THE EFFECT OF INTENSIVE TENS THERAPY ON MUSCLE BLOOD BIOMARKERS AND ACTIVITIES OF DAILY LIVING IN HAEMORRHAGIC STROKE PATIENTS: A PRELIMINARY STUDY

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With the conclusion of this thesis, we would be able to further our understanding and the mechanisms of transcutaneous electrical stimulation on motor-impaired haemorrhagic stroke patients.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

ADL	Activities of Daily Living
ANOVA	Analysis of Variance
AVM	Arteriovenous Malformation
BBB	Blood-Brain Barrier
CK	Creatine Kinase
CK-MB	Creatine Kinase – Myocardial Band
COVID-19	Novel Coronavirus 2019
CVD	Cerebrovascular Disease
DALY	Disability-Adjusted Life Years
ECG	Electrocardiogram
fMRI	Functional Magnetic Resonance Imaging
HPA	Hypothalamus-Pituitary-Adrenal Axis
HREC	Human Research Ethics Committee
ICD-11	International Classification of Diseases - 11
ITT	Intensive TENS Therapy
LDH	Lactate Dehydrogenase
LMICs	low- and middle-income countries
MCO	Movement Control Order
RTT	Repetitive Task Training
SD	Standard Deviation
SOPD	Surgical Outpatient Department
TENS	Transcutaneous Electrical Nerve Stimulation
TIA	Transient Ischemic Stroke
USM	Universiti Sains Malaysia
WHO	World Health Organization

KESAN TERAPI TENS INTENSIF TERHADAP BIOMARKER DARAH OTOT DAN AKTIVITI KEHIDUPAN HARIAN PADA PESAKIT STROK HAEMORHAGIK: KAJIAN AWAL

ABSTRAK

Strok, khususnya strok hemoragik, adalah keadaan yang sangat melemahkan dengan beberapa masalah pemulihan. Transcutaneous Electrical Nerve Stimulation (TENS) telah lama menjadi alat yang sangat diperlukan dalam pemulihan strok. Oleh itu, kami bertujuan untuk mengetahui kesan TENS, khususnya terapi TENS intensif selama 2 minggu, pada pemulihan pesakit strok hemoragik bermotor yang pulih, di atas 6 minggu fisioterapi konvensional. Kami juga ingin menjelaskan kesan fisioterapi konvensional sahaja pada serum Creatine Kinase dan serum Troponin T, kerana kedua-duanya belum pernah dipelajari dalam literatur semasa. Ukuran hasil kami adalah serum Creatine Kinase, serum Troponin T, dan skor Indeks Barthel yang diubah. Ini adalah kajian intervensi terkawal sebelum pengambilan sampel yang mudah dilakukan, yang dilakukan di Jabatan Neurosains dan Unit Perubatan Rehabilitasi Hospital Universiti Sains Malaysia, yang melibatkan 10 subjek kawalan dan 10 subjek intervensi. 1 daripada 10 subjek intervensi memutuskan untuk berhenti dari kajian kerana masalah kesihatan, dan 2 subjek intervensi dan 1 subjek kawalan tidak mengikut rawatan susulan. Kumpulan intervensi menjalani terapi intensif TENS dan fisioterapi konvensional selama 2 minggu, kemudian 4 minggu fisioterapi konvensional. Kumpulan kawalan menjalani fisioterapi konvensional selama 6 minggu. Ketiga-tiga ukuran hasil setiap peserta diukur sebelum dan selepas 2 minggu terapi intensif TENS dan 6 minggu terapi konvensional. Kami kemudian

membandingkan dan menganalisis nilai pra dan pasca untuk kepentingan. Hasil kajian menunjukkan bahawa serum Creatine Kinase dan serum Troponin T relatif tidak terjejas oleh terapi TENS intensif 2 minggu dan 6 minggu fisioterapi konvensional (serum Creatine Kinase: $p = 0.521$) (serum Troponin T: $p = 0.632$). Perkara yang sama juga berlaku untuk 6 minggu fisioterapi konvensional sahaja (serum Creatine Kinase: $p = 0.572$) (serum Troponin T: $p = 0.921$). Sebaliknya, perbandingan skor Indeks Barthel yang diubah menunjukkan peningkatan yang signifikan pada subjek intervensi ($p = 0.040$) berbanding dengan subjek kawalan. Kajian ini pada masa ini bertindak sebagai kajian rintis dan keputusan kajian ini, yang menunjukkan tiada perubahan dalam biomarker darah tetapi peningkatan aktiviti skor kehidupan harian dalam pesakit strok hemoragik selepas ITT, akan dianggap sebagai keputusan awal untuk penyelidikan masa depan mengenai terapi TENS intensif. .

THE EFFECT OF INTENSIVE TENS THERAPY ON MUSCLE BLOOD BIOMARKERS AND ACTIVITIES OF DAILY LIVING IN HAEMORRHAGIC STROKE PATIENTS: A PRELIMINARY STUDY

