UNIVERSITI PUTRA MALAYSIA

CORRELATION BETWEEN THE VOLUME OF MRI BRAIN INFARCT AND INFLAMMATORY MARKERS AMONG ACUTE ISCHEMIC STROKE PATIENTS IN HPUPM

GROUP 29

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Executive Summary

Introduction: Stroke is one of the top five leading causes of death in Malaysia and increases in the mortality rate between 2016 and 2019. There is growing evidence that inflammation plays an important role in acute ischemic stroke. The level of inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are strongly correlated with the volume of brain infarct detected using Magnetic Resonance Imaging (MRI). These laboratory and radiological findings will eventually predict the mortality and recurrent vascular events in patients with acute ischemic stroke. **Objective**: This cross-sectional study conducted in Hospital Pengajar Universiti Putra Malaysia aims to determine the correlation between the volume of MRI brain infarct with inflammatory markers among patients with acute ischemic stroke in HPUPM. **Methodology**: Patients' sociodemographic information and level of inflammatory markers were extracted from the Hospital Information System (eHIS), the MRI images were accessed through the Picture Archiving and Communication System (PACS) whereas the MRI reports for each sample were searched from the Reporting Information System (RIS). In addition, the MRI sequences DWI/ADC and FLAIR axial were selected. The findings of the site and size of brain ischemia were studied. **Results:** This study was based on 60 data sets of patients diagnosed with acute ischemic stroke in Hospital Pengajar Universiti Putra Malaysia, Serdang, Selangor. The majority of the patients were male (68.3%), Malay (51.7%) and ranging from age group 61-75 years old. Most underlying medical conditions recorded among the patients are hypertension (73.3%), dyslipidemia (50%) and diabetes mellitus (50%). Patients admitted to the hospital with markedly high CRP level. The ESR level was normal, the most recorded among the 28 AIS patients. Middle cerebral artery infarct was the most affected area among the patients (38.3%) and the mean volume of MRI brain infarction is 27.3268 ± 55.6957cm3. A statistically significant

association was found between CRP and volume of brain infarction whereas no association was found between ESR and volume of brain infarction.

Conclusion: A significant association was found between CRP and volume of brain infarction. No association was found between ESR and volume of brain infarction.

Recommendation: We would like to suggest other researchers to involve larger sample sizes with longer duration and also include with another stroke medical centre in Malaysia

Keywords: acute ischemic stroke, inflammatory maker, volume brain infarct, MRI

CHAPTER 1

INTRODUCTION

1.1 Background

Stroke is a medical condition that increases in incidence and causes disability not only in Malaysia but worldwide. It can affect various ages or sex, with underlying risk factors, comorbidities (hypertension diabetes, hyperuricemia, dyslipidemia, previous TIA, smoking, cardiac troubles, etc.) [1] or even without any underlying health problem. According to the World Health Organization (WHO), stroke is the second most major cause of death after heart disease, involving almost seven million deaths in 2012 worldwide, which is 11.1% of total deaths [2].

Incidents of stroke recorded the most in east Asia, followed by the eastern European region, whereas the lowest rates were in central Latin America. Based on previous research, in Malaysia, stroke is one of the top five leading causes of death after ischemic heart disease, septicaemia, malignant neoplasm, and pneumonia [3]. There was also an increase in the overall stroke mortality rate between 2016 and 2019; from 74-105 Standardized Mortality Rate (SMR)/ 100,000 [4].

To diagnose acute ischemic stroke, several tests will be done carefully to confirm and determine the cause of it. The test includes a physical examination, blood test, and also neurological examination. During the physical examination, blood pressure, mental alertness, numbness or weakness, or trouble speaking, seeing, or walking will be examined. Through blood tests, much information regarding the causes of stroke symptoms could be obtained. This is also including the level of inflammatory markers such as C-Reactive Protein (CRP), Electrolyte Sedimentation Rate (ESR), and also Platelet Volume (PV). Using these

inflammatory markers, we can picture the prognosis and decide the best treatment for the patients. Along with all those procedures, a neurological examination is also essential since any bleeding or blood flow problem can be seen using Magnetic Resonance Imaging (MRI). It can also rule out other problems such as tumours that patients usually come with the same symptoms as a stroke.

1.2 Problem statement

Stroke represents a global health issue that remains a major cause of death among the population with a significant morbidity rate, making it the second major cause of death worldwide as well as a leading cause of disability. [5] Prognostic markers could potentially be beneficial in the management of patients who have suffered from a stroke. The prognosis of stroke depends on the etiology, location, and size of the infarction. Markers such as epicardial fat tissue (EFT), neutrophil-lymphocyte ratio (NLR) and Von Willebrand factor (VWF) have been previously studied to be able to predict stroke severity and prognosis. [6] Large infarct volumes which can be measured using MRI can explain death in the first months after stroke, whereas lesions of specific supratentorial structures, mostly in the left hemisphere, also contribute to poor functional outcomes. [7] Thus, establishing a association between the volume of MRI brain infarct and laboratory markers such as inflammatory markers CRP and ESR represents a valuable area of research.

1.3 Significance of the study

This research focuses on the significance of the correlation between the volume of MRI brain infarct with inflammatory markers among acute ischemic stroke patients. Thus, it will enable us to understand the importance of the inflammatory markers used in patients with stroke. This research plays a fundamental role in allowing people to learn about how

strongly the inflammatory markers correlate with stroke severity and independently predict mortality and recurrent vascular events in patients with acute ischemic stroke. Fundamentally, the goal of this study is to clarify the relationship between inflammatory markers and stroke severity using the volumetric measurement of infarct size.

1.4 Research questions

The main research questions examined may be expressed as follows:

I. What are the demographic factors (age, sex and race), risk factors and inflammatory markers in the patient presenting with acute ischemic stroke?
II. Where is the site of the brain infarct and how to measure the volume of the brain infarct?
III. What is the association between demographic factors and inflammatory markers?
IV. What is the correlation between inflammatory markers with the volume of MRI brain infarct?

1.5 Study objective

1.5.1 General objectives

To determine the association between the volume of MRI brain infarct with the inflammatory marker (CRP and ESR) among acute stroke ischemia patients.

1.5.2 Specific objectives

I. To describe the demographic factors (age, sex), smoking, medical conditions (diabetes mellitus, dyslipidemia and hypertension) and inflammatory markers in the patient presenting with acute stroke

II. To describe the site of brain infarct and measuring the volume of brain infarct.

III. To determine the association between a demographic factor and inflammatory markers

IV. To determine the correlation between the level of inflammatory markers with the volume of MRI brain infarct.

1.6 Hypothesis

1.6.1 Null Hypothesis

There is no significance in the correlation between the volume of MRI brain infarct with inflammatory markers among acute ischemic stroke patients in HPUPM.

1.6.2 Alternative hypothesis

There is a significance in the correlation between the volume of MRI brain infarct with inflammatory markers among acute ischemic stroke patients in HPUPM.

CHAPTER 2

LITERATURE REVIEW

2.1 Acute Ischemic Stroke

2.1.1 Definition and classification of stroke

Stroke is a clinical syndrome characterized by rapidly developing clinical syndromes and/or signs of focal and at times global, loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin. [8] However, from another study by Sacco et al, stated that stroke is a neurological deficit attributed to an acute focal injury of the central nervous system (CNS) by a vascular cause. [9] This condition happens when there is blockage of blood to some part of the brain causing brain cell death and loss of some neural abilities [10]. There are two main types of strokes which are ischemic stroke and haemorrhagic stroke. Ischemic stroke is due to insufficient blood flow, thrombosis, or even embolism. Meanwhile, a haemorrhagic stroke happens because of bleeding in the brain.

An ischemic stroke is a clinical syndrome that is characterized by an acute loss of focal cerebral or monocular function with symptoms lasting less than 24 hours and may be because of inadequate cerebral or ocular blood supply as a result of blockage of the vessel [8].

2.1.2 Etiology of acute ischemic stroke

The etiology of acute ischemic stroke can be due to either a thrombotic or embolic event. In the case of thrombosis, the dysfunction lies within the blood vessel itself in which blood flow to the brain is obstructed. In the case of embolism, the blood flow in the vessel is obstructed due to debris from elsewhere in the body. [11]

Ischemic stroke can also be classified as large vessel stroke, small vessel stroke (Lacunar stroke), and cardioembolic stroke. [12] Atherothromboembolism, intracranial small vessel disease, and cardiogenic embolism is said to be the main causes of ischemic stroke. Other causes include arterial dissection, trauma, vasculitis (primary/secondary), metabolic disorders, congenital disorders, and other less common causes such as migraine, pregnancy, oral contraceptives, etc. [8]

The atherothrombotic plaque can grow to obstruct a vessel and cause occlusion or embolization which occludes smaller distant vessel(s). Lacunar strokes occur often due to the involvement of smaller or perforating blood vessels supplying the deeper structures of the brain. [12] Cardiogenic embolism is most commonly due to atrial fibrillation and valvular heart disease. [8]

2.1.3 Pathophysiology of acute ischemic stroke

Ischemic stroke's main mechanism is the reduction of blood flow to the brain, which leads to injury to the tissue. The insult is usually focal due to the complex vessels of the brain. When injury to the brain tissue occurs, the central region receives almost no blood flow which leads to rapid death of that area within minutes. The area surrounding this region is termed the "penumbra", in which the tissue is still not completely dead. The duration in which the tissues of the penumbra can survive is very limited. Thus, clinicians need to restore blood flow to the brain within this period.

