aims to determine if there is a significantly different of the AHC and STG in TRS compared to non-TRS patients. The focus would be on evaluating the structure, signal intensity, and volume of AHC and STG. By identifying changes in these structures using MRI, we would be able to understand the aetiology of developing treatment resistant among schizophrenia patients more clearly and could eventually develop possible preventive strategies and new treatments for TRS patients.

1.2 **Objectives**

1.2.1 General Objective

To compare MRI Brain findings between TRS and non-TRS patients.

1.2.2 Specific Objectives

1. To determine the association between anatomical and signal intensity changes in the AHC in TRS and non-TRS patients.
2. To determine the association between anatomical and signal intensity changes in the STG in TRS and non-TRS patients.
3. To compare the mean volume of the AHC in TRS with non-TRS patients.
4. To compare the mean volume of the STG in TRS with non-TRS patients.

1.3 Hypothesis

1. There is a significant association between anatomical changes and signal intensity of AHC in TRS and non-TRS patients.
2. There is a significant association between anatomical changes and signal intensity of STG in TRS and non-TRS patients.
3. There is a significant difference in the mean volume of the AHC in TRS and non-TRS patients.
4. There is a significant difference in the mean volume of the STG in TRS and non-TRS patients.

1.4 Research Question

1. What is the association between anatomical and signal intensity changes of AHC in TRS and non-TRS patients?
2. What is the association between anatomical and signal intensity changes of STG in TRS and non-TRS patients?
3. Is there any significant difference in the mean volume of the AHC between TRS and non-TRS patients?
4. Is there any significant difference in the mean volume of the STG between TRS and non-TRS patients?

CHAPTER 2: LITERATURE REVIEW

2.1 Brain

The brain is the largest organ in the human body and serves many important functions. It is composed of three main parts: cerebrum, brainstem and cerebellar (Clark, Boutros and Mendez, 2010). The largest part is the cerebrum, which consists of two cerebral hemispheres. Both cerebral hemispheres consist of the cerebral cortex and the underlying white matter, connected to each other by the corpus callosum (Clark, Boutros and Mendez, 2010).

Each cerebral hemisphere is divided into six lobes: frontal, parietal, occipital, temporal, limbic lobe, and insular (Overview of Cerebral Function - Neurologic Disorders - MSD Manual Professional Edition, 2022). Each lobe serves specific functions in the human body. Sulci separate the individual lobes. The central sulcus separates the frontal lobe anteriorly and the parietal lobe posteriorly. The lateral sulcus divides the frontal and parietal lobes with the temporal lobe, which lies inferiorly. The parietal lobe is distinguished from the occipital lobe by the imaginary line drawn from the parieto-occipital sulcus to the preoccipital notch (Buchsbaum *et al.*, 1992; Clark, Boutros and Mendez, 2010). The insula is seen under the Sylvian fissure, while the limbic lobe is the most medial structure of each cerebral hemisphere (Overview of Cerebral Function - Neurologic Disorders - MSD Manual Professional Edition, 2022).

2.1.1 Temporal lobe

The temporal lobe occupies the middle cranial fossa and lies inferior to the parietal lobe (Khashper, Chankowsky and Del Carpio-O'Donovan, 2014). It has two surfaces, the lateral surface and the medial surface. The lateral surface is formed by the superior, middle, and inferior temporal gyri delineated by the superior and inferior temporal sulci. Meanwhile, the medial temporal lobe consists of five structures: amygdala, hippocampus, uncus, dentate gyrus, and parahippocampal gyrus (Bronen and Cheung, 1991; Mark *et al.*, 1993). The temporal cortex consists of small areas involved with the auditory, olfactory, vestibular, visual senses and the perception of spoken and written language (Barta *et al.*, 1990; Buckley, 2005).