ABSTRACT

Stroke, in particular haemorrhagic stroke, is a highly debilitating condition with several rehabilitations' problems. Transcutaneous Electrical Nerve Stimulation (TENS) has long been an indispensable tool in stroke rehabilitation. Thus, this study aimed to discern the effects of TENS, specifically intensive TENS therapy for 2 weeks, on a motor-impaired haemorrhagic stroke patient's recovery, on top of 6 weeks of conventional physiotherapy. We want to elucidate the effect of TENS and conventional physiotherapy on serum Creatine Kinase and serum Troponin T, as the roles of each biomarker is unclear in informing the outcome of stroke rehabilitation. Our outcome measures were serum Creatine Kinase, serum Troponin T, and modified Barthel Index scores. This was a pre-post convenient sampling controlled interventional study, conducted in the Department of Neurosciences and Rehabilitation Medicine Unit of Hospital Universiti Sains Malaysia, with 10 control subjects and 10 TENS-therapy subjects. 1 of the 10 interventional subjects decided to withdraw from the study due to health issues, while another 2 interventional subjects and 1 control subject were lost to follow up. The intervention group underwent both intensive TENS therapy and conventional physiotherapy for 2 weeks, then 4 weeks of conventional physiotherapy. The control group underwent 6 weeks of conventional physiotherapy. All three outcome measures of each participant were measured before and after 2 weeks of intensive TENS therapy and 6 weeks of conventional therapy. We then compared and analysed the pre and post values for significance. Results indicated

that serum Creatine Kinase and serum Troponin T were relatively unaffected by 2 weeks of intensive TENS therapy and 6 weeks of conventional physiotherapy (serum Creatine Kinase: $p = 0.521$) (serum Troponin T: $p = 0.632$). The same also applied to 6 weeks of conventional physiotherapy alone (serum Creatine Kinase: $p = 0.572$) (serum Troponin T: $p = 0.921$). On the contrary, modified Barthel Index score comparisons did show significant increases in intervention group ($p = 0.040$) as compared to control group. This study currently acts as a pilot study and the results of this study, which demonstrated no change in blood biomarkers but improved activity of daily living scores in haemorrhagic stroke patients post-ITT, shall be treated as preliminary results for future research on intensive TENS therapy.

CHAPTER 1

INTRODUCTION

1.1 Definition of Stroke:

The International Classification of Diseases - 11 (ICD-11) defines stroke as the presence of acute neurological dysfunction, which includes cerebral ischemic stroke, intracerebral hemorrhage, subarachnoid hemorrhage, and stroke not known to be ischemic or hemorrhagic (World Health Organization, 2018). In essence, stroke is defined as a rapidly developing syndrome of neurological deficit, involving nerves of the brain, spinal cord or retina. This deficit is a result of either ischemia or haemorrhage; it can be sensory or motor in nature or both, and this is determined by imaging or pathology (Sacco R. L., 2013).

1.2 Epidemiology of Stroke

The impact of stroke to world health has become more and more worrying, with an increase of 8.2% in estimated global lifetime risk of stroke for those aged 25 years and above, from 1990 to 2016 (GBD, 2018). In developing countries, the disability-adjusted life years of stroke was 2,189 per 100,000 while the mortality rate was 137 per 100,000 (Feigin V. L. et al, 2015). Throughout the world, the latest stroke statistics revealed an age-adjusted incidence of stroke of 76 to 119 per 100,000 population (Thrift et al, 2017). Worldwide stroke burden is among the five diseases with the greatest disease burden, based on disability-adjusted life years (DALY). Low- and middle-income countries (LMICs) as well as haemorrhagic stroke sufferers represent the majority of the global burden of stroke, in the form of deaths and DALYs lost.

The data on the burden of stroke in Malaysia is not as detailed as those of the global population. The Malaysian Ministry of Health 2018 announces stroke to be the third leading cause of death due to health disease at 7.1% of deaths, superseded by ischemic heart diseases (13.9%) and pneumonia (12.7%) (MOH, 2009). Regarding the incidence of stroke, the first and so far only stroke incidence study in Malaysia (as of December 2019) was done in a small region of Penang in 2010, with the result being 67 per 100,000 population (Neelamegam M. et al, 2013). Though inappropriate, if we extrapolate this statistic to the rest of Malaysia, it would make Malaysia among countries with the least incidence of stroke. The mortality of stroke is better documented by the Malaysian Stroke Registry, which revealed an increase in stroke mortality rate from 74 per 100,000 in 2009, up to 105 in 100,000 in 2016 (Abdul Aziz Z. et al, 2016).

The mean age of Malaysian stroke patients is within the range of other Asian countries at 62.5 years old. Patients more than 60 years old comprised the majority of stroke patients (60%) while the 50-59 age group make up another 26%. In terms of gender, males make up the majority of stroke patients; however, females at 70 years and above are at a higher risk of stroke, theorized to be due to generally longer lifespans compared to males (Abdul Aziz Z. et al, 2016).

The distribution of stroke subtypes in Malaysia is 76% ischemic stroke, 17% haemorrhagic stroke, and 7% transient ischemic stroke (TIA) or unknown cause of stroke. Among each subtype, the rate of deaths directly or indirectly due to ischemic stroke is 8.6%, but for haemorrhagic stroke it is as high as 26.6%. This shows the lethality of haemorrhagic stroke and the importance of quality care for stroke sufferers.

Based on data from the World Health Organization (WHO), Malaysia has 101.8 DALYs lost per 1000 population due to non-communicable diseases, with stroke

being one of these diseases (Loo K. W. et al, 2012). No one has yet done a study on stroke burden on the Malaysian population, as of December 2019.

1.3 Rehabilitation of Stroke Survivors

The WHO described rehabilitation as "a set of measures that assist individuals who experience, or are likely to experience disability to achieve and maintain optimal functioning in interaction with their environments". To achieve this, rehabilitation aims to anticipate and alleviate complications or impairments borne by a patient's disease, as well as maximize their physical, cognition, communication and emotional function, ultimately allowing those patients to integrate into society (Winstein C. J. et al, 2016; Kristensen HK et al, 2016; Hankey G. J., 2017). More specifically in this study, physical rehabilitation for stroke sufferers with motor impairment utilizes repetitive task training (RTT), with various forms of active and passive musculoskeletal, neurophysiological, and cardiopulmonary interventions, along with the associated assistive devices and modalities. RTT is a long used, tried-and-true component of post-stroke rehabilitation, with patients having increased upper and lower limb motor function compared to no RTT, as researched in the systemic reviews by Thomas et al in 2017 and de Sousa et al in 2018 (Thomas et al., 2017; de Sousa, Harvey, Dorsch and Glinsky, 2018).