The cell death that occurs in the centre area can be explained by the continued usage of adenosine triphosphate (ATP) by the neurons. However, the synthesis of the adenosine triphosphate (ATP) is insufficient. This leads to a decreased level of adenosine triphosphate (ATP) and an increased level of adenosine diphosphate (ADP). Furthermore, the disturbance of the ionic homeostasis leads to lactic acidosis. These events represent the genesis for the

cascade of ischemic events which include multi step and multi cell downstream mechanisms. Neurotransmitter release and inhibition of uptake are also involved in the mechanisms of tissue injury in the brain. The main neurotransmitter involved is glutamate, the main excitatory neurotransmitter involved. Glutamate can bind to ionotropic N-Methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (iGluRs), which causes a major influx of calcium. When the receptors are hyperstimulated, the massive influx of calcium activates phospholipases, lipases, nucleases, and proteases that destroy the essential proteins and membranes of the cell. Glutamate can also cause water and high sodium influx which leads to cell swelling and oedema and ultimately shrinks the extracellular space. [13]

The events outlined above results in an increased production of free radicals through the mitochondria as well as other sources such as degradation of hypothanxine and prostaglandin synthesis. The free radicals will damage nucleic acid, carbohydrates, lipids, and proteins. The severity of the effect of the reactive oxygen species is exacerbated due to the inability of antioxidant enzymes and scavenging mechanisms to counteract the toxicity. Other mechanisms of neuronal death include lipoxygenase cascade, polyADP-ribose polymerase (PARP), mitochondrial transition pore formation, and amplified ionic imbalance. The amplified ionic imbalance is due to the secondary recruitment of calcium-permeable Transient Receptor Potential Melastatin (TRPM) ion channels. Along with ROS, reactive nitrogen species can modify the functions of proteins, in which the proteins may function to produce neuroprotective effects. These cascades of events lead to cell necrosis, apoptosis, and autophagy which ultimately lead to neuronal death. [13]

White matter also represents a factor of ischemic stroke. The white matter of the brain receives significantly less blood flow compared to the grey matter, with little collateral blood

supply. This results in increased severity of ischemia in this area as well as tissue edema and rapid cell swelling. Furthermore, it activates many proteases, which further weaken the myelin sheath and the structural integrity of axons. [14]

The inflammatory response in ischemic stroke is particularly interesting due to its significance in this proposed research. The inflammation cascades initiate primarily after the stroke, all over the ischemic area. The modulation of the immune system may lead to a decreased infarct size. Important inflammatory cells include microglial cells, macrophages/monocytes, neutrophils, and T-cells. Microglial cells, which increase in the infarcted area, appear to be the most significant inflammatory cell involved. Its production of neurotrophic substances including Brain-Derived Neurotrophic Factor (BDNF), insulin-like growth factor I (IGF-I), and other growth factors provide neuroprotective effects. However, microglial cells also release several pro-inflammatory cytokines which include interleukin-1β (IL-1β), TNF-α, and IL-6, along with Nitric Oxide (NO), ROS, and prostanoids which can be destructive to neighbouring tissues. These cells can also possibly cause more harm by recruiting other inflammatory cells to the penumbras. Inflammatory cells such as IL-6 and Toll-like receptor 4 have been suggested for the assessment of the severity of stroke. [15]

2.1.4 Demographic and medical factors associated with acute ischemic stroke

Ischemic stroke is associated with risk factors which may be modifiable or nonmodifiable. Nonmodifiable demographic factors include age and sex in which age affects the proportion of men and women developing ischemic stroke. Women have a high or higher risk of stroke compared to men at younger ages, however, at older ages the relative risk is slightly higher for men. [16] According to the Malaysian National Stroke Registry, among 11,284 cases of ischemic stroke cases reported from 2004-2016, 55% were male. They also found that in almost all age stratifications, the number of men transcended women except for age over 70

years old. With regards to age, generally, as age increases the risk of stroke increases. The Malaysian National Stroke Registry also reports the mean age for stroke in Malaysia to be 62.5 years with a standard deviation of 12.5 years. [17]

Many lifestyle factors and genetic factors play a role in the development of chronic diseases such as hypertension, diabetes mellitus and dyslipidemia. The presence of these conditions may predispose an individual to develop ischemic stroke. Hypertension is the most significant risk factor of stroke with a strong, direct, linear, and continuous relationship between blood pressure and stroke risk. Diabetes mellitus is also another risk factor of stroke, with the risk of stroke doubling in patients with diabetes mellitus. [16,19] Smoking exhibits a strong dose–response relationship between the number of cigarettes smoked daily and ischemic stroke. [18] Dyslipidemia and its association with stroke are complex, with increased total cholesterol increasing risk for ischemic stroke and elevated high-density lipoprotein cholesterol decreasing the risk for ischemic stroke. [20] The Malaysian National Stroke Registry also reports hypertension (67.0%), diabetes (39.6%), cigarette smoking (25.2%), and hyperlipidemia (23.0%) to be the commonest risk factors in their stroke population. [17]

2.2 Role of inflammation and inflammatory markers involved in acute ischemic stroke

2.2.1 Inflammation

Inflammation is a process triggered by tissue injury secondary to infection, trauma, or ischemia and orchestrated by leukocytes and several molecules belonging to different mediators such as cytokines, chemokines, and adhesion molecules in an attempt to restore tissue homeostasis. Inflammation plays an important role in the pathogenesis of acute ischemic stroke.

Process of Inflammation can be an appropriate potential target for assessment of acute ischemic stroke, prognosis, and its management. Based on a previous study, the study of pre-clinical stroke shows that inhibition of inflammatory response could lead to the decline of brain injury and improve neurological outcomes. [21] For clinical studies, systemic inflammation causes the vulnerability of patients to stroke and following prognosis. A previous study stated that demographic factors, such as ethnicity also can influence the relationship. [22]

Several inflammatory markers are commonly used in primary care for diagnosis and monitoring of inflammatory conditions which are C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and plasma viscosity (PV). In this study, we aimed to determine the influence of the level of CRP and ESR in predicting the prognosis in patients with acute ischemic stroke.

2.2.2 Process of inflammation in ischemic stroke

As stated before, an ischemic stroke happens when there is a transient or permanent reduction in local cerebral blood flow and the most often area affected is the middle cerebral artery. [23] Based on some research, focal cerebral ischemia creates time-dependent recruitment and activation of inflammatory cells. The inflammatory cells that cause this inflammation process are neutrophils, T cells, and macrophages, and slow down the inflammatory response, decrease infarct size and enhance neurological deficit in experimental stroke.

Phases of inflammation after ischemic stroke can be divided into acute (minute to hours), subacute (hours to days), and chronic (days to months) phases. For acute phases, Reactive Oxygen Species (ROS) proinflammatory chemokines which are cytokines and chemokines

are secreted rapidly from injured cerebral tissue into the extracellular compartment. [23] These mediators stimulate brain endothelial cells and cause the expression of adhesion molecules on the cerebral endothelium. Then, it will promote the adhesion and transendothelial migration of circulating leukocytes from the blood-brain barrier into the brain parenchyma. For the subacute phase, penetrating leukocytes secrete cytokines and chemokines, ROS, Matrix Metalloproteinase (MMP) which then emphasize the brain inflammatory responses and then lead to more activation of brain resident cells and penetration of leukocytes, eventually cause damage of the BBB, brain oedema, neuronal death and haemorrhagic transformation. [24] However, these proinflammatory factors play a dual role at the early and late stage which will promote tissue repair and be useful in recovery during the chronic phase after chronic stroke.

From all these processes, white blood cells which are macrophages and T cells will be secreted and cause CRP rise in circulation due to inflammation. Meanwhile, for ESR, high proportions of fibrinogen circulating in the blood during inflammation caused red blood cells to stick to each other. Thus, the level of ESR is also elevated during acute ischemic stroke.

2.2.3 C-reactive protein in acute ischemic stroke

CRP is an acute-phase reactant that is produced in response to tissue injury or any infection. This inflammatory marker is involved in the pathophysiological process during the early response to brain injury. [1] CRP has prothrombotic properties and will increase platelets activity. Contrarily, CRP and platelet adhesion were stated as factors that influencing the severity of the cardiovascular disease. Some studies stated that CRP has been related to stroke recurrence [25] while others do not say so. [26] Dahshan et al stated that levels of CRP and cell adhesion molecules showed elevation in stroke patients at onset [1] and may stay stable for more than 28 days. [27] Purfoy et al suggested that the higher level of CRP

observed, the more useful it to be used as markers to predict more severe ischemic disease in the future. [28]

Even though some studies reported that there is no association between the level of CRP and the clinical outcome of ischemic stroke [29-31], many studies also show contradicting results. [32]

Caia et al. (2019) reported that the CRP level that was taken during admission was related to neurological deterioration and poor prognosis. This shows that the higher the level of CRP during admission, the more neurological deterioration and resulting in more unfavourable prognosis. [10] This statement has also been proven by various researchers to show that CRP level during admission can predict next stroke recurrence in patients with acute ischemic stroke. [33,34]

Moreover, levels of this inflammatory marker are correlated with the severity that is measured using NIH Stroke Scale/Score (NIHSS) and infarction size at the onset. [1] Moreover, a study showed that dead patients have higher CRP levels during the first 24 hours compared to those who survived. [27]

2.2.4 Erythrocyte Sedimentation Rate (ESR) in acute ischemic stroke

ESR is the oldest inflammatory marker compared to CPR and Plasma viscosity (PV). It is determined by the distance in millimetres that erythrocytes resolve in anticoagulated whole blood in one hour. Westergren's technique is used to measure the ESR level within 12 hours of stroke recurrence since it was first introduced by Westergren in 1921. [35] The gravitational settling occurs since erythrocytes normally have net negative charges while high molecular weight proteins are positively charged, therefore repel each other. Fibrinogen is increased during acute-phase reaction therefore ESR increases too since it leads to rouleaux formation.

Since haematological and biochemical results are easily available and cheaper, the analysis is very useful for prognosis and has vast value for stroke patients. Various researches conducted in the last few years also suggested the importance of haematological parameters like ESR, platelet count and leukocyte count in full blood count during admission to determine the stroke outcome. [36] This is because it is sensitive to fibrinogen and immunoglobulins. Furthermore, ESR is a fast and low cost test and can be used to assess the strength of inflammatory response corresponding to atherosclerosis and a study by Nayak et.al already confirmed that an increase of ESR at the time of admission correlates with poor outcome. [37]

During ischemic stroke, the proteins that play an important role are fibrinogen, immunoglobulins, lipoprotein and alpha-2 macroglobulin. [35] The proteins will eventually prevent the negative electrical forces which usually prevent the erythrocyte from sticking together and lead to accelerated erythrocyte aggregation. This study also confirmed a study by Singh et al which shows the relation between serum fibrinogen and ESR. [38]

A study by Emsley et al., shows an increment of ESR value in patients with stroke compared to those non-stroke patients with atherosclerosis. [39] This also confirms the statement that atherosclerosis is one of the common causes of ischemic stroke and this can be assessed using ESR.