a) Amygdala-hippocampal complex

Amygdala has a crucial function in the processing of emotion. It is found anterosuperior to the temporal horn tip, just lateral to the uncus of the parahippocampal gyrus. The amygdala and uncus are inseparable on MRI (Bronen and Cheung, 1991). Meanwhile, the hippocampal complex is necessary for declarative or episodic memory (Elizabeth A Phelps, 2004; Yang *et al.*, 2021). It is composed of the cornu ammonis (hippocampus proper), subiculum, dentate gyrus, subsplenial gyrus, supracallosal gyrus, alveus, fimbria, and fornix. It is bounded by choroidal fissures superiorly (Bronen and Cheung, 1991). The entire complex protrudes into the medial wall of the temporal horn and is divided into three parts: the bulbous anterior head, the body, and the posterior tail. The notching of the head is called the pes hippocampus. The tail is seen posterior to the brain stem and consists of the cornu ammonis and dentate gyrus (Bronen and Cheung, 1991).

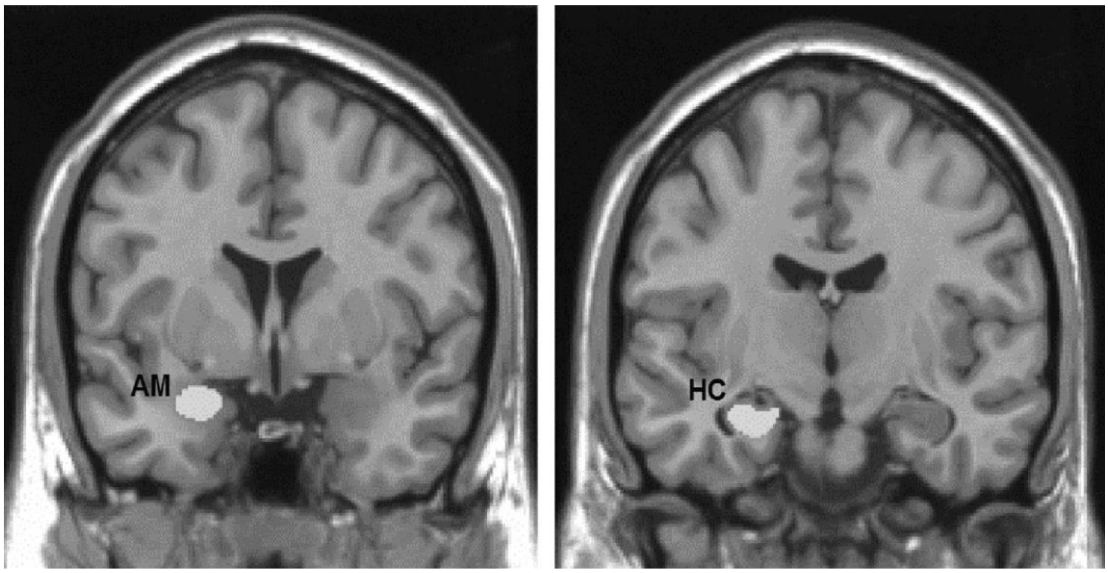

Figure 1: Bilateral amygdala-hippocampal complex in coronal view (Scorzin *et al.*, 2008)

b) Superior temporal gyrus

The superior temporal gyrus (STP) is a lateral temporal lobe structure and is further subdivided into two surfaces, the lateral surface and the dorsal surface (Kim *et al.*, 2004). The STP is in the Sylvian fissure. The upper surface of STP has a transverse temporal gyrus or Heschl gyrus (HG), which contains the primary auditory cortex that plays a significant role in translating and processing all sounds and tones (Daly *et al.*, 2007; Yoshida *et al.*, 2009). There is an important area adjacent to the STP called Wernicke's area, which is responsible for speech perception and comprehension (Yoshida *et al.*, 2009; Shenton *et al.*, 2010; Kubera *et al.*, 2014). The lateral surface of the STG is thought to be the secondary auditory cortex that also plays functions in interpreting sounds (Kim *et al.*, 2004; Daly *et al.*, 2007).