Physical recovery from stroke is not a simple process. Each patient's rate of recuperation is influenced by their genetic, pathophysiologic, sociodemographic and clinical features (Alawieh, Zhao and Feng, 2018). Most rehabilitation centre guidelines for post-stroke recovery of motor impairment stresses on RTT (de Sousa, Harvey, Dorsch and Glinsky, 2018). However, recently a new rehabilitative modality called transcutaneous electrical nerve stimulation (TENS) is growing in prominence as an

imperative tool to combat limb paresis and spasticity that commonly plagues post-stroke patients.

In this study, we aim to determine the suitability of intensive TENS therapy for chronic stroke patients by measuring the changes in 3 outcomes: activities of daily living via modified Barthel index, skeletal muscle improvement via serum Creatine Kinase, and cardiovascular disease risk via serum Troponin T.

1.4 Research Question

1. What is the effect of a 2 week period of daily intensive transcutaneous electrical nerve stimulation (TENS) therapy in addition to 6 weeks of conventional physiotherapy on activities of daily living using modified Barthel Index (MBI)?
2. What is the effect of a 2 week period of daily intensive TENS therapy with 6 weeks of conventional physiotherapy on serum Creatine Kinase?
3. What is the effect of a 2 week period of daily intensive TENS therapy with 6 weeks of conventional physiotherapy on cardiac Troponin?
4. What are the changes in serum Creatine Kinase caused by 6 weeks of conventional physiotherapy in post-haemorrhagic stroke patients?
5. What are the changes in cardiac Troponin caused by 6 weeks physiotherapy in post-haemorrrhagic stroke patients?
6. How different are the changes in modified Barthel Index scores compared against the changes in the 2 muscle blood biomarkers after a 2 week period of daily intensive TENS therapy and 6 weeks of conventional physiotherapy in motor-impaired post-haemorrhagic stroke patients.

1.5 Study Hypothesis and Rationale

Null Hypothesis: There are no differences in pre and post study values of serum Creatine Kinase, serum cardiac Troponin, and modified Barthel Index (MBI) between subjects receiving 2 weeks of daily intensive TENS therapy with 6 weeks of conventional physiotherapy, and subjects receiving just 6 weeks of conventional physiotherapy

Alternate Hypothesis: There are significant differences in pre and post study values of serum Creatine Kinase, serum cardiac Troponin, and modified Barthel Index (MBI) between subjects receiving 2 weeks of daily intensive TENS therapy with 6 weeks of conventional physiotherapy, and subjects receiving just 6 weeks of conventional physiotherapy

1.6 Study Significance and Rationale

Among the stroke subtypes, the one with the far more debilitating prognosis has always been haemorrhagic stroke. Despite being far less in prevalence at only 17% of all strokes compared to the 76% of ischemic stroke in Malaysia, its mortality rate of 26.6% is triple that of ischemic stroke sufferers at only 8.6%. Most rehabilitation protocols in current medical practice are based on results derived from studies on ischemic stroke patients primarily, with little to no research on haemorrhagic stroke patient rehabilitation. Consequently, the experience of recovery and rehabilitation of haemorrhagic stroke patients from their predicament are more challenging than those of ischemic stroke patients. This makes it all the more impertinent that effective rehabilitation techniques such as transcutaneous nerve stimulation (TENS) be thoroughly tested to be more precise towards haemorrhagic stroke recovery.

What is the significance of using TENS in our study? As later demonstrated in the literature review (Chapter 2), TENS has already shown various physical benefits to a stroke patient's rehabilitation in the aspects of spasticity and paresis. However, no study has yet objectively measured the effect of TENS on muscle growth in the form of biomarkers, nor measure any improvements it might have on activities of daily living. The concept of TENS improving muscle growth in motor-impaired haemorrhagic stroke patients is that repeated efferent peripheral nerve stimulation and the resultant repeated muscle contraction will act as a form of repeated exercise, causing increased muscular strength. This would explain why TENS complements conventional physiotherapy, which most commonly involves repeated task training, a form of exercise in of itself. This premise was first explored by Azman and Azman in 2017, though their focus was more on direct electrical muscle stimulation, they acknowledged that the same concept can be applied to TENS (Azman and Azman, 2017).

1.7 General Objective:

To evaluate the changes in serum Creatine Kinase and cardiac Troponin against changes in activities of daily living (ADL) using 2 weeks of intensive TENS therapy (ITT) on motor-impaired post-haemorrhagic stroke patients.

1.8 Specific Objectives:

1. To study the effect of 2 weeks of intensive TENS therapy with 6 weeks of conventional physiotherapy on activities of daily living using modified Barthel Index (MBI).

2. To study the effect of 2 weeks of intensive TENS therapy with 6 weeks of conventional physiotherapy on serum Creatine Kinase using ARCHITECT C8000 Clinical Chemistry Analyzer.
3. To study the effect of 2 weeks of intensive TENS therapy with 6 weeks of conventional physiotherapy on cardiac Troponin using Roche Cobas e411 Chemistry Analyzer.
4. To investigate the changes in serum Creatine Kinase caused by 6 weeks of conventional physiotherapy in post-haemorrhagic stroke patients.
5. To investigate the changes in cardiac Troponin caused by 6 weeks physiotherapy in post-haemorrhagic stroke patients.
6. To compare changes in modified Barthel Index scores with changes in the 2 muscle blood biomarkers after 2 weeks of intensive TENS therapy and 6 weeks of conventional physiotherapy in motor-impaired post-haemorrhagic stroke patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Stroke and its Subtypes

A stroke of the brain, in formal terms also known as a cerebrovascular accident (CVD) is among the primary causes of mortality in the modern world, as explored in detail in Chapter 1. Stroke is broadly classified into ischemic and haemorrhagic stroke.