ESR is not a specific marker for inflammation and may be affected by other factors; underlying risk for atherosclerosis such as diabetes mellitus, hypertension or even smoking. [38] Thus, we need to do this test along with other clinical findings to find an accurate prognosis for the patient.

2.3 Magnetic Resonance Imaging (MRI)

2.3.1 MRI Definition and Function

Magnetic resonance imaging (MRI) is a non-invasive and painless procedure which uses a large magnet with a strong magnetic field and radio waves to create very specific images of tissues and organs in the body. [40] However, the MRI does not involve damaging ionizing radiation. It is often used for disease detection, diagnosis, and treatment monitoring. The MRI detects changes in protons found in the water that make up living tissues and harness powerful magnets which produce a strong magnetic field that forces protons in the body to align with that field. When a radiofrequency current is then pulsed through, the protons are stimulated, and spin out of equilibrium, straining against the pull of the magnetic field. When the radiofrequency field is turned off, the MRI sensors are able to detect the energy released as the protons realign with the magnetic field. [41] The time it takes for the protons to realign with the magnetic field, as well as the amount of energy released, changes depending on the environment and the chemical nature of the molecules.

2.3.2 MRI Findings in Patients with Acute Ischemic Stroke

According to research, ischemic stroke is one of the most popular neurological disorders that may lead to severe dysfunction and eventually death. The MRI can uncover the biology of stroke and this can be very helpful for the clinical management and treatment of the patients, particularly to the most severe cases. However, when the onset of stroke is known, that is when treatment is crucial. This implies the clinical trials which have brought the stroke treatment used time and noncontrast CT scans for patient inclusion, [42] and will change only

after clinical trials demonstrate that MRI is useful to identify patients who will benefit from treatment.

To illustrate the present circumstances in the use of MRI in acute ischemic stroke, consider the imaging data from 2 patients (Figure 1). [43] In the first case, a man presented with severe neurological symptoms but with an unknown time of stroke onset. Imaging revealed an occlusion of a major cerebral artery, but that only a small part of the brain had undergone irreversible damage. Based on the physiology revealed by imaging, intra-arterial removal of the occlusion was executed, and the patient made a full recovery. In the second case, a man presented with moderate symptoms, but unlike the first case, the time of stroke onset was known. Once again, a major artery was occluded, but only a small portion of the territory at risk was irreversibly injured at the time of initial imaging. The decision not to intervene was made because the patient fell outside the established time windows for treatment. The infarction grew accompanied by substantial worsening of symptoms. These cases illustrate the current dominance of time over the information provided by imaging in stroke management. This review presents evidence on how MRI-revealed stroke biology may lead to better outcomes in spite of the time of stroke onset. But, routine use of MRI to guide treatment requires verification in clinical trials.

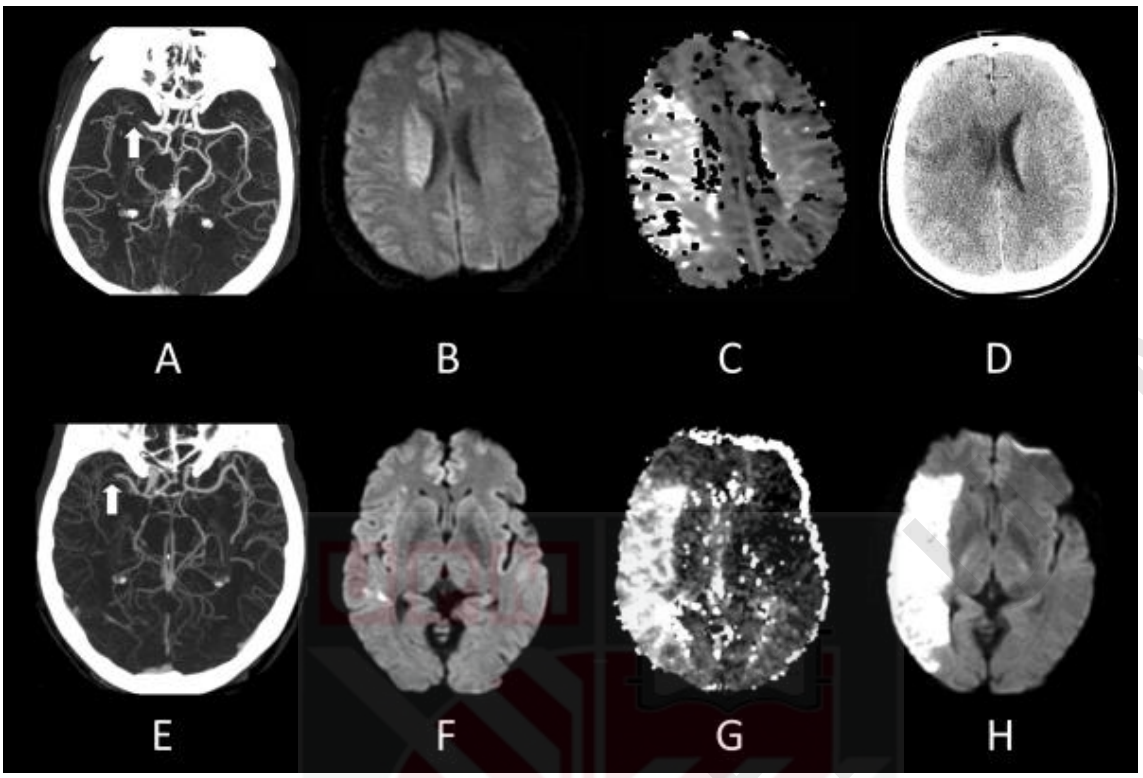


Figure 1 which was depicted from González R. G. (2012). Clinical MRI of Acute Ischemic Stroke shows:

2 patients with right middle cerebral artery ischemic stroke

(First row) A 38-year-old man was presented with severe left-sided hemiplegia of unknown onset time. In *A*, CT angiography, the arrow shows occlusion of the right middle cerebral artery. In *B*, Diffusion MRI demonstrates a small diffusion anomaly in the right hemisphere. In *C*, Perfusion MRI displays anomalies involving the whole right middle cerebral region.

The time-to-minimum-perfusion map at the same level as the DWI image demonstrates a perfusion deficit that is much larger than the DWI abnormality. Because of the large diffusion/perfusion mismatch and the likely poor long-term outcome, the decision was made to proceed to intra-arterial thrombolysis, which was successful. D, A follow-up head CT scan shows infarction in the region of the right corona radiata and a small portion of the cerebral cortex. The patient made a complete recovery.

(Second row). A 61-year-old man was presented with a mild left-sided weakness which started 12.7 hours prior to his admittance. In *E*, CTA, the arrow shows a proximal right middle cerebral artery occlusion. In *F*, the initial DWI reveals multiple punctate foci of diffusion anomaly in the right hemisphere region. In *G*, the mean transit time (MTT) MR perfusion map shows a large volume of poor perfusion involving the majority of the right middle cerebral artery region. The patient did not undergo thrombolytic therapy due to the lengthened duration between the stroke onset and imaging. *H*, The patient's condition exacerbated into severe paralysis, and the follow-up MRI revealed a large area of infarction.

2.3.3 Method in Measuring of Infarct Volume in Magnetic Resonance Diffusion-Weighted Image

Infarct volume (cm3) was defined as a hyperintense area visible from the b = 1000 mm/s2 images and produced apparent diffusion coefficient maps.

The boundary of acute infarct was delineated on DWI sequences by using a proprietary segmentation algorithm developed in the Computational Image Analysis (CAD) in the Department of Radiology. For the first step of the volume measurement, the entire infarct volume was separated from surrounding anatomic structures by using a segmentation algorithm that combines the image analysis techniques of active contours and a level set approach. Once the segmentation was completed on an image, the infarct contour was propagated to its neighboring images, serving as an initial region of interest for subsequent segmentations on the neighboring images. This process was continued iteratively until all the infarct images were segmented. Once the segmentation was finalized, infarct volumes were automatically calculated (Figure 2) depicted from Filippi, C. G. et al. (2015). For brain volume, we determined the contour of the brain cortical surface from which ventricular volume is subtracted by contouring the ventricular lining. [44]

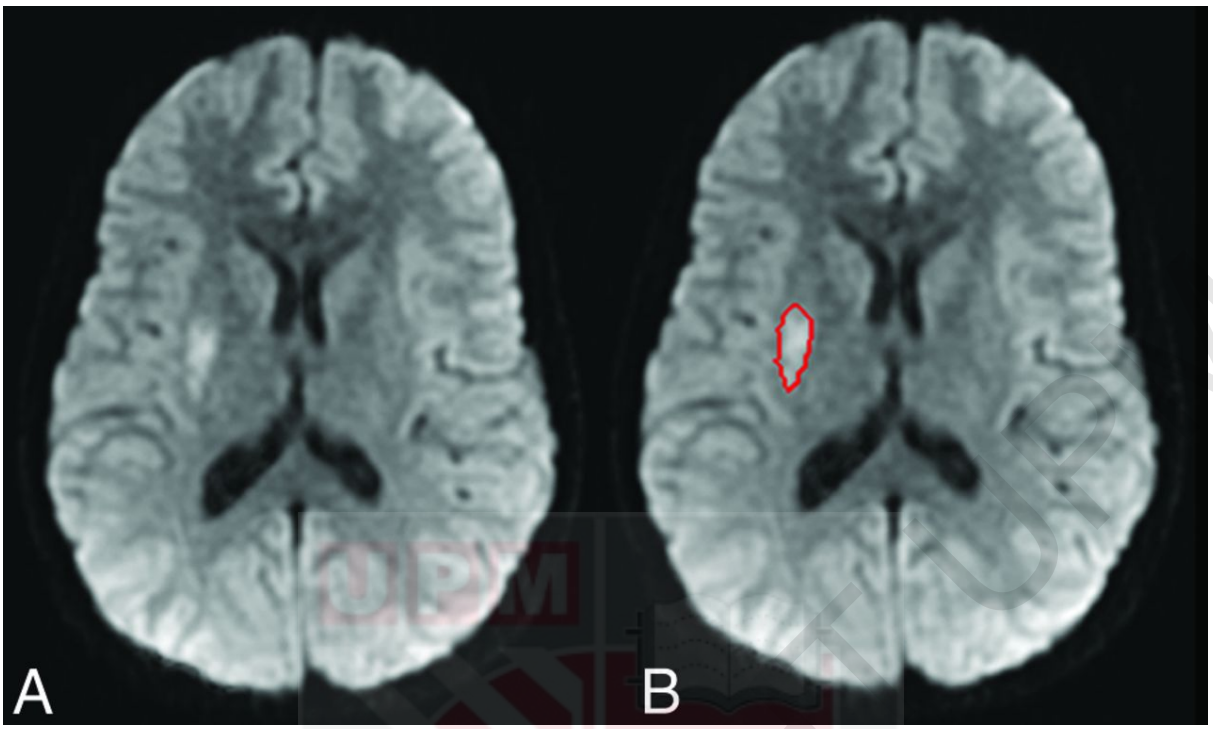


Figure 2: A, Reduced diffusivity within the right putamen consistent with an area of acute infarction. B, the contour derived from the semi-automated computer segment mentation software that is used to derive infarct volume.