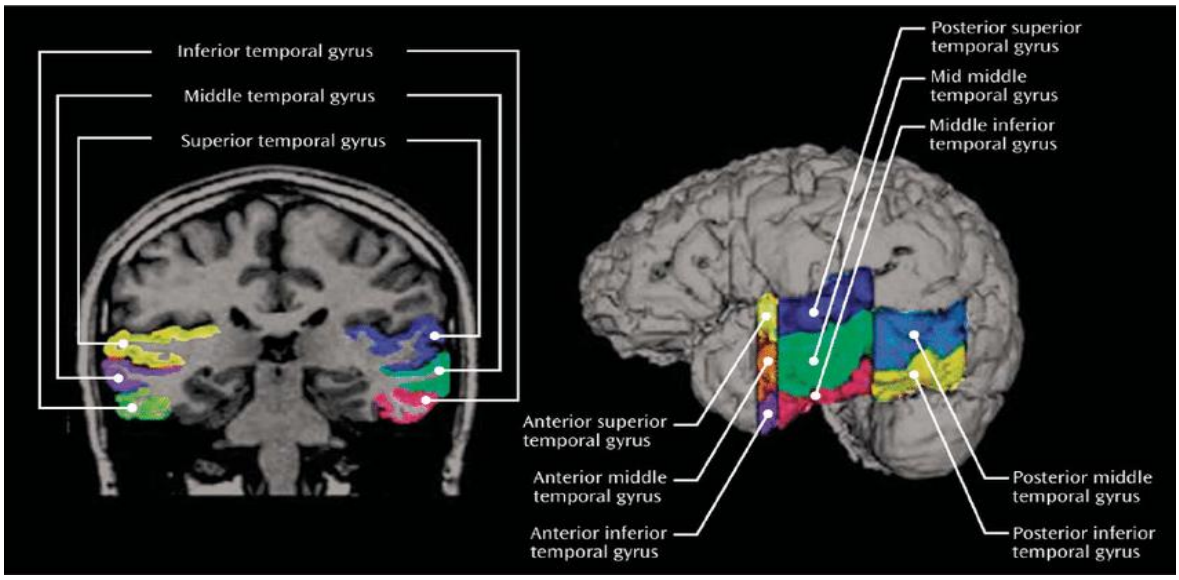

Figure 2: Superior temporal gyrus (Kuroki et al., 2007)

2.2 Schizophrenia

Schizophrenia is a chronic, disabling mental disorder that affects approximately 0.5–1% of the population (Jinhua Sun *et al.*, 2009; Zugman *et al.*, 2013). Symptoms of schizophrenia are typically seen in adolescence and early adulthood (J Sun *et al.*, 2009; Jinhua Sun *et al.*, 2009; Shenton *et al.*, 2010). The symptoms of schizophrenia might be variable at the presentation and are categorised as positive, negative, or cognitive symptoms (Liu *et al.*, 2014). The symptoms of schizophrenia involve a range of cognitive, behavioural, and emotional dysfunctions, but no single symptom is pathognomonic of schizophrenia (Li *et al.*, 2012; Mouchlianitis, Mccutcheon and Howes, 2018). The clinician must recognise the symptoms in order to distinguish schizophrenia from other psychotic disorders (Patel *et al.*, 2014).

Schizophrenia is part of the schizophrenia spectrum and other psychotic disorders. Others include schizotypal (Personality) disorder, delusional disorder, brief psychotic disorder, schizophreniform disorder, schizoaffective disorder, substance/medication-induced psychotic disorder, and psychotic disorder due to another medical condition (Schizophrenia, 2007; Patel *et al.*, 2014; Wheeler and Voineskos, 2014). They are defined by abnormalities in one or more of the following five domains: delusions, hallucinations, disorganised thinking or speech, grossly disorganised or abnormal motor behaviour (including catatonia), and negative symptoms (ZamZam *et al.*, 2011).