Ischemic stroke is the development of an obstruction in the cerebral vasculature resulting in infarction of the distally perfused cerebral area. The most prevalent etiology arising from either acute local thrombus formation over an existing atherosclerotic plaque, or emboli formation from a proximal circulatory source (most typically from the heart) leading to obstruction of the cerebral circulation.

Haemorrhagic stroke itself can occur due to direct trauma and damage to intracerebral blood vessels, or due to spontaneous bursting or leaking of cerebral blood vessels secondary to an acute hypertensive crisis, or a vascular malformation such as an aneurysm or arteriovenous malformation (AVM).

Because of their contrasting etiologies and pathophysiologies, both subtypes of stroke have opposing management principles. However, the clinical result is almost similar: loss of blood circulation to the part of the brain distal to the site of vascular insult, death of the cerebral cells in the involved region, and ultimately loss of neurological function of that cerebral region (Lindsay, Bone and Lindsay, 2010; FitzGerald, Gruener and Mtui, 2012; Waxman, 2017).

2.2 The Importance of Haemorrhagic Stroke

Quite unsurprisingly, most post-stroke rehabilitation studies have primarily focused on ischemic stroke sufferers (Perna and Temple, 2015; Kitago and Ratan,

2017). This is in part due to the assumption that regardless of etiology, both subtypes of stroke result in ischemia of the affected region, despite much of the literature agreeing that several differences in pathophysiology between the two subtypes warrant a different approach to post-stroke recovery (Perna and Temple, 2015, Kitago and Ratan, 2017). However, another important factor is the abundance of ischemic stroke cases relative to haemorrhagic stroke cases, making subject recruitment of ischemic stroke sufferers easier.

Two large studies comparing the functional outcome of rehabilitation between ischemic and haemorrhagic stroke sufferers have arrived to a similar conclusion: haemorrhagic stroke sufferers have the propensity for greater long-term functional improvement compared to ischemic stroke (Paolucci et al., 2003; Chu et al., 2020). However, they also agree that improvement of this cohort manifests at a slower rate as compared to ischemic stroke sufferers. Certain chronic disabilities will persist despite well-implemented rehabilitation plans, possibly until the rest of the patient's life (Kim, 2018). Furthermore, one study pointed out that due to the higher mortality rate of haemorrhagic stroke, studies on the recovery of haemorrhagic stroke may not accurately present the full picture (Perna and Temple, 2015). With regular advancements in haemorrhagic stroke management, the rate of haemorrhagic stroke mortality may decrease, leading to escalating numbers of haemorrhagic stroke survivors, and perhaps leading to worse post-haemorrhagic stroke morbidity overall (Dadlani and Agrawal, 2017). This makes it more essential to improve our rehabilitation services with increasingly efficient methods and techniques, which are best tailored to haemorrhagic stroke (Kitago and Ratan, 2017), and perhaps better able to improve lives of haemorrhagic stroke survivors. Therefore, we decide to focus our efforts on

haemorrhagic stroke sufferers, as the first step to a rehabilitation protocol more attuned to haemorrhagic stroke patients in the future.

2.3 TENS: The Revolutionary Rehabilitation Device

Transcutaneous electrical nerve stimulation, or TENS, encompasses delivering pulses of electrical current to the skin of a patient via a device, with the goal of inducing the production of nerve impulses in afferent nerves under the skin. A battery-powered, hand-held device generates the electrical current and passes through conductive pads to reach the skin. The procedure is non-invasive, and the strength (amplitude) of the electricity given can be increased or decreased to achieve a balance between painful overstimulation of the muscles and overt lack of stimulation. One can also modulate the frequency, duration and pattern of electrical current pulses, depending on the patient's clinical rehabilitative needs. Three different techniques of TENS are regularly used: conventional TENS (low amplitude, high frequency), acupuncture-like TENS (high amplitude, low frequency) and intense TENS (high amplitude, high frequency) (Sluka, 2007; Johnson M., 2007; Banerjee and Johnson, 2013).

Conventionally, TENS aims to alleviate different forms of chronic pain. Lately, due to increasing evidence of its benefit to motor impaired patients, more and more recovery centres began adopting TENS in their rehabilitation regimes for stroke patients. With its relatively economical price, convenience and ease of handling, TENS would be a boon to any motor recovery programme. Therefore, our principal goal is to evaluate the use of intensive TENS therapy in the rehabilitation of motor-impaired stroke patients.

2.3.1 Spasticity

Multiple sessions of TENS has been shown to improve a stroke patient's spasticity, reflex control and motor impairment since 1992 (Levin M. F. and Hui-Chan C. W., 1992). Spasticity in stroke arises due to corticoreticular fibre damage at the dorsal horn of the spinal cord, leading to hyperexcitability of the peripheral nerves. This directly results in simultaneous contraction of agonist and antagonist muscle pairs, causing loss of dexterity and paresis of the limb (Sheean G. and McGuire J. R., 2009). Recent evidence suggests that TENS relieves spasticity via two mechanisms: increasing presynaptic inhibition of the spastic muscle, as well as decreasing inhibition of corticospinal nerve signals to the antagonist muscle (Levin M. F. and Hui-Chan C. W., 1992; Martins F. L. et al, 2012). Furthermore, the use of TENS on paretic antagonist muscles also directly improves spasticity (Kim T. H. et al, 2013). Researchers have found that TENS alone is able to provide immediate, short term benefits to stroke patients suffering from spastic limbs (Cho H.Y. et al, 2013), while patients using TENS as an adjunct to conventional physiotherapy will experience longer lasting motor improvement than either conventional physiotherapy or TENS alone (Ng S. S. M. and Hui-Chan C. W. Y., 2007).