Segmentation was performed by manual tracing of regions of reduced diffusion on DWI sequences. All manual segmentations were performed at a dedicated workstation.

2.3.4 Correlation of Inflammatory Marker With Size Of Brain Infarction

M. A. Shoaeb et al. found that serum CRP only can predict the severity and prognosis in ischemic stroke but not in haemorrhagic stroke [48]. As stated before this, atherosclerosis of coronary, carotid, and peripheral arteries is correlated with elevated CRP levels in the patients. Many studies found that C-reactive protein is an independent indicator to determine future cerebrovascular events [49-51]. Heidi et al conducted a study and found out that CRP was positively correlated with the infarct volume, whereas IL-6 did not have a significant relation to it since IL-6 is more sensitive to measurement timing. A higher level of CRP is

associated with underlying processes that will cause severe strokes. The elevation could trigger the activation of coagulation through the important role of tissue factor expression. [52] The activation of this process will increase mortality in stroke patients. [53]

Smith CJ et al. and Pedersen ED et al., found that there is a correlation between CRP and cerebral infarct volume [53,54] meanwhile Waje-Andreassen U et al., did not find a correlation between them. [55] But findings by Marquardt L, supported the finding of higher plasma CRP level only in patients with larger infarct. [56] Other than that, an animal study found that human CRP increases the cerebral infarct size after middle cerebral artery occlusion. [57] From all of this evidence, we are strongly confirmed that higher CRP levels are associated with larger infarct volumes in acute ischemic stroke.

Acute-phase proteins play an important role in promoting a decrease in the survivability of neurons subjected to ischemia. This includes fibrinogen that has the greatest effect on erythrocyte aggregation compared to other acute-phase proteins. Elevation of fibrinogen will eventually increase the plasma viscosity and erythrocyte aggregation, hence decreasing the blood flow in the small blood vessel and causing ischemia and infarction [58]. Erythrocyte aggregation that occurs can be evaluated indirectly by ESR [59].

Jin R et al. and Brait VH et al. stated that the pathophysiology of cerebral injury in acute stroke is contributed by the brain inflammatory process and has been reported to be associated with infarct size [60,61]. This statement is supported by studies that show a high level of ESR is associated with a larger volume of infarct size and poor outcome in the future. [53,62] Other than that, Kisialiou et al. conducted a study that observed the values of ESR as soon after AIS that reflect the infarct size and the extent of local brain damage in the patient. They found that the higher level of ESR indicates larger infarct size with long-term poor outcome, but not with short-term outcome. [63]

Thus, there are positive correlations presented between ESR and CRP values and the volume of brain infarct. This is supported by previous studies that we stated before that proved the higher level of inflammatory markers in stroke patients were associated with more extensive brain infarcts that can be seen through MRI.

2.3.5 Cerebral Infarct Volume Measurements to Improve Patient Selection for Endovascular Treatment.

The early reperfusion treatment is one of the best ways for reducing dysfunction. [64] Endovascular treatments for patients with acute ischemic stroke have improved over the years. [65] Several randomized control studies have succeeded in showing that endovascular treatment surpasses medical treatment based on intravenous recombinant tissue plasminogen activator (rt-PA) in patients with intracranial artery occlusions, especially in the anterior circulation. [66] Baseline computed tomographic (CT) or magnetic resonance (MR) angiography are the primary tools used for patient selection for endovascular treatment. However, additional imaging protocols varied widely between studies, and therefore, it is not clear which imaging protocols should be used for optimal results. For further patient selection, some trials have used additional imaging protocols to exclude patients who already had large infarcts prior to initiating endovascular treatment. [67] Considering the variety of protocols successfully used, it is imperative to determine the simplest and most effective one. Thus, a study done by (Miran Han et al., 2016) has evaluated the prediction power of these protocols for clinical outcomes in patients with endovascular treatment, especially focusing on imaging protocols to determine infarct volumes by including 79 patients (45 men; 34 women) selected from a database of 201 consecutive patients with ischemic stroke who had undergone endovascular treatment at Ajou University Hospital, Suwon, Korea.

The difference in infarct pattern on the DWI may be useful to differentiate in situ thrombosis of intracranial atherosclerotic disease from cardioembolism. [68,69] In addition,

multimodal MRI can be useful for proper patient selection for endovascular treatment beyond the usual time window (> 6 hours). MRI protocols such as DWI stroke volume, infarct core volume, and DWI ASPECTS system that measure the infarct volume in acute ischemic stroke showed high inter-reader reliability and good prediction power for clinical outcomes. The performance of these MRI protocols appeared to be superior to CT ASPECTS systems. However, the time delay from taking MRI is one of the major concerns for acute ischemic stroke treatment so reducing the time required to perform these MRI protocols is a challenging issue that will need to be addressed. [70,71]

2.4 Conceptual framework

Conceptual framework is the visual presentation in flow charts that explains the key factors, concepts or variables to be studied in this study. It also provides research direction as well as limits the boundaries of research. The conceptual framework of this study is shown in Figure 1.

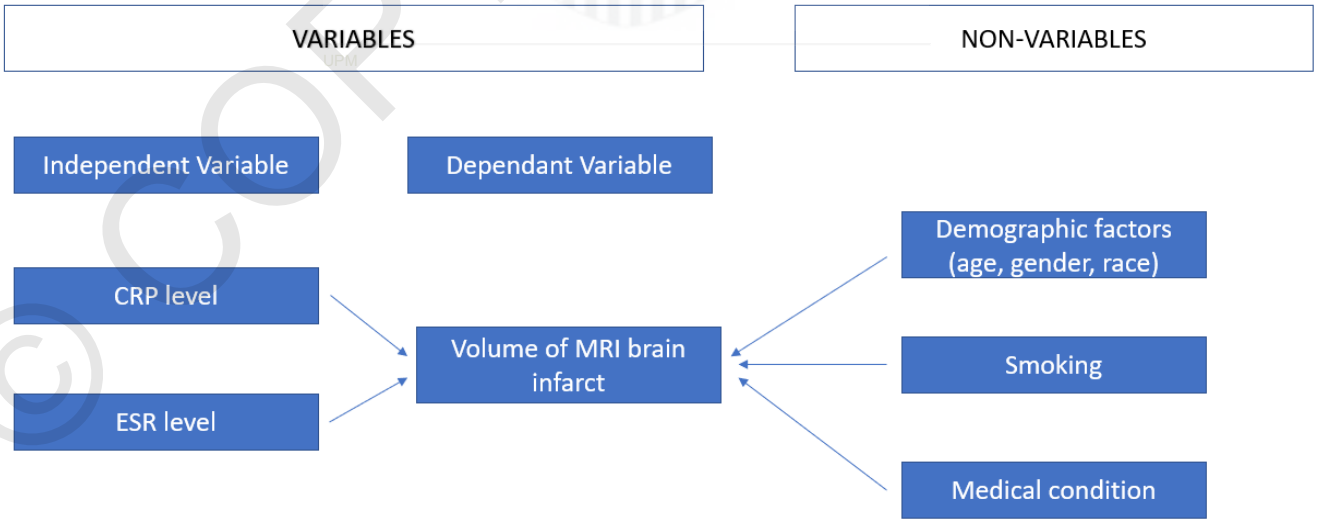


Figure 1 Conceptual Framework

CHAPTER 3

METHODOLOGY

3.1 Study Location

This study was conducted in Hospital Pengajar Universiti Putra Malaysia, which is located in Serdang, Selangor, near Putrajaya. It is planned to serve approximately 570 000 people in Serdang, Putrajaya, Kajang and Bangi. The hospital which occupies 400 beds serves as a teaching hospital for students from University Putra Malaysia. It commenced its operation in March 2019.

3.2 Study Design

This was a cross-sectional study using secondary data, in which the data was obtained from patients who came to Res Q (Stroke Centre) in HPUPM and research purposes which is within inclusion criteria.

3.3 Study Duration

The study duration was six months which starts from January 2021 until June 2021. The data collection was taken from January 2020 until February 2021.

3.4 Sampling

3.4.1 Study Population

The study population are all patients with acute ischemic stroke who went for MRI brain examination.

3.4.2 Sampling Frame

The lists of patients who went for MRI brain examination retrieved from the PACS and Radiology Information system (RIS).

The sociodemographic and clinical assessment of the patients were assessed via the Hospital Information System (eHIS).

3.4.3 Sampling Unit

The sampling unit is a patient from HPUPM who met the inclusion and exclusion criteria, underwent MRI brain examination within the period of January 2020 until February 2021.

3.4.4 Sample Size Estimation

The sample size (n) for this study was calculated using the formula:

$$n = \frac{\{[z_{(1-\alpha/2)} * \sqrt{2\bar{P}(1-\bar{P})}] + [z_{(1-\beta)} * \sqrt{P_1(1-P_1) + P_2(1-P_2)}]\}^2}{(P_1-P_2)^2}$$

P = (P1 + P2)/ 2 = 0.097

P1 = Most significant outcome of ischemic stroke in 2014 (NIHSS 5-20) = 0.482 (Z.A. Aziz et al., 2015)

P2: Least significant outcome of ischemic stroke in 2014 (NIHSS >21) = 0.171 (Z.A. Aziz et al., 2015)

$Z(1 - \alpha/2)$= 1.96 for 95% CI

$Z(1 - \beta/2)$= power = 80%= 0.84

Therefore, the sample size obtained is n=34 subjects per group x 2 groups

= 68 subjects (Total subjects in this study)

An additional adjustment was made and the final sample size is 82 subjects.