According to DSM-5, patients will be diagnosed with schizophrenia when they fulfil the diagnostic criteria below (Adapted from American Psychiatric Association. (2013), *Diagnostic and statistical manual of mental health disorders* (5th ed.):

A. Two (or more) of the following symptoms present for a significant portion of time for one month (or less if successfully treated). At least one of these should include (1), (2), or (3):
    1) Delusions
    2) Hallucinations
    3) Disorganised speech
    4) Grossly disorganised or catatonic behaviour
    5) Negative symptoms (i.e., diminished emotional expression or avolition)
B. One or more major areas of functioning, such as work, are markedly below the level achieved before the onset.
C. Continuous signs of the disturbance persist for at least six months. This six-month period must include at least one month of symptoms (or less if successfully treated).

D. Schizoaffective disorder and depressive and bipolar disorder with psychotic features have been ruled out.
E. Substance abuse or other general medical condition are excluded.
F. Suppose there is a history of autism spectrum disorder or other communication disorder of childhood onset. In that case, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least one month (or less if successfully treated).

2.3 Treatment-resistant schizophrenia

Schizophrenia patients are treated with antipsychotic treatment. However, the response to antipsychotic drugs is highly variable. The study shows that approximately 30% of the patients have a poor response to the treatment and continue to have persistent symptoms (Conley and Kelly, 2001; Farooq *et al.*, 2013; Patel *et al.*, 2014). These patients are classified as treatment-resistant schizophrenia (TRS). A study done by Kane *et al.* in 1988 characterised TRS patients as those who show no improvement of positive symptoms (delusions, hallucinations, disorganised speech and behaviour) despite adequate antipsychotic medications for an adequate duration (Kane *et al.*, 2014).

According to Kane *et al.*, to establish the diagnosis of TRS, the patients must demonstrate all these criteria (Kane *et al.*, 2014):

- At least two trials with drugs of different chemical classes with doses equivalent to 1000mg/day of chlorpromazine for six weeks without significant relief.
- No period of good function in the preceding five years.

2.4 Brain changes in treatment-resistant schizophrenia on MRI

Literature reviews have highlighted that TRS patients have alterations in the neurobiology process (Phelps, 2004; Demjaha *et al.*, 2014; Liu *et al.*, 2014; Tarcijonas and Sarpal, 2018). This process occurs to a greater degree in TRS patients than in non-TRS patients, so antipsychotic treatment has little benefit to this group. A cross-sectional study has found an association between brain abnormalities and poor outcomes, including in chronic schizophrenia patients (Wheeler and Voineskos, 2014; Vita *et al.*, 2019).

A few previous studies also show progressive volume reduction of bilateral temporal lobes and hippocampus in TRS patients compared to non-TRS (Jinhua Sun *et al.*, 2009; Narayanaswamy *et al.*, 2015; Mouchlianitis, Mccutcheon and Howes, 2016; Yang *et al.*, 2021). In the study done on TRS patients by Yoshida *et al.*, the left anterior AHC, left parahippocampal gyrus, and left STG are significantly smaller in this group (Selby, Joe V., Friedman, Gary D., Quesenberry Charles P., Weiss, 1992; Yoshida *et al.*, 2009). They also highlighted the association between the volume reduction of STG with thought disorder score. In another study, volume reduction in STG has been associated with a degree of auditory hallucination (Barta *et al.*, 1990; Nakajima *et al.*, 2015; Ganella *et al.*, 2016).

There are very limited studies available that look into the signal intensity changes on MRI scanning in schizophrenia patients, specifically in TRS patients. A previous publication by Kong et al. found prolonged T1 and T2 relaxation time in the right temporal neocortex and the right amygdala. Both structures appear hypointense on T1W and hyperintense on T2W in schizophrenia patients compared to healthy controls (Kong

et al., 2012). There is one preliminary study published in 2016 by Jorgensen et al. addressed the issue of signal intensity changes at the lateral temporal gyrus among schizophrenia and bipolar patients by using contrasted MRI to measure the grey/white matter contrast ratio on T1 weighted images (Jogensen *et al.*, 2016). They reported that there is increased grey/white-matter contrast on T1 in a few sensory and motor regions including in the STG, among the schizophrenia patients his team studied on. Alteration of the signal intensity in both grey and white matter in schizophrenia patients can also happen due to changes in myelin content (Jogensen *et al.*, 2016).