Figure 2.1: A Trancutaneous Electrical Nerve Stimulation unit

2.3.2 Paresis

Tekeoolu Y. et al was among the first pioneers of the use of TENS in chronic stroke patient rehabilitation when he demonstrated that high-intensity TENS greatly improves the quality of life of hemiplegic patients (Tekeoolu Y. et al, 1998). There was also improvement in motor and sensory function even if sensory stimulation is below the sensory threshold (Peurala S. H. et al, 2002). Furthermore, chronic stroke sufferers experience superior improvement in paretic limb performance when combining TENS with conventional physiotherapy, leading to speculation that TENS causes consolidation of the effects of RTT physiotherapy (Wu C. W. et al, 2006; Conforto A. B. et al, 2007; Kwong, Ng, Chung and Ng, 2018). In fact, it is possible that using TENS along with everyday life activities is sufficient to produce the positive effects when combined with conventional physiotherapy. Tyson S. F. et al showed this when they implemented TENS in day-to-day activities of lower limb-impaired patients for a few hours a day, resulting in improved strength, proprioception, balance and mobility compared with no stimulation (Tyson S. F. et al, 2013).

Functional magnetic resonance imaging (fMRI) has shown that somatosensory stimulation increases cerebral blood flow not just to the somatosensory cortical area of hemiparetic patients, but also to the primary and secondary motor areas. This means somatosensory stimulation induces cortical plastic reorganization of adjacent intact brain tissues and results in improved voluntary movement of the paretic hand and arm (Golaszewski S. et al, 1999; Wu C. W. et al, 2005; Kim T. H. et al, 2013). This could very well explain the effectiveness of using TENS in post-stroke rehabilitation studies.

2.4 Exercise-induced Increase in Muscle Protein Biomarkers

After decades of research on the benefits of exercise, there is now little doubt that exercise, can extend a person's longevity and significantly reduce the risk of cardiovascular diseases, regardless of the type of exercise and proportional to the intensity or amount (Lee D. C. et al, 2014, Eijsvogels T. M. H. et al, 2016a). Interestingly however, studies as early as 1984 demonstrated increases in muscle protein biomarkers Creatine Kinase and Troponin post-exercise (Apple F. S. et al, 1984; Cummins P. et al, 1987). As most forms of traditional physiotherapy are essentially exercise-based, an important question arises: What is the significance of these biomarkers in exercise?

2.4.1 Exercise and Creatine Kinase

CK has long been a general marker of muscle damage, found in the cytoplasm and mitochondria of cells with high-energy demand such as skeletal and cardiac muscles. (Cummins P. et al, 1987; Baird M. F. et al, 2012; Palacios G. et al, 2015). CK-MB, in particular, is clinically correlated to the degree of cardiac injury and has until recently, commonly been utilized in diagnosing the presence of myocardial infarction.

Although rising CK levels are usually associated with muscle damage secondary to diseases such as myocardial infarction and rhabdomyolysis, many studies demonstrated significant but milder increases in CK levels post-exercise, with the level of CK increase being influenced by exercise type, exercise volume, age, genetics, gender, muscle mass, and level of training (Baird M. F. et al, 2012). The physiological elimination from myocytes to prevent accumulation in the cytoplasm is currently the most recent theoretical mechanism for serum CK increase after mild or moderate

exercise, without the presence of muscle trauma (Baird M. F. et al, 2012). CK values typically peak less than 24 hours post-exercise, ranging 200-300 IU/liter after running or cycling, or up to 7000 IU/liter in stepping exercises (involving eccentric contractions) (Newham D. J. et al, 1983). Astonishingly, there were also increased CK-MB levels, though these were later found to be released mostly from skeletal muscles instead of cardiac tissues, despite only 2% of CK is in the MB isoform in skeletal muscles. (Apple F. S. et al, 1984; Cummins P. et al, 1987; Baird M. F. et al, 2012; Eijsvogels T. M. H. et al, 2016a).

Chronic stroke patients are common victims of cachexia and muscle wasting, especially without proper rehabilitation and physical therapy. The decrease in their muscle mass can be reflected through the decrease in serum CK compared to non-ill people (Rosalki S. B., 1998). Fortunately, modern stroke management commonly involves physiotherapy for those experiencing motor impairment, thus allowing stroke sufferers to achieve a more manageable level of functioning (Langhorne P. et al, 2009). A previous study demonstrated proportional increases in serum CK after short-term and long-term exercise (Steinhagen-Thiessen E. & Reznick A. Z., 1987), while modern athletes commonly measure serum CK to monitor their muscle status (Lee E. C. et al, 2017). Thus, it is for these reasons that we wish to use CK to monitor a stroke sufferer's progress through physiotherapy with and without TENS.

2.4.2 Exercise and Troponin

There are two known isoforms of cardiac Troponin, Troponin I and Troponin T. Both isoforms are elevated specifically in the presence of cardiac injury (Palacios G. et al, 2015). In fact, Troponin assays have largely replaced CK-MB as the primary diagnostic and risk assessment biomarker for myocardial infarction, primarily due to its

higher specificity to myocardial damage (Saenger A. K., 2010; Scirica B. M. & Morrow D. A., 2004).