P1: Group of patients that experienced a moderate neurological deficit

P2: Group of patients that experienced a severe neurological deficit

3.5 Selection Criteria

3.5.1 Inclusion criteria

a. Age >18 years old.

b. All patients with acute ischemic stroke.

c. MRI brain done <24 hours from onset of stroke in HPUPM between January 2020 until February 2021.

3.5.2 Exclusion criteria

a. Pregnant patient

b. Patient with ischemic stroke >24 hours

c. Trauma patients

d. Patients who have a history of previous brain surgery

e. Patients with intracranial bleed, venous sinus thrombosis, infection or underlying malignancy

3.6 Study instruments and data collection technique

3.6.1 Study instruments

This study uses secondary data which were extracted from the Hospital Information System (eHIS), Reporting Information System (RIS) and Picture Archiving and Communication System (PACS). No questionnaire was given to the patients.

The sociodemographic data of the selected patients were obtained from the eHIS while the MRI images were accessed via the PACS whereas the MRI reports for each sample were searched from the Reporting Information System (RIS).

MRI sequences DWI/ADC and FLAIR axial were selected. The findings of the site and size of brain ischemia will be studied. The reference for the techniques of measurement is taken from the previous study by Ahmed Dashan et al 2019.

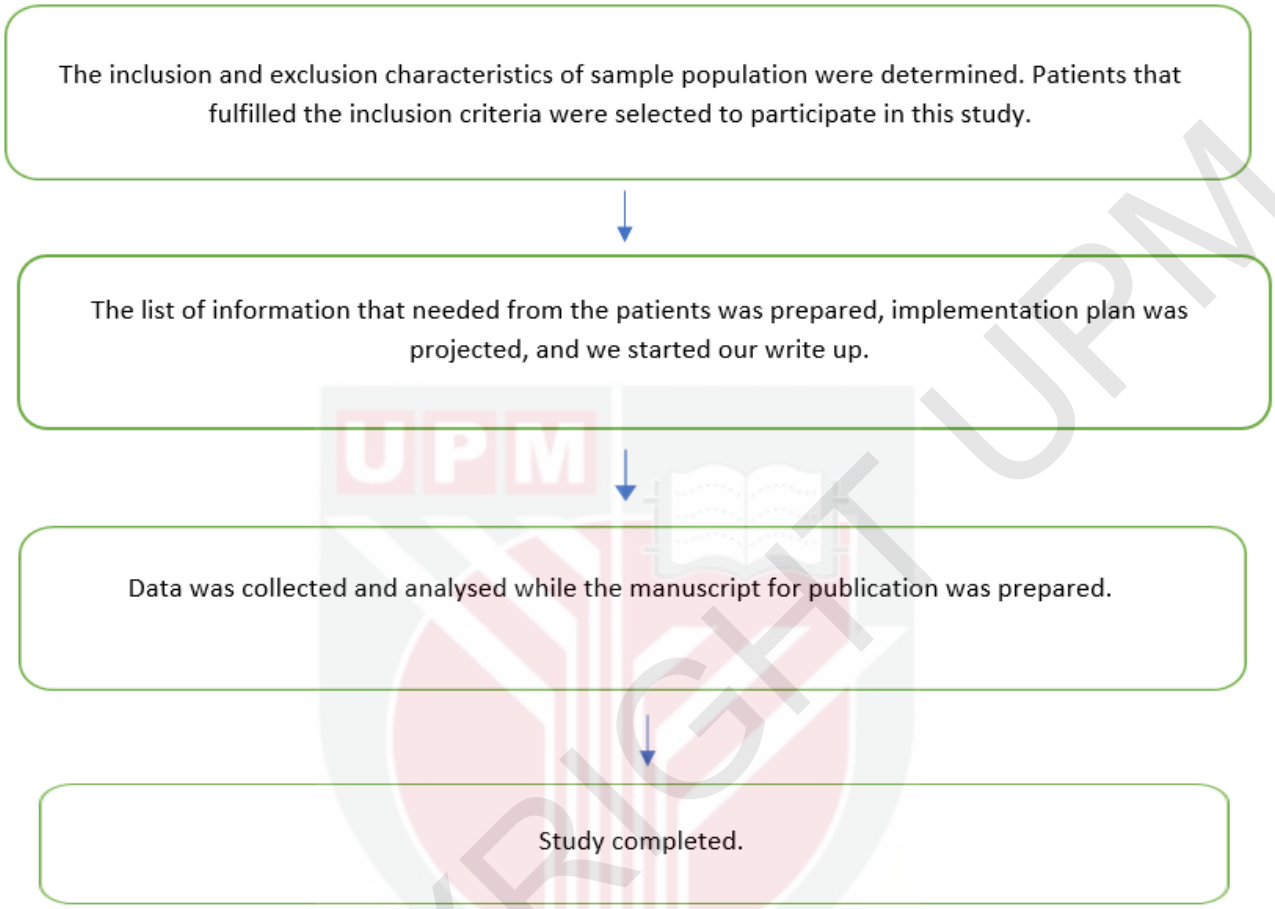
3.6.2 Data collection process

The collected data which include age, race, gender, medical history (diabetes, hypertension, ischemic heart disease, et al), smoking, CRP, ESR and MRI findings were recorded in the data collection form as attached in the Appendix. All the data will then be transferred to the SPSS.

3.6.3 Risk of Bias Assessment

The measurements of the brain ischemia size were done by two investigators. The measurements of the brain ischemia size are also taken three times and the average figures were taken as the final measurement to reduce the bias measurement.

3.7. Study Flow Chart



3.7 Data Analysis

Data were analysed using Statistical Analysis of Social Sciences System (SPSS) Version 22. The descriptive statistic was used to report the mean, standard deviation (SD), frequency, and percentage (%) of all data. A p-value less than 0.05 (<0.05) will be taken to indicate statistical significance.

Objectives	**Tests**
To describe the demographic factors (age, sex), smoking, medical conditions (diabetes mellitus, and hypertension) and inflammatory markers in the patient presenting with acute stroke	Descriptive analysis
To describe the site of brain infarct and measuring the volume of brain infarct.	Descriptive analysis
To determine the association between demographic factors with inflammatory markers	Pearson Chi-Square Test Spearman correlation Independent T-test Mann Whitney U Test
To determine the correlation between the level of inflammatory markers with the volume of MRI brain infarct.	Pearson correlation Spearman correlation

3.8 Variables

3.8.1 Dependent variables

The dependent variable in this study is the MRI brain findings.

3.8.2 Independent variables

a. ESR

b. CRP

c. Sociodemographic: age, gender

d. Smoking

e. Medical history: diabetes, hypertension

3.10 Definitions of variables

3.10.1 Independent variables

a. Age is defined as the number of years that a participant has lived or existed.

b. Ethnicity is defined as the type of race that a participant belongs to which is either Malay, Chinese or Indian.

3.10.2 Dependent variables

a) Acute brain infarct in MRI brain shows a hyperintense area from the b = 1000 mm/s2 images and produces apparent diffusion coefficient maps.

b) The measurement using the Computational Image Analysis (CAD) in Radiology Department, HPUPM.

3.11 Ethics approval

Ethical approval for this study will obtain from Ethics Committee for Research Involving Human Subjects Universiti Putra Malaysia or Jawatankuasa Etika Universiti untuk Penyelidikan Melibatkan Manusia (JKEUPM).

All data and information gathered from this study are kept and handled in a confidential manner that has followed the applicable laws and regulations. All the data collected would be kept for a minimum of three years for data analysis and after that, all of it would be destroyed.

3.12 Publishing policy

In publishing and presenting the study result, all the participants' identities would not be revealed without consent from participants.

CHAPTER 4

RESULT

4.1 Response Rate

This study was conducted to identify the relationship between demographic factors (age, sex), smoking, medical conditions (diabetes mellitus, and hypertension) and inflammatory markers in the patient presenting with acute stroke in Hospital Pengajar Universiti Putra Malaysia. A cross sectional study and a minimum of 68 patients were required. This study uses secondary data which were extracted from the Hospital Information System (eHIS), Reporting Information System (RIS) and Picture Archiving and Communication System (PACS). No questionnaire was given to the patients. We managed to obtain data from 70 AIS patients but only 60 patients met this study criteria. Therefore, the response rate was 88.2% since we were unable to meet 68 respondents.

4.2 Descriptive Analysis

4.2.1 Demographic Factors

A total of 60 acute ischemic stroke patients were studied. There were 41 (68.3%) male and 19 (31.7%) female patients (Figure 3). According to Table 1, there were 4 patients (6.7%) of the lowest frequency who were below 40 years old. The majority of the patients, 35% (21) were in the age group of 61 to 75 years old.Among the patients, 31 (51.7%) are Malay, 15 (25.0%) are Chinese and 14 (23.3%) are Indian (Figure 4)

