THE BIOMARKERS' CHANGES AFTER CONVENTIONAL REHABILITATION THERAPY IN ISCHEMIC AND HEMORRHAGIC STROKE PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA.

KHIATI HADJER

UNIVERSITI SAINS MALAYSIA

2022

THE BIOMARKERS CHANGES AFTER CONVENTIONAL REHABILITATION THERAPY IN ISCHEMIC AND HEMORRHAGIC STROKE PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA.

by

KHIATI HADJER

Dissertation is submitted in partial fulfilment of the requirements for the degree of Master of Neuroscience

March 2022

AKNOWLEDGMENT

All thanks to almighty Allah for the knowledge and strength to carry out this study despite the challenges of Covid19 pandemic that disrupted my plans for data collection.

I sincerely appreciate the chairman of my supervisory team, Associate Prof Dr Muhammad Hafiz Hanafi for his support all through the processes of this study. My appreciations go to my co-supervisor and INP Programme Coordinator Associate Prof Muzaimi Mustapha for his thorough scrutiny, supports and statistical critics that made this study worthwhile. I heartily appreciate my co-supervisory team members, Dr Nur Karyatee Bt Kassim for her expert contributions and timely responses despite her busy engagements.

My gratitude and appreciation go to staff at neurophysiological gym for their help and support. I wish also to appreciate the Department of Neuroscience , school of health sciences Universiti Sains Malaysia for their warm reception and provision of enabling environment all through my stay in the University.

I am indebted to my parents Mansour and Dr. Fatiha and my siblings Asmaa, Mohamed, Abd Alhamid and Mounib and to all my friends and colleagues in USM too to be written down in this confined space, thanks for your supports and encouragement. My grandfather Madani who I have lost during my journey.

Lastly, deep thanks to myself as well.

Hadjer Mansour Khiati

Alhamdulillah

TABLE F CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ADL	Activities of Daily Living
AF	Atrial Fibrillation
BBB	Blood Brain Barrier
BI	Barthel Index
CIMT	Constraint-Induced Movement Therapy
CK	creatine Kinase
CK-MB	Creatine Kinase-MB
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT Scan	Computed Tomography scan
cTnT	Cardiac Troponin T
cTnT-hs	Cardiac troponin T High sensitive
d/wk	Day/week
ECLIA	Electrochemiluminescence Immunoassay
HUSM	Hospital Universiti Sains Malaysia
IC	Confidence Interval
ICH	Intracerebral hemorrhage
IL-6	Interleukin-6
M	Mean
M1	Motor cortex
Max	Maximum
MBI	Modified Barthel Index
Min	Minimum
N	Number

OT	Occupational therapists
PT	Physiotherapists
ROM	Range of Motion
ROS	Reactive Oxygen Species
SAH	Subarachnoid hemorrhage
SD	Standard Deviation
SOPD	The Surgical Outpatient
SPSS	Statistical Package for the Social Sciences

PERUBAHAN BIOMARKER SETELAH TERAPI REHABILITASI KONVENSIONAL DALAM PESAKIT ANGIN AHMAR ISKEMIK DAN ANGIN AHMAR PENDARAHAN DI HOSPITAL UNIVERSITI SAINS MALAYSIA.

ABSTRAK

Pendahuluan: Angin Ahmar adalah penyakit heterogen yang mempunyai pelbagai sebab dan faktor risiko, dan merupakan salah satu penyebab utama kematian di dunia. Patofisiologi angin ahmar adalah proses yang kompleks yang membawa kepada rembesan biomarker darah yang berbeza ke dalam peredaran darah, seperti enzim Creatine kinase (CK), Cardiac Troponin T (cTnT) dan proinflammasi Interleukins-6 (IL-6). Terapi pemulihan konvensional bertujuan untuk mengimbangi rembesan biomarker ini pada pesakit dengan angin ahmar dengan meningkatkan aktiviti jantung dan otot. Terapi pemulihan juga, membantu individu meningkatkan kualiti hidup, kebergantungan hidup dan memudahkan semula integrasi sosial.