The phenomenon of post-exercise Troponin release was first hinted at in a 1987 study on post-exercise muscle enzyme changes, where a small percentage of subjects were found to have increased Troponin levels despite having no history of cardiac disease (Cummins P. et al, 1987). With the advent of high sensitivity Troponin assays, evidence of post-exercise Troponin increases becomes much clearer (Eggers K. M. et al, 2019). Several factors have been identified to predispose to higher post-exercise Troponin, but some studies also show conflicting evidence: underlying cardiovascular or renal disease, higher exercise intensity, less training experience, late sampling and low assay sensitivity. Among the factors with conflicting associations are age and exercise duration (Eijsvogels T. M. H. et al, 2015; Gresslien T. & Agewall S., 2016; Eijsvogels T. M. H. et al, 2016b; Aengevaeren V. L. et al, 2019).

A systemic review concluded that there is no imaging or anatomical evidence of permanent cardiac damage with elevated Troponin levels (Eijsvogels T. M. H. et al, 2016b). Nonetheless, multiple studies in the last decade has confirmed that increased serum Troponin from baseline is associated with an increased future risk of morbidity and/or mortality from cardiovascular diseases, regardless of how small the increase in serum Troponin, or the presence of an underlying chronic illness (Everett B. M. et al, 2015; Sze J. et al, 2016; Roos A. et al, 2017; Eggers K. M. et al, 2019). Recent evidence also suggests that elevated post-exercise Troponin unmasks underlying cardiovascular disease that cannot be detected in normal resting conditions (Skadberg O.et al, 2017; Aengevaeren V. L. et al, 2019).

The mechanism of this increase has yet to be fully elucidated and is still a topic of debate among researchers and physiologists. At present, the most popular mechanism

is physiological release of Troponin degradation products during exercise (Eijsvogels T. M. H. et al, 2015; Gresslien T. & Agewall S., 2016; Aengevaeren V. L. et al, 2019; Aakre K. M. & Omland T., 2019).

Due to this phenomenon of post-exercise Troponin release, we wish to assess for any change in Troponin levels after intensive TENS therapy, as any increase in serum Troponin of stroke patients might worsen their survival prognosis since stroke itself is also a risk factor of future morbidity and mortality secondary to cardiovascular disease.

2.5 CK and Troponin in Stroke

An important element that must be taken into account in our study is the release of CK and Troponin in acute stroke patients, which can affect the accuracy of our findings.

The natural history of stroke with motor impairment is known to involve gross muscle wasting and denervation secondary to disuse, which further aggravates the weakness already experienced by stroke sufferers (Patten C. et al, 2004). Raised CK-MB in acute stroke was first attributed to the generalized lytic state of skeletal muscles secondary to repeated injections and decreased oral intake (Ay H. et al, 2002). However, mounting evidence of increased Troponin as well as electrocardiographic (ECG) changes immediately post-stroke has raised the possibility of neurologically induced cardiac damage (Norris J. W. et al, 1979; James P. et al., 2000). Indeed, increases in both CK-MB and Troponin levels post-stroke is strongly associated with mortality from cardiovascular diseases, with Troponin T being more predictive than CK-MB. The larger the stroke lesion volume, the higher the level of Troponin, and the higher the risk of death in the short term (Apak I. et al, 2005; Suleiman H. M. et al, 2017). Elevation

of serum myocardial enzymes is reported in 11% to 21% of SAH patients and in 1% to 17% of ischemic stroke patients (Bugnicourt et al., 2010).

It is somewhat common medical knowledge that cardiovascular or cardiac disease, specifically atrial fibrillation, highly increases the risk of ischemic stroke, up to roughly 20% (Wolf, Abbott and Kannel, 1991), but can the inverse be true, where a cerebrovascular stroke induces severe enough cardiac damage to greatly affect cardiac function?

2.6 Stroke and Cardiac Damage

As old as 1984, evidence of cardiac damage resulting from an acute stroke has emerged, without the presence of risk factors or pre-existing cardiac damage (Kolin and Norris, 1984). The interaction between the brain and the heart has been so thoroughly investigated, that it has birthed a new field of research: neurocardiology (Samuels, 2007).

During acute stroke, electrocardiography has identified nearly 37.5% of subarachnoid haemorrhage patients and 22% of ischemic stroke patients develop some form of cardiac arrhythmia (Frontera et al., 2008; Park et al., 2016). Among intracerebral or subarachnoid haemorrhage patients, 40-100% were found to have some kind of electrocardiographic abnormality within a year of their acute event (Frontera et al., 2008; Junttila et al., 2013). Worse still, a Japanese 2013 study demonstrated a 7.2% rate of acute cardiac dysfunction in both spontaneous and traumatic cerebral haemorrhage patients (Lee et al., 2016), not to mention left ventricular diastolic dysfunction developing in more than half of subarachnoid haemorrhage patients (Chen et al., 2017). In the case of ischemic stroke, worsening cardiac dysfunction secondary to stroke inevitably leads to increased risk of more composite vascular events within a

year, including stroke itself (Park et al., 2016), forming an ever-worsening positive feedback loop of ischemic stroke worsening cardiac function.

Mild cardiac damage triggered by stroke can take the form of neurogenic stress cardiomyopathy and Takotsubo cardiomyopathy, both of which manifest as ventricular wall motion abnormalities visible through echocardiography (van der Bilt et al., 2015; Ghadri et al., 2016). Various forms of stress, physical or emotional, admittedly can induce these two conditions, and Takotsubo cardiomyopathy in particular has been postulated to be reversible post-stroke (Chen et al., 2017; Park et al., 2016).