Figure 3: Bar chart shows gender of patients

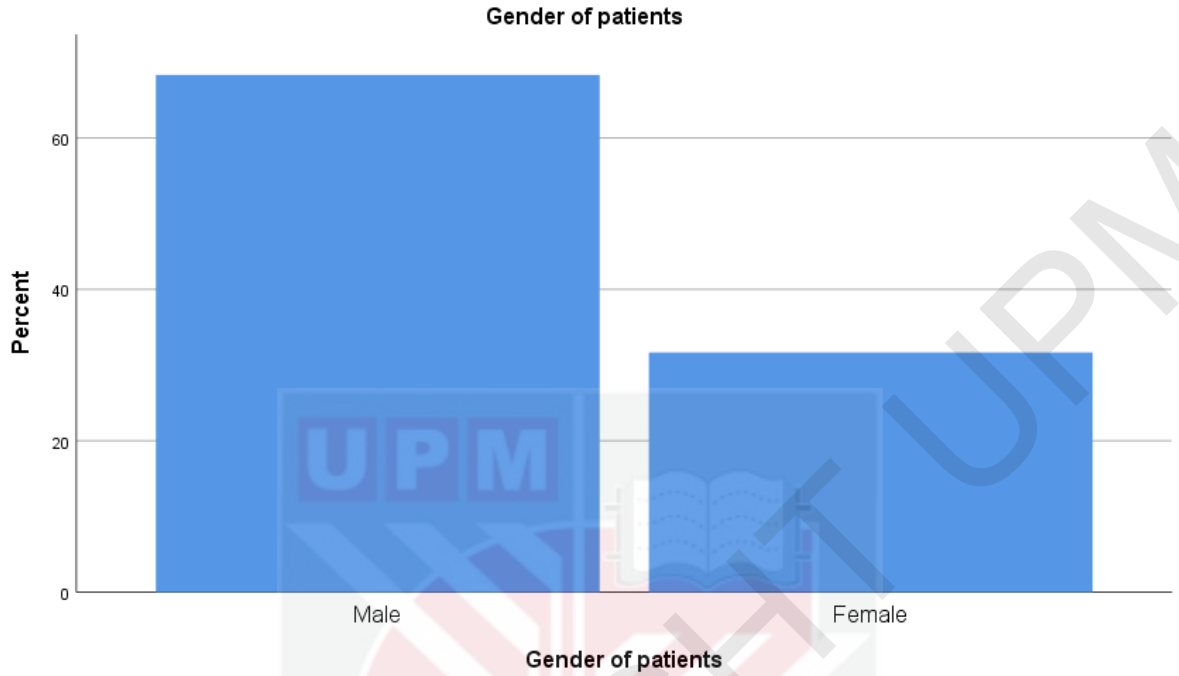
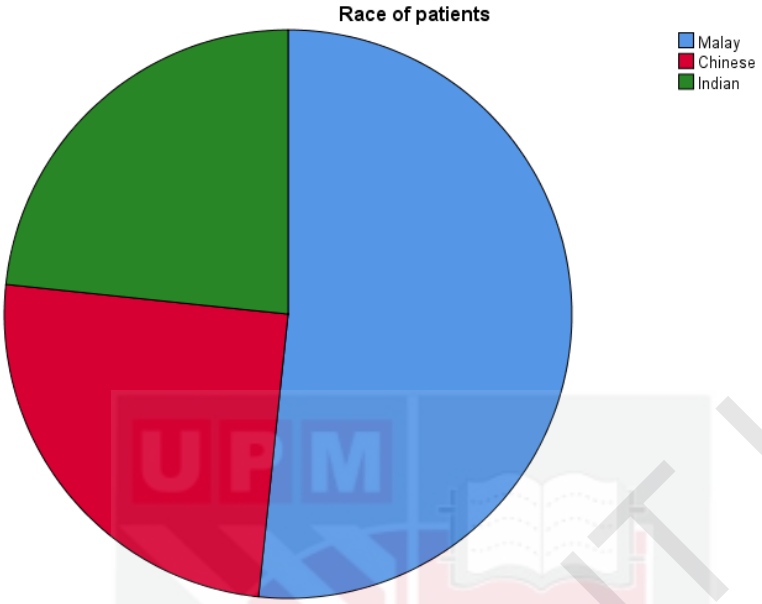


Table 1: Age group of patients.

Age group	Frequency (n)	Percentage (%)
<40	4	6.7
41-55	12	20.0
56-60	11	18.3
61-75	21	35.0
76-85	12	20.0

Figure 4: Pie chart shows races of patients



4.2.2 Risk factors

Among the patients, only 8 persons (13.3%) are smokers. Other than that, 2 patients (3.3%) are having hyperlipidemia. 5 AIS patients (8.3%) are having ischemic heart disease. 30 patients (50%) have underlying diabetes mellitus and 30 patients (50%) for dyslipidemia. Hypertensive patient recorded the highest frequency which is 44 people (73.3%) (Table 2)

Table 2: Underlying medical conditions

Underlying medical conditions	Frequency (n)	Percentage (%)
Smoker	8	13.3
Hyperlipidemia	2	3.3
IHD	5	8.3
DM	30	50
Dyslipidemia	30	50
Hypertension	44	73.3

4.2.3 Inflammatory Markers in Acute Ischemic Stroke Patient

Among the 60 patients, CRP levels were reported in 57 of the patients whereas ESR levels were reported in 28 of the patients. CRP levels have a mean of 42.85 ± 42.1436 mg/dl and a median of 19 mg/dl with IQR of 60.5 mg/dl as well as positively skewed (non-normal distribution) ESR levels has a mean of 32.321 ± 33.1618 mm/h and a median of 24.5 mm/h with IQR of 36.0 mm/h as well as positively skewed (non-normal distribution)

Table 3: CRP and ESR levels in acute ischemic stroke (AIS) patients

	CRP (mg/dl)	ESR (mm/h)
n	57	28
Minimum	0.5	3.0
Maximum	207.8	120.0
Mean	42.85 ± 42.1436	32.321 ± 33.1618
Median	19.0	24.5
Interquartile Range	60.5 (6.15 - 66.65)	36.0 (5.0 - 41.0)
Skewness	1.765	1.531

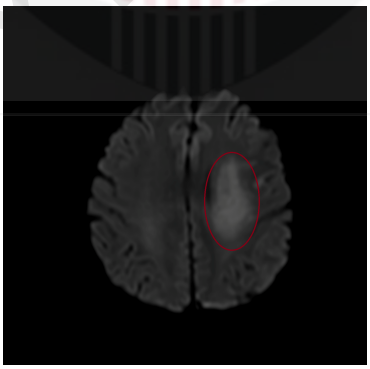
4.2.4 Site of Brain Infarct

Among the 60 ischemic stroke patients, 23 (38.3%) had a middle cerebral artery (MCA) infarction, 19 (31.7%) had a lacunar infarction, 4 (6.7%) had a Posterior cerebral artery (PCA) infarction and 2 (3.3%) had an Anterior cerebral artery (ACA) infarction. Watershed or border zone infarcts occurred in 3 (5.0%) patients. Infarctions affecting the precentral or postcentral gyrus supplied by the ACA and MCA occurred in 3 (5.0%) whereas cerebellar infarction also occurred in 4 (6.7%) patients. In 2 (3.3%) patients, the infarction site was not specified. (Table 4)

Table 4: Infarct Site

Infarct Site	Frequency	Percentage
MCA infarct	23	38.3%
Lacunar Infarct	19	31.7%
PCA infarct	4	6.7%
ACA infarct	2	3.3%
Watershed infarct	3	5.0%
Pre/Postcentral Gyrus infarct	3	5.0%
Cerebellar Infarct	4	6.7%
Unspecified	2	3.3%

Figure 5: MRI-DWI of AIS patient with left MCA infarction. Hyperintense areas (red circle) are denoted as infarctions.



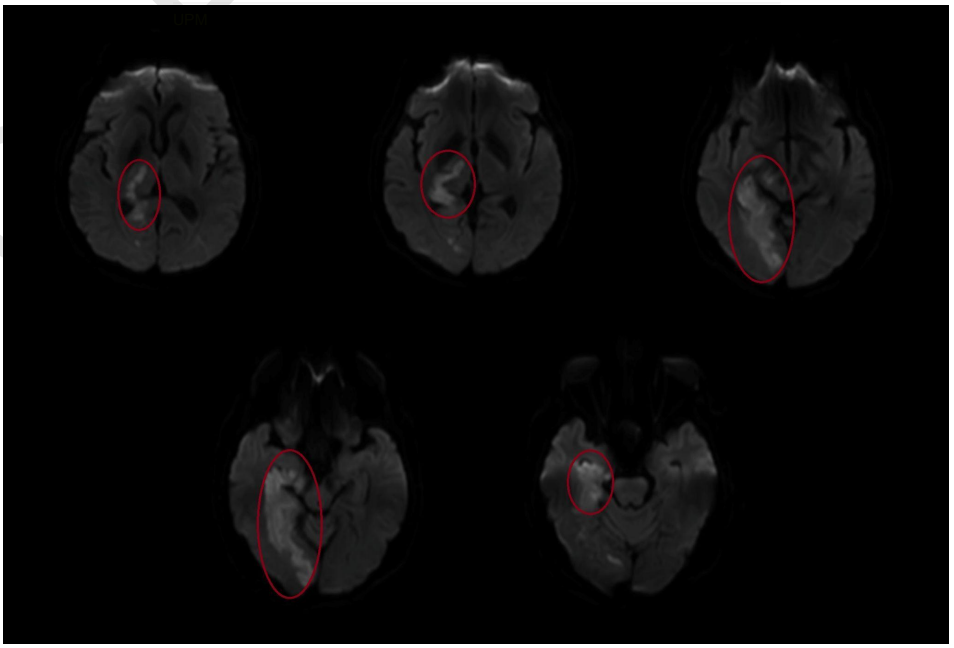
4.2.5 Volume of Brain Infarct

The volume of infarction measured from the 60 total patients using MRI-DWI ranged between 0.06cm3 and 323.57cm3 with a mean of 27.9268 ±56.6957 and a median of 6.1350cm3. The data was significantly skewed to the right with significant kurtosis indicating non-normal distribution. (Table 5)

Table 5: Volume of Infarct

Volume of Infarct (cm3)	
Minimum	0.06
Maximum	323.57
Mean	27.9268
Median	6.1350
Standard Deviation	56.6957
Skewness	3.413
Kurtosis	13.986
Percentiles 25 50 75	 0.8782 6.1350 19.2672

Figure 6: MRI-DWI slices used to calculate volume (red circles) of AIS patients with right PCA infarct.



4.3 Association Between Demographic Factors with Inflammatory Markers

4.3.1 CRP level data

57 patients (n=57) with CRP levels measured were studied. The association between CRP and age was tested using the Spearman correlation test and resulted in a correlation coefficient of r_s = 0.133 and a p-value of 0.328 indicating no statistical association. Correlation between CRP and gender was tested using the Mann Whitney U test and resulted in U = 339.5 and p=0.843 indicating no correlation. Lastly, an association between CRP and race was tested using the Kruskal Wallis H test and resulting in H(2) = 4.955 and p-value = 0.084 indicating a non-statistically significant association.