Matlamat: Kajian ini menilai perubahan tahap plasma serum CK, cTnT, IL-6 dan Modified Barthel Index (MBI) setelah enam minggu terapi pemulihan konvensional pada pesakit angin ahmar iskemia dan angin ahmar pendarahan di Hospital USM.

Kaedah: 19 peserta (Lelaki = 12, Wanita = 7, Umur M = 52.79; Iskemia = 9, Pendarahan = 10) menerima sesi fisioterapi konvensional selama enam minggu. Sesi ini berdasarkan terapi pemulihan konvensional yang dilakukan oleh sekumpulan ahli fisioterapi yang serupa. Alat analisis automatic sepenuhnya digunakan untuk mengukur tahap serum CK (The Architect c8000), Serum cTnT (Cobas e411) serum IL-6 (Cobas e601), dan MBI digunakan untuk mengukur kesan biologi dan fungsi sebelum intervensi dan selepas enam minggu intervensi.

Keputusan: Tidak ada perubahan yang signifikan secara statistik dalam CK, cTnT, IL-6 dan MBI sebelum dan selepas enam minggu pemulihan konvensional pada kumpulan angin ahmar iskemia dan pendarahan. Perbandingan antara kumpulan angin ahmar iskemia dan kumpulan angin ahmar pendarahan tidak menunjukkan perbezaan yang signifikan p> 0.05 (significant = 0.05 tahap signifikan), pada semua biomarker setelah terapi pemulihan konvensional selama 6 minggu. Walau bagaimanapun, terdapat perbezaan yang signifikan dalam skor MBI jenis angin ahmar iskemik dan pendarahan sebelum dan setelah terapi selama 6 minggu. (p = .006)

Perbincangan: Hasil kajian menunjukkan bahawa perubahan CK, dan cTnT, IL-6 mempunyai mekanisme yang berbeza, dan dipengaruhi oleh faktor yang berbeza yang berkaitan dengan keparahan angin ahmar, dan pemulihan fungsi yang lebih baik pada pesakit ahmar adalah bergantung kepada keterukan kecederaan sistem saraf dan kehilangan fungsi. Hasil pemulihan fungsi asalah lebih positif yang diukur dan dilaporkan oleh pesakit sendiri menggunakan MBI.

Kesimpulan: Dari penemuan kami, kajian ini menunjukkan bahawa terapi pemulihan konvensional mempunyai kesan positif terhadap perubahan fungsi pada pesakit angin ahmar. Walaubagaimanapun, perubahan tahap biomarker darah dan MBI adalah terhad kerana ukuran sampel yang kecil. Bilangan sampel yang lebih besar disyorkan untuk kajian masa depan untuk mengesahkan pengaruh pemulihan konvensional terhadap perubahan fisiologi pada pesakit angin ahmar iskemia dan pendarahan.

THE BIOMARKERS CHANGES AFTER CONVENTIONAL REHABILITATION THERAPY IN ISCHEMIC AND HEMORRHAGIC STROKE PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA.

ABSTRACT

Introduction: Stroke is a heterogeneous disease with multiple causes and risk factors and a major cause of death globally. The pathophysiology of stroke is a complex process that leads to the release of different blood markers into blood circulation, such as Creatine kinase (CK) enzyme, Cardiac Troponin T (cTnT) and pro-inflammatory Interleukins-6 (IL-6). A conventional rehabilitation therapy aimed to balance the release of these biomarkers in patients with stroke by increasing the activity of the heart and muscles. Rehabilitation therapy also helps individuals to improve their quality of life, dependency and facilitate social reintegration.

Aim: This study evaluated the changes in the serum concentration level of plasma CK, cTnT, IL-6 biomarkers and Modified Barthel Index (MBI) scores after six weeks of the conventional rehabilitation therapy in ischemic and hemorrhagic stroke patients in Hospital Universiti Sains Malaysia (HUSM).