Neurogenic heart syndrome, as the phenomenon of post-stroke cardiac damage has been termed, has borne various theories to its cause. One well-known postulation is catecholamine surge hypothesis, where a spike in serum epinephrine occurs secondary to insular damage (Chen et al., 2017). This ultimately leads to excessive sympathetic activation of the cardiac tissue, with the damage being in the form of myocytolysis (Smith K. E. et al, 1986; Hachinski V. C. et al, 1986; Barber M. et al, 2006; Dous G. V. et al, 2017). Evidence for this can be seen in a 2006 study, whereby 88% of ischemic stroke affecting the right-hemispheric insular cortex eventually develop myocardial injury within just 3 days of the acute event (Ay et al., 2006). Furthermore, the literature has an abundance of alternative theories regarding neurogenic heart syndrome, which is easily summarised in Figure 2.2.

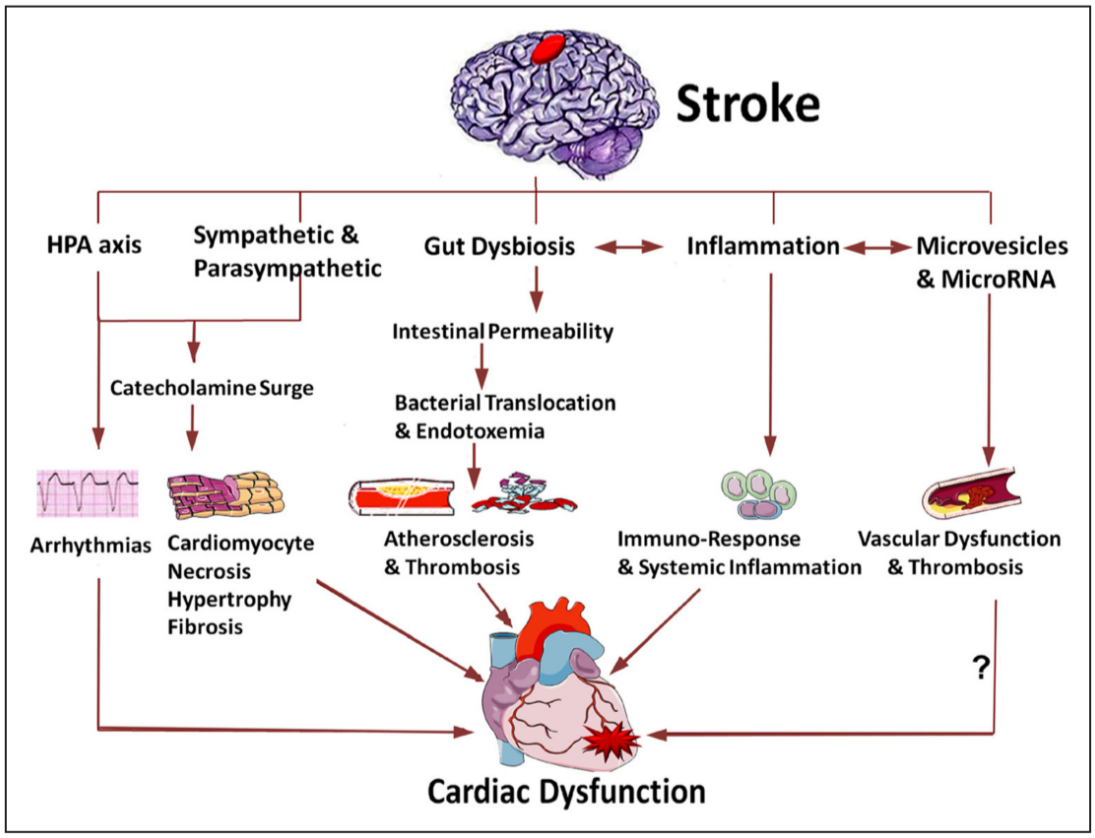

Figure 2.2: Summary of the theorised mechanisms of post-stroke brain-heart interaction. HPA = hypothalamus-pituitary-adrenal axis; ? = future studies required (Source: Chen et al., 2017)

It is for this reason that we decide to include only stroke patients whose condition has stabilized, at least 6 months after the incident of stroke.

2.7 TENS, CK and Troponin

Kang, Jeon and Lee published a study in 2015 regarding the effects of microcurrent stimulation and TENS on muscle fatigue, using several parameters including serum CK and lactate dehydrogenase (LDH) levels. Though the study concluded that TENS have no effect on muscle fatigue, this is one of the few studies that included both TENS intervention and serum CK analysis.

CHAPTER 3

METHODOLOGY

3.1 Research design

Pre-post convenience sampling single-blinded controlled interventional study

3.2 Study area

Department of Neurosciences and Rehabilitation Medicine Unit, Hospital Universiti Sains Malaysia

3.3 Study population

Outpatient haemorrhagic stroke patients with motor impairments in Hospital Universiti Sains Malaysia, from November 2020 to March 2021

3.4 Subject Inclusion Criteria

- Females and males
- Age above 18 years old, or below 18 years old with the informed consent by a legal guardian
- Stabilized first haemorrhagic stroke at least 6 months duration, evidenced from CT scan from admission
- Having mild to moderate motor impairment of limbs, spastic or paresis
- Not involved in other ongoing research for stroke.

3.5 Subject Exclusion Criteria

- Patients who had underwent intensive physiotherapy/exercise in the previous 3 weeks before recruitment screening
- Patients who are medically unstable, have upper limbs co-morbidities, or have problems associated with inability to sit still for at least 20 minutes.
- Patients with severe paresis impeding use of TENS
- Patients with history of cardiac disease, or evidence of it as shown from electrocardiogram
- Patients with any form of cognitive dysfunction or unlikely to be willing or able to comply with study procedures.
- Patients who have a pacemaker, spinal or bladder stimulator, had previous skull opening surgery or trauma, history of epilepsy (family relative), and possess metallic foreign body implants.