Table 6: CRP- Demographic factor tests

	Coefficients	P-value
CRP-Age	r_s = 0.133	0.328
CRP-Gender	U = 339.5	0.843
CRP-Race	H(2) = 4.955	0.084

(p<0.05 indicates statistical significance)

4.3.2 ESR level data

28 patients (n=28) with ESR levels measured were studied. The association between ESR and age was tested using the Spearman correlation test and resulted in a correlation coefficient of r_s = 0.354 and a p-value of 0.64 indicating no statistical association. Correlation between ESR and gender was tested using the Mann Whitney U test and resulted in U=31.5 and p=0.116 indicating no correlation. Lastly, the association between ESR and race was tested using the Kruskal Wallis H test and resulting in H(2) = 1.088 and p-value = 0.580 indicating no association.

Table 7: ESR- Demographic factor tests

	Coefficients	P-value
ESR - Age	$r_s = 0.354$	0.640
ESR - Gender	U = 31.5	0.116
ESR - Race	H(2) = 1.088	0.580

($p<0.05$ indicates statistical significance)

4.4 Correlation Between the Level of Inflammatory Markers With The Volume Of MRI Brain Infarct

4.4.1 CRP level data

The patients with recorded CRP values (n=57) were tested for association of CRP and volume of infarction. The Spearman's Correlation test was used. There is a weak correlation between CRP level and volume of MRI brain infarct with r=0.297. This correlation between CRP level and volume of MRI brain is statistically significant with p=0.025 which is less than the significant value ($p<0.05$)

Table 8: Correlation between CRP and volume of brain infarct

			CRP level (mg/dl)	Volume of brain infarct (cm^3)
Spearman's rho	CRP level	Correlation coefficient	1.000	0.297
		Sig. (2-tailed)		0.025
		N	57	57
	Infarct volume	Correlation coefficient	0.297	1.000
		Sig. (2-tailed)	0.025	
		N	57	57

4.4.2 ESR level data

The patients with recorded ESR values (n=28) were tested for association of ESR and volume of infarction. The Spearman's Correlation test was used. There is a negligible correlation between ESR level and volume of MRI brain infarct with r=0.192. This correlation between ESR level and volume of MRI brain is not statistically significant with p=0.326 which is larger than the significant value ($p<0.05$) (Table 9)

Table 9: Correlation between ESR and volume of brain infarct

			ESR level (mm/h)	Volume of brain infarct (cm^3)
Spearman's rho	CRP level	Correlation coefficient	1.000	0.192
		Sig. (2-tailed)		0.326
		N	28	28
	Infarct volume	Correlation coefficient	0.192	1.000
		Sig. (2-tailed)	0.326	
		N	28	28

CHAPTER 5

DISCUSSION

5.1 The demographic factors (age, sex), smoking, and underlying medical conditions in the patient presenting with acute stroke.

Based on Figure 3, the categorization of patients based on their gender; there are 41 (68.3%) male and 19 (31.7%) female patients. The male to female ratio for this study was 2.2:1. This shows that male patients outnumbered female patients. Research conducted by Dahshan et al. (2019) that involved 33 patients also showed the number of male patients was more than female; 19 males (57.6%) and 14 females (42.4%). This shows that male have a higher risk of getting acute ischemic stroke compared to females.

From 60 acute ischemic stroke patients in Hospital Pengajar UPM, the majority of patients are between 61-75 years old, which is 35.0% (n=21). The second highest age group is 76-95 years old and 41-55 years old, which is 20% (n=12) For the age group 56-60 years old, it was 18.3% (n=11). The age group below 40 years old is the least, which is 6.7% (n=4).

According to Figure 4, the majority of patients with acute ischemic stroke that were admitted to HPUPM were Malay which is 51.7% (n=31). Meanwhile for Chinese it is 25 (n=15) and Indians it is 23.3% (n=14). Other research conducted by Keat et al. (2012), out of 246 stroke patients admitted to Penang Hospital, the majority were Chinese (55.7%), Malays (28.9%), and Indians (14.2%), while other races accounted for 6%. This difference may reflect the local population as Malays are the majority among the population in Selangor, meanwhile, there are many Chinese found in Penang.

Table 2 showed that the majority of patients with a diagnosis of acute ischemic stroke have hypertension as the risk factor which is 73.3% (n=44) followed by dyslipidemia with 50% (n=30) and diabetes mellitus with 50% (n=30). Other than that, risk factors for acute ischemic

stroke that presented among these patients were smoking with 13.3% (n=8), ischemic heart disease with 8.3% (n=5) and the least patients were having hyperlipidemia with 3.3% (n=2). According to research by Hotter et al. (2019), from 91 acute ischemic stroke patients, hypertension, dyslipidemia and diabetes mellitus also recorded the three highest risk factors with 85.1% (n=74),63.2 % (n=55) and 28.7% (n=25) respectively. From this, we could see that patients that had hypertension, dyslipidemia and diabetes mellitus had a higher chance to get acute ischemic stroke and this prevalence also had been found from research conducted by Lu et al. (2019). These three vascular risk factors can cause pathologic changes to the blood vessels and will lead to stroke if cerebral vessels are affected.

5.2 The site of brain infarct and the volume of brain infarct in acute ischemic stroke patients

Among the 60 patients that have been presented with ischemic stroke at Hospital Pengajar Universiti Putra Malaysia (HPUPM), the majority experienced a Middle Cerebral Artery (MCA) infarction which is epidemiologically expected as MCA infarctions are the most common site of ischemic stroke. [75] In a study conducted by Yee Sien Ng et al, in which 2213 individuals who sustained first-ever ischemic strokes were analysed and categorized found similar findings to our study that MCA and lacunar strokes predominate the infarction areas. [76] Furthermore, other similar findings include single digit percentages of PCA and ACA infarctions, the former of which is greater in number. Cerebellar infarctions being slightly relatively increased compared to Yee Sien Ng et al's study. Watershed is said to account for 5-10% of all cerebral infarction, [77] which is in line with our study. Differences in the definition and classification of infarction sites may cause discrepancies in our results.

From the 60 patients who suffered a brain infarction, their volumes ranged from 0.06cm3 to 323.57cm3 with a median of 6.1350cm3 and IQR of 18.389cm3 whereas the mean was 27.3268 ± 55.6957cm3. The data was non-normally distributed, with data being positively skewed with significant kurtosis indicating extreme outliers. Our results are similar to previous studies with non-normally distributed volumes ranging from <1 to >100 and medians favouring lower infarct volumes. [78,79] The largest infarct volumes [>100cm3] were associated with MCA infarction consistent with the literature [78] while the largest infarct volume of 323.67cm3 was associated with MCA infarction and brain oedema.

5.3 The association between demographic factors with the inflammatory markers

The current study shows that there was no association between the demographic factors (age, gender and race) and the CRP levels. Spearman's correlation showed there was no association between CRP levels and age in patients with acute ischemic stroke with ($r=0.133$, $p=0.328$). This result contradicts the findings by Paczek et al. (2016) which shows that ageing is associated with a state of chronic low-grade inflammation and increased serum levels of inflammatory markers which includes the CRP levels. The incompatibility in our study results may be caused by the small sample size which is 60 acute ischemic patients from HPUPM. Mann Whitney U test showed $U=339.5$ and $p=0.843$ which indicates no association between CRP levels and gender of patients with acute ischemic stroke. In our study, the majority of patients with acute ischemic were male (68.3%) and the rest were females (31.7%). According to a study by Khera et al. (2005), significant race and gender differences exist in the population distribution of CRP. The results in our study and the study conducted by Khera et al. (2005) contradicts because of the location of where our study was conducted which was in HPUPM only and thus the result cannot be used to determine the association between CRP levels and gender of patients as a study supposed to include more locations. Kruskal Wallis H test which showed $H(2)=4.955$ and p-value=0.084 indicates there was no

association between CRP levels and race of patients with acute ischemic stroke. In our study, the majority of the patients were Malays (51.7%), followed by Chinese (25%) and Indians (23.3%). A study conducted by Nazmi et al. states that race or ethnicity was independently associated with CRP levels of African, Latin or South Asian descent were at higher risk for elevated CRP than subjects of European descent. However, the results in our study show there is no association and this may be due to the fact that our study only compares the race among Malaysians and no other ethnicities.

The current study also shows that there was no association between demographic factors (age, gender and race) and ESR levels. Spearman's correlation test showed there was no association between ESR levels and age in patients with acute ischemic stroke with ($r=0.354$, $p=0.640$). Mann Whitney U test was used for ESR levels and gender in patients with acute ischemic stroke and showed no association with $U = 31.5$ and p-value=0.116. Kruskal Wallis H test showed no association between ESR levels and race of patients with acute ischemic stroke with $H(2)=1.088$ and p-value=0.580. There is no significant association of demographic factors and ESR levels as well since this study has many disadvantages of not being able to get a large sample size, a proper location of the study and slight biases in choosing patients. Most of the studies that were done to measure the inflammatory markers with the demographic factors have shown significant results or unclear results, thus this study proves to be insufficient to determine the association between these variables. [83]

In conclusion, there was no association between demographic factors (age, gender and race) with inflammatory markers (CRP and ESR levels) in acute ischemic stroke patients in this study.

5.4. The correlation between inflammatory markers with the volume of MRI brain infarct

Spearman's Correlation test in this study showed there was a negligible correlation between ESR level and the volume of MRI brain infarct (r=0.192, p=0.326) This result is similar to the findings by Comoglu et al. (2013), in which this study revealed that ESR level is not specific enough to predict the prognosis of patients with acute ischemic stroke. However, many researchers found that there is a correlation between ESR level and the volume of MRI brain infarct. As an example, Kisialiou et al. (2012) reported that a high ESR value is associated with larger infarct size. Similarly, a study done by Nayak et al. (2011) found that higher ESR in the acute phase of a stroke may indicate a greater increase in fibrinogen concentration and a more significant reduction in cerebral blood flow which revealed a higher volume of brain infarct. This contrast finding might relate to the fact that ESR is a nonspecific marker of infection and inflammation. Other than that, ESR is an indirect method to reveal the red blood cell aggregation. Another possible reason ESR level cannot be used to determine the volume of brain infarct in our study may be related to the timing of ESR measurement. This study was focused on ESR level during admission and this might be more informative if the serial measurement were performed for the consecutive peak value following the insult.