Neuroimaging studies such as MRI have been widely used to detect the underlying neurobiology abnormality such as structural, signal intensity, and volume changes resulting in such resistance and identify these patients earlier (Guilherme, Rocha and Elkis, 2010). Compared with computed tomography (CT), MRI has higher sensitivity, exceptional soft-tissue contrast, multiplanar imaging capability, and lack of ionising radiation, making it a primary modality of choice in evaluating TRS patients (Narayanaswamy *et al.*, 2015; Robert, Eisenhauer and Altschuler, 2015).

All the studies mentioned above were done mainly using the 1.5 Tesla MRI machine. We are moving from 1.5 Tesla to 3 Tesla machines, which have a better signal-to-noise ratio. Therefore, we expect better image resolution and be able to appreciate more changes in the AHC and STG. Quantitative MR imaging is capable of delineating the AHC and STG more precisely than those achieved with visual inspection in clinical MR imaging studies. The combination of quantitative techniques (T1 volumetric with inversion-recovery) and FLAIR will optimise the diagnosis of TRS by using MRI. The AHC and STG volumes are manually traced on anatomical images (Kim *et al.*, 2004).

2.5 Conceptual framework

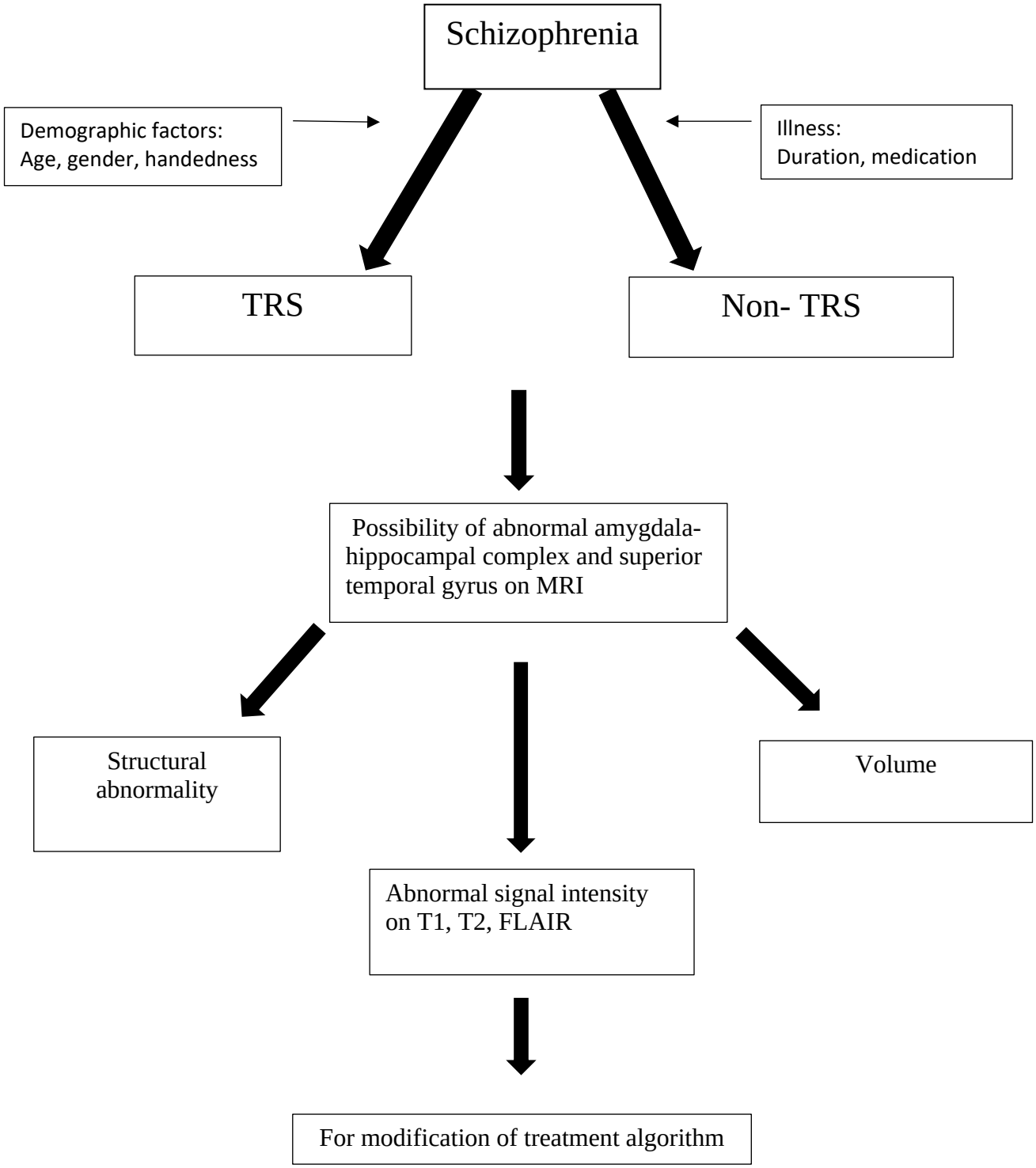

Figure 3: Conceptual framework

2.6 Study rationale

Schizophrenia is a major mental disorder that affects approximately 0.5–1% of the population. There is an increase in the number of patients diagnosed with schizophrenia not only in Malaysia but globally. Despite the increasing number of schizophrenia patients, limited studies exploring brain changes in TRS patients are currently available.

This study aims to determine whether any structural and signal-intensity brain abnormality is detected on MRI in treatment-resistant schizophrenia patients compared to non-TRS. In this study, we are focusing on the anatomical changes in the AHC and STG because the structural changes in these two structures are important in the pathophysiology of TRS, and the earliest brain changes after treatment can be detected in both AHC and STG. By identifying changes in those structures from the neuroimaging, we would be able to understand more clearly the aetiology of TRS and could eventually develop possible preventive strategies. Besides that, we can also consider the possibility of developing new treatments for TRS patients.