3.6 Sample size estimation and method

We used the OpenEpi online toolkit to estimate sample size, based on previous relevant studies. The Figures 3.1, 3.2, 3.3, 3.4, 3.5 and 3.6 below illustrate the different calculated sample size for each study objective stated in Chapter 1.

Sample Size For Comparing Two Means

Input Data

Confidence Interval (2-sided)	95%
Power	80%
Ratio of sample size (Group 2/Group 1)	1

	Group 1	Group 2	Difference*
Mean	80.4	60.4	20
Standard deviation	10	13.3	
Variance	100	176.89	

Sample size of Group 1	6
Sample size of Group 2	6
Total sample size	12

*Difference between the means

Results from OpenEpi, Version 3, open source calculator--SSMean

Figure 3.1: Objective 1 - ITT + physiotherapy on Barthel Index

Sample Size For Comparing Two Means

Input Data

Confidence Interval (2-sided)	95%
Power	80%
Ratio of sample size (Group 2/Group 1)	1

	Group 1	Group 2	Difference*
Mean	234.8	182.3	52.5
Standard deviation	24.6	20.9	
Variance	605.16	436.81	

Sample size of Group 1	3
Sample size of Group 2	3
Total sample size	6

*Difference between the means

Results from OpenEpi, Version 3, open source calculator--SSMean

Figure 3.2: Objective 2 - ITT + physiotherapy on Creatine kinase

Sample Size For Comparing Two Means

Input Data

Confidence Interval (2-sided)	95%		
Power	80%		
Ratio of sample size (Group 2/Group 1)	1		
	Group 1	**Group 2**	**Difference***
Mean	355	63	292
Standard deviation	54	100	
Variance	2916	10000	
Sample size of Group 1	2		
Sample size of Group 2	2		
Total sample size	4		

*Difference between the means

Results from OpenEpi, Version 3, open source calculator--SSMean

Figure 3.3: Objective 3 - ITT + physiotherapy on Troponin T

We used non-probability convenience sampling method to recruit subjects for our study, from 1st January to 30th June 2020. We recruited 20 haemorrhagic stroke patients with half of the participants in the intervention group and the other half in the control group. Unfortunately, 1 of the 10 interventional subjects decided to withdraw from the study due to health issues, while another 3 subjects were lost to follow up.

3.7 Research tools

Miutar Mini Massager: applied on arm of affected limb, using 2 conductive pads (2 pads: origin and insertion of the extensor carpi radialis), pulse width 230 microseconds, frequency 115 Hz, duration 20 minutes per session (See Figure 3.4 and 3.5)

Modified Barthel Index Activities of Daily Living: representative of subject's activities of daily living (See Appendix B)

Cardico 1215 Electrocardiogram Machine: Pre-intervention screening for myocardial health issues (See Figure 3.6)

ARCHITECT C8000 Clinical Chemistry Analyzer: for serum Creatine Kinase quantification; owned by Department of Chemical Pathology, Hospital Universiti Sains Malaysia; regularly serviced and maintained (See Figure 3.7)

Roche Cobas e411 Chemistry Analyzer: for serum Troponin T quantification; owned by Department of Chemical Pathology, Hospital Universiti Sains Malaysia; regularly serviced and maintained (See Figure 3.8)

Pro forma of subjects: demographics, medical history and record of study progression. We retrieved each patient's medical history from his or her personal medical record, with approval from the Director of Hospital (See Appendix C)

Figure 3.4 Miutar Mini Massager

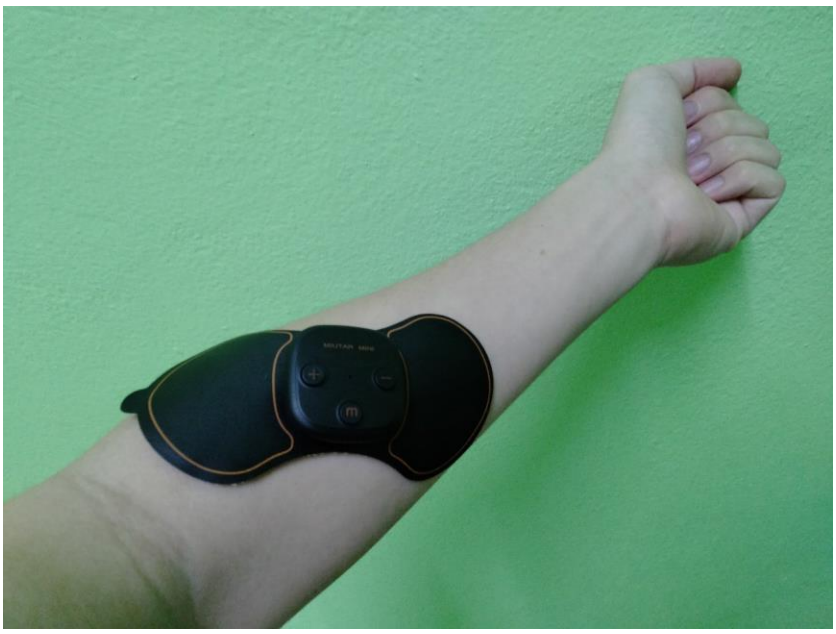

Figure 3.5 Method of Use of the Miutar Mini Massager

Figure 3.6 Cardico 1215 Electrocardiogram Machine

Figure 3.7 ARCHITECT C8000 Clinical Chemistry Analyzer