Spearman correlation test showed a weak correlation between CRP levels and volume of brain infarct in acute ischemic acute stroke patients in HPUPM which is statistically significant (r = 0.297, p = 0.025). CRP plays an important role in the human immune system. Since CRP is a marker for inflammation, this shows that inflammation may play a significant role in acute ischemic stroke. These findings are similar to a study by Youn et al (2012) in which they described an association between CRP levels and the volume of brain infarct (r = 0.239, p = 0.010) [80]. These findings also offer a slight contrast to the results found by Heidi et al (2011) in which they found a moderate correlation between CRP and volume of brain infarct (r = 0.47., p = 0.005) [79] Moreover, CRP level and volume of infarct are both

individually correlated with the severity of stroke. [81, 82] Slight discrepancies in results may be due to measurement differences especially in volume measurements as radiologically determined infarct sizes may be subject to slight subjectivity. From these findings, it can be concluded that levels of CRP may be correlated with volumes of brain infarctions, however in what way and to what extent is not yet clear.

CHAPTER 6

CONCLUSION

6.1 SUMMARY OF RESEARCH

This study's main objective was to determine the correlation between the level of inflammatory markers (CRP and ESR) and the volume of MRI brain infarct in Hospital Pengajar Universiti Putra Malaysia (HPUPM). This was a cross-sectional study which used retrospective data of patients admitted with acute ischemic stroke in HPUPM.

There were 60 samples collected from HPUPM for this study. Based on the socio-demographic factor socio-demographic profile, the majority of the patients were male (68.3%), Malay (51.7%) and ranging from age group 61-75 years old. Most underlying medical conditions recorded among the patients are hypertension (73.3%), dyslipidemia (50%) and diabetes mellitus (50%).

Patients admitted to the hospital with markedly high CRP level with a mean value of 42.85 ± 42.1436. However, the normal level of ESR was seen among most of the 28 AIS patients.

MCA infarction was the most affected area among the patients (38.3%) and the mean volume of MRI brain infarction was 27.3268 ± 55.6957cm3. A weak statistically significant association was found between CRP and volume of brain infarction whereas no association was found between ESR and volume of brain infarction.

6.2 LIMITATION

This study has a few limitations because Hospital Pengajar Universiti Putra Malaysia is a new teaching hospital with service experience of fewer than five years. Besides, data were collected from only one hospital which reported single institution experiences, hence this study contains inherent biases, such as patient selection. Due to covid-19 and time constraint, HPUPM is chosen as a study site while it is expected to have less number. Firstly, the data

we obtained did not manage to reach the calculated sample size of 68 patients, only 60 patients were included in our study, 88.2% of our expected sample size. Secondly, among the 60 patients, only 28 patients had recorded ESR levels, only 41% of our expected sample size.

6.3 RECOMMENDATIONS

Based on our study, some recommendations can be suggested to both clinicians and researchers. Firstly, CRP levels may be used as a general indicator of the extent of brain infarction in acute ischemic stroke patients. Further research regarding the association between the volume of infarction and inflammatory markers that involves larger sample sizes with longer duration may improve accuracy and may present more significant and accurate results. Research involving the volume of infarction is currently scarce, thus studies exploring the significance and the association of volume of infarction with other relevant laboratory markers in ischemic stroke patients may help further our understanding on this matter.

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APPENDIX

PROFORMA FORM

Section A : Patient Information Sheet

I. Demographic Data

SDNo. :

Age :

Race :

Malay ___ Chinese ___ Indian ___ Others (Please state) _____

II. Medical history

NIH Stroke Scale Score :

Underlying Medical Problem :

Section B : Level of Inflammatory Markers (On Admission)

CRP level	
ESR level	

Section C : MRI Data (On Admission)

Area of infarct	
MRI findings	

GANTT CHART

ACTIVITY	2021											
	J	F	M	A	M	J	J	A	S	O	N	D
Preparing proposal												
Literature review												
Presentation proposal												
Approval of Ethical Committee												
Data collection												
Data analysis												
Writing thesis												
Submission of Poster and Scientific Article												
Poster Competition												
Publication												

Ref. no: UPM/TNCPI/RMC/JKEUPM/1.4.18.2 (JKEUPM)

Date: 28 March 2021

Dear Prof./Dr./Mr./Ms.,

APPLICATION FOR JKEUPM ETHICAL CLEARANCE: APPROVED

With reference to the above, I am pleased to inform you that your application for ethical clearance for the research project entitled **'Correlation Between the Volume of MRI Brain Infarct and Inflammatory Markers among Acute Ischemic Stroke Patients in HPUPM'** has been approved.

Please note that the official letter of approval will be issued as soon as possible. However, the ethical clearance is considered effective from the date of this email, and you may now proceed with your research.

Kindly remind the ethical approval is required in the case of amendments/ changes to the study documents/ study sites/ study team.

Researchers should also complete a Study Final Report upon study completion. The form can be obtained from the Ethics Committee for Research Involving Human Subjects (JKEUPM) website (http://www.tncpi.upm.edu.my/faildokumen).

If you have any enquiries, please contact Ms. Nurulhasanah Ishak (03-97691605) or Ms. Nor Ellia Abd Ajis (03-97691244).

Note: Please use this reference number for any transaction:- **JKEUPM-2021-103**

Thank you.

Yours faithfully,

Prof. Dr. Zamberi Sekawi
Chair
Ethics Committee for Research Involving Human Subjects
Universiti Putra Malaysia

FORM 3.2 STUDY FINAL REPORT

1.	JKEUPM Ref. No.	**JKEUPM-2021-103**
2.	Study Title	**Correlation Between the Volume of MRI Brain Infarct and Inflammatory Markers among Acute Ischemic Stroke Patients in HPUPM**
3.	i. Principal investigator a. Name b. Address c. Tel.No d. Email ii. List of co-investigators	i) Principal investigator Name: Prof Madya Dr. Suraini Binti Mohamad Saini (Supervisor) Address: Department of Radiology, Faculty of Medicine and Health Sciences. Universiti Putra Malaysia, 43400 Serdang, Selangor Tel: 03-89472512 Email: surainims@upm.edu.my ii) Co-investigators Name: Prof. Madya Dr. Sabariah Binti Md Noor (Co-supervisor) Address: Department of Pathology, Faculty of Medicine and Health Sciences. Universiti Putra Malaysia, 43400 Serdang, Selangor Tel : 03-8947 2761 Email: md_sabariah@upm.edu.my Name: Anna Maisarah Bt Mohamad Ariff (Student) Address: Faculty of Medicine and Health Sciences, Universiti Putra Malaysia Tel: 010-5114397 Email: 201061@student.upm.edu.my Name:Tharani A/P G. Baramesvaran (Student) Address: Faculty of Medicine and Health Sciences, Universiti Putra Malaysia Tel: 012-2842801 Email: 204134@student.upm.edu.my Name: Muhammad Syakir Bin Azmi (Student) Address: Faculty of Medicine and Health Sciences, Universiti Putra Malaysia Tel: 01123816449 Email: 202623@student.upm.edu.my

4.	Name Of Funding Agency	-
5.	Study Site	Hospital Pengajar Universiti Putra Malaysia
6.	Total number of eligible subjects in study site	157 patients
7.	Recruitment of subjects in study site i. Number of participants recruited: ii. Number of participants completing trial/ study: iii. Proposed in original application: iv. Number of withdrawals from trial to date due to: a) withdrawal of consent b) no response from participants c) loss to follow-up d) death (not the primary outcome) Total study withdrawals: v. Number of treatment failures to date (Prior to reaching primary outcome) due to: a) adverse events b) lack of efficacy Total treatment failures:	i) 72 patients ii) 60 patients iii) 86 patients iv) 12 patients - lack of data and unable to meet the inclusion criteria Total study withdrawals: 12 patients v) 0 patient

8.	Duration of study	January 2021 to May 2021
9.	Protocol Violation or Deviation	
10.	Executive summary *(Summary of research background, objectives, methodology, findings and conclusion of the research project) - maximum 500 words)* *Committee may request additional information if required.	**Background**: Stroke is one of the top five leading causes of death in Malaysia and increases in the mortality rate between 2016 and 2019. There is growing evidence from the previous study that inflammation plays an important role in acute ischemic stroke. The concentration of inflammatory markers which are C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are strongly correlated with the volume of brain infarct that could be detected using Magnetic Resonance Imaging (MRI). All of these will eventually predict the mortality and recurrent vascular events in patients with acute ischemic stroke. **Objective**: This cross-sectional study in Hospital Pengajar Universiti Putra Malaysia aims to determine the correlation between the volume of MRI brain infarct with inflammatory markers among acute ischemic stroke patients in HPUPM. **Methodology**: This study involved 60 participants from Hospital Pengajar Universiti Putra Malaysia, Serdang, Selangor. Patients were selected based on the included and excluded criterias. No questionnaire was given to the patients. However, the patients' sociodemographic information and level of inflammatory markers were extracted from the Hospital Information System (eHIS), the MRI images were accessed through the Picture Archiving and Communication System (PACS) whereas the MRI reports for each sample was searched from the Reporting Information System (RIS). In addition, the MRI sequences DWI/ADC and FLAIR axial were selected. The findings of the site and size of brain ischemia were studied. **Findings:** This study was based on 60 data sets of patients diagnosed with acute ischemic stroke in Hospital Pengajar Universiti Putra Malaysia, Serdang, Selangor. The majority of the patients were male (68.3%), Malay (51.7%) and ranging from age group 61-75 years old. Most underlying medical conditions recorded among the patients are hypertension (73.3%), dyslipidemia (50%) and diabetes mellitus (50%). Patients admitted to the hospital with markedly high CRP level. The ESR level was normal, the most recorded among the 28 AIS patients.Middle cerebral artery infarct was the most affected area among the patients (38.3%) and the mean volume of MRI brain infarction is 27.3268 ± 55.6957cm3. A statistically significant association was found between CRP and

		volume of brain infarction whereas no association was found between ESR and volume of brain infarction. **Conclusion:**: A significant association was found between CRP and volume of brain infarction. No association was found between ESR and volume of brain infarction.		
11.	Signature of Principal Investigator	*Suraini*	Date	5/6/2021