The data for this study may help in future research related to TRS. Hopefully, this study will add useful information to the limited knowledge available concerning brain changes in TRS patients. Furthermore, this pilot study may help determine the feasibility of recruitment and new methods in assessing brain structural involvement in TRS and non-TRS patients prior to a larger-scale study.

CHAPTER 3: METHODOLOGY

3.1 Research Design

This research is a comparative cross-sectional study using MRI comparing brain findings between TRS and non-TRS patients in Hospital Universiti Sains Malaysia (HUSM) from 1 September 2020 until 31 October 2021.

3.2 Sample Population

- Reference population – Schizophrenia patients who get treatment in Kelantan.
- Target population – Schizophrenia patients diagnosed as TRS and non-TRS in HUSM.
- Source population – Schizophrenia patients who get treatment in HUSM.
- Sampling frame – Schizophrenia patients diagnosed as TRS and non-TRS in HUSM and fulfil the inclusion and exclusion criteria.

3.3 Sample Size Calculation

1. Objective 1 & 2

   Our literature review could not elicit any study examining the association between anatomical and signal intensity changes in the AHC and STG in TRS and non-TRS patients using MRI.

2. Objective 3 & 4

Since the study is to find associations and compare the difference between TRS and non-TRS patients, the sample size was calculated using the formula for two independent means (Najib my, 2015).

The calculation is based on the study by Lawrie et al., which found a significant association between amygdala-hippocampal complex and superior temporal gyrus changes in TRS and non-TRS (Lawrie *et al.*, 1995). According to Cocks and Torgerson's research, a pilot trial should have at least 9% of the sample size of the main planned study (Cocks and Torgerson, 2013).

Table 1: Sample size calculation

Parameter	Effect Size, d	Sample size, n	Adjusted Sample Size, n+ 10%	Source	Sample Size for Pilot Study, n x 10%	Source
Whole-brain	0.40	198	220	Lawrie *et al.*, 1995	22	(Cocks and Torgerson, 2013
Left temporal lobe	0.40	198	220		22	
Right temporal lobe	0.50	126	150		15	
Left hippocampus	0.30	350	390		39	
Right hippocampus	0.30	350	390		39	

Table 2: Sample size calculation for two independent means.

Sample Size Calculator for Two Independent Mean (based on effect size)	
Instruction: Enter values in green cells Read output in gray cells	
Effect size, d =	0.3
Type I error, α =	5.0%
Number of tail, τ =	2
Type II error, β =	20.0%
(power of study =)	80.00%
Ratio between conrol to case, m =	1
Sample size calculated, n =	175
Sample size for control =	175
Sample size for case =	175
Total sample size =	350
Anticipated dropout rate =	10.0%
Corrected sample size, n_c =	195
Sample size for control =	195
Sample size for case =	195
Total sample size =	390

The proposed sample size for this study is 39. However, in this study, we could only recruit 30 participants (14 for TRS and 16 for non-TRS) due to budget limitations (please refer to the budget proposal). Post- hoc power was calculated after the analysis to measure the power of the study.

3.4 Sampling Method

A convenience sampling technique was used for this study. All patients who received treatment in the HUSM's psychiatric clinic and ward and fulfilled the inclusion and exclusion criteria were recruited for the study. The data was collected by exporting the subjects' images from the PACS system into Philips Intellispace Portal software for further data analysis.

3.5 Subject Criteria

In this study, all the TRS and non-TRS patients who fulfilled the inclusion and exclusion criteria were referred by the psychiatrist to our research team. We gave a preview of the procedures and research activity before taking consent from patients/relatives.

3.5.1 Inclusion Criteria

1. All patients diagnosed with schizophrenia
2. Age between 20 to 59 years old

3.5.2 Exclusion Criteria

1. Patients with intellectual disability
2. Patients who substance-abuse
3. Patients with cognitive disorders (dementia, delirium)
4. Patients with other central nervous system organic diseases (epilepsy, brain trauma)
5. Severe or unstable somatic disease (malignant disease, autoimmune disease)
6. Patients with contraindications to MRI.

3.6 Research tools

1. MRI Machine - Philips 3 Tesla Achieva MR Scanner, Best, The Netherlands
2. Workstation – Philips MR Release 5.4 Achieva TX 3.0.
3. BARCO MDNC-3421 V1.00.03.
4. Philips Intellispace Portal software for measuring the brain's regional volume

3.7 Operational Definition

- Treatment-resistant schizophrenia (TRS) (Kane *et al.*, 2014)
    - TRS is characterised by no improvement of positive symptoms (delusions, hallucinations, disorganised speech and behaviour) despite adequate antipsychotic medications with adequate duration.
    - To establish the diagnosis of TRS, the patients must demonstrate all these:
        a) At least two drug trials of different chemical classes with doses equivalent to 1000mg/day of chlorpromazine for six weeks without significant relief.
        b) No period of good function in the preceding five years

- Non-treatment-resistant schizophrenia (Non-TRS)
    - Patients that show improvement of positive symptoms with antipsychotic medications.

- Amygdala-hippocampal complex (AHC) (Bronen and Cheung,1991)

    - the amygdala is crucial for emotion processing, while the hippocampal complex is necessary for declarative or episodic memory.
    - Both are medial temporal lobe structures
    - Hippocampal complex:
        - Composed of the cornu ammonis (hippocampus proper), subiculum, dentate gyrus, subsplenial gyrus, supracallosal gyrus, alveus, fimbria, and fornix
        - Bounded by choroidal fissure superiorly
        - The entire complex protrudes into the medial wall of the temporal horn,
        - Divided into three parts- the bulbous anterior head, the body, and the posterior tail.
        - The notching of the head is called the pes hippocampus.
        - The tail is seen posterior to the brain stem
        - Appears as two U-shaped interlocking grey matter structures on coronal sections, consisting of the cornu ammonis and dentate gyrus
    - Amygdala:
        - Found anterosuperior to the temporal horn tip
        - Just lateral to the uncus of the parahippocampal gyrus
        - The amygdala and uncus are unseparated on MRI

- Superior temporal gyrus (STG) (Kim *et al.*, 2004)
    - Is a lateral temporal lobe structure and is further subdivided into two surfaces, the lateral surface and the dorsal surface.
    - Is a long structure, located along the Sylvian fissure dorsally and the superior temporal sulcus (STS) ventrally.
    - Plays an important role in the production and interpretation of language and communication.

3.8 Data Collection

3.8.1 Patient cohort

This cross-sectional study was conducted in Hospital USM, Kota Bharu, Kelantan Malaysia, for one year, from November 2020 to October 2021. We included 14 subjects treated as TRS and 16 non-TRS patients (control group) to undergo MRI brain imaging. Subjects were screened against the inclusion/exclusion criteria. Subjects/caretakers who agreed to participate were asked to sign written informed consent forms. This study obtained ethical approval from the Human Research Ethics Committee of USM (JEPem code: USM/JEPem/20010070).

3.8.2 MRI Protocol

All subjects undergo imaging in the MRI room in the Radiology Department, HUSM using Philips 3 Tesla Achieva MR scanner, Best, The Netherlands. The imaging took about 45 minutes to 1 hour. In cases where the patient was not cooperative, sedation IV midazolam was given to the patient prior to examination by the psychiatric team.

The side effects of IV Midazolam, such as nausea, vomiting, hypotension, or respiratory depression, were explained to the subject/caretaker (Gibson, 2018). The subjects only need to undergo one-time MRI scanning. All subjects undergo a similar non-contrasted epilepsy protocol. Subjects were positioned on the gantry with a standard head coil (SENSE-HEAD-32). Scout sequences were obtained to ensure subjects' heads were adequately in the field of view. Sequence parameters are listed in the table below. The patients were monitored in the MRI recovery bay post-procedure for at least 30 minutes.

Table 3: MRI brain sequences for the images used in the study.

	Slice thickness (mm)	FOV (mm)	TR/TE (ms)
Sagittal T1-weighted	5	230 x 183 x 143	500/10
Axial T1-weighted	5	230 x 183 x 154	600/10
Axial T2-weighted	5	230 x 184 x 143	3000/80
3D Axial FLAIR	321	250 x 250 x 180	4800/340
Oblique coronal T1 IR	3	200 x 209 x 79	600/100
Oblique coronal TSE-T2	5	79 x 159 x 200	1987/100
Oblique coronal FLAIR	5	230 x 183 x 143	600/10
DTI	55	240 x 240 x 138	7562/73

3.8.3 Image viewing

The data was collected by viewing T1W, T2W, and FLAIR sequences using the Picture Archiving and Communication System (PACS) (PACS Universal Viewer Version 5.0 SP6). The monitor used is BARCO MDNC-3421 (V1.00.03) monitor. Subjects' images were then transferred from PACS systems into Philips Intellispace Portal 9.0 Software (version 9.0) for volumetric measurement.

3.8.4 Image analysis and Manual tracing of AHC and STG volumes:

Image analysis and measurements were performed using a PACS and Philips Intellispace Portal 9.0 Software (version 9.0), which were available in MRI Suite, Radiology Department, Hospital Universiti Sains Malaysia. The MR imaging sequence used for qualitative visual analysis (structural abnormality and signal intensity) was the oblique coronal T2 weighted image. Thresholds were selected for each structure to optimise grey-white contrast, and the same thresholds were used for all patients. For volumetric analysis, the DTI and 3D axial FLAIR sequences were used to measure the volumes of each structure. Each structure was measured in two dimensions. For AHC, it was measured in axial and coronal views; meanwhile, for STG, it was measured in sagittal and coronal views. The volumetric analysis was performed by a single observer and was validated by an experienced radiologist with more than five years of experience. The analysts were blinded to subjects. The intra-class correlation was performed for measurement validation.

The bilateral AHC and STG volumes were measured by manually tracing those structures using landmarks, as shown in Figures 6 to 9. It was based on a previous study (Pruessner, J. C.,2000)