Method: This was a prospective study with 19 participants (Males= 12, Females= 7, Age M= 52.79; Ischemic= 9, Hemorrhagic= 10) who underwent conventional physiotherapy sessions over a period of six weeks. The sessions were based on conventional rehabilitation therapy conducted by similar sets of physiotherapists. A fully automated analyser, was used to measure the concentration level of serum CK (The Architect c8000), Serum cTnT (Cobas e411) serum IL-6 (Cobas e601), and MBI was used to measure the biological and functional outcomes before the intervention and after the six weeks intervention.

Results: There were no statistically significant changes in CK, cTnT, IL-6 and MBI scores before and after six weeks of conventional rehabilitation in ischemic and hemorrhagic stroke groups. Comparison between the ischemic and hemorrhagic stroke groups showed no significant difference $p > 0.05$ ($\alpha = 0.05$ significant level) in all biomarkers' levels after six weeks of conventional rehabilitation therapy. However, there was a significant difference in MBI scores between ischemic and hemorrhagic stroke types after six weeks of therapy ($p=.006$).

Discussion: The findings suggest that the changes in CK and cTnT, IL-6 are likely to be regulated by different mechanisms and influenced by different factors related to the severity of the stroke. Better functional recovery in stroke patients is related to the initial nervous system severity and dysfunction. Functional recovery outcome has been more positive, which was measured and self-reported by the patients using MBI.

Conclusion: From our findings, this study suggested that conventional rehabilitation therapy positively affects functional outcomes in stroke patients. However, the changes in the concentration level of blood biomarkers (CK, cTnT, IL-6) and MBI in this study were limited due to the small sample size. Larger sample size is recommended for future studies to confirm the influence of conventional rehabilitation on physiological changes in ischemic and hemorrhagic stroke patients.

CHAPTER 1

INTRODUCTION

1.1 Background

A cerebrovascular accident or a "Stroke" is a disturbance in the function of the brain, occurs when the blood supply to the brain is interrupted, preventing from getting oxygen and nutrients, leading to cells death. The lesion and clinical signs depend on the severity and location of the disturbance (Ackerley & Stinear, 2010). The two major causes of stroke are blockage or rupture of arterial supplies. Ischemic stroke is the result of either insufficient or direct loss of blood flow to a part of the brain from either temporary or permanent arterial occlusion of vessels supplying that area (Moro et al., 2004), and hemorrhagic stroke is due to the rupture of the brain vessels supplied the brain that leads to neurological deficits and permanent disabilities (Khoshnam et al., 2017; Tozlu et al., 2019). The incidence of ischemic stroke is higher, but hemorrhagic stroke causes more deaths and is responsible for more lifetime disabilities (Feigin et al., 2015).

Stroke is the second cause of death worldwide, and it has a significant cause of disabilities as well (Moro et al., 2004; Sherrie M Jean, 2012). The stroke's prevalence increased because of the age of the population. However, in low and middle-income countries, the incidence of stroke has increased in the young population. Strokes' mortality and incidence are different between countries, races and ethnicity. Prevention, early treatment, and rehabilitation have positively influenced decreasing the prevalence of stroke burden in high-income countries over the past 30 years (Feigin et al., 2015). Stroke patients who need professional care by clinicians

with expertise in neurological conditions continue to increase in the coming decades (Aqueveque et al., 2017a).

Stroke is a heterogeneous disease with various or multiple subtypes. The purpose of classifying stroke is to describe the characteristics of patients, group patients, therapeutic and medical decision-making. Most clinical researchers used Trail of Org 10172 in Acute Stroke Treatment (TOAST) system by Trail of Org 10172 to classify stroke. TOAST stroke classification system is based on the stroke etiologic (Amarenco et al., 2009a; Marbun et al., 2018).

TOAST distinguished between two major categories, i.e., Ischemic stroke that is divided into large artery thrombosis, small penetrating artery thrombosis, cardiogenic embolic, cryptogenic and other determinate causes, and hemorrhagic stroke and its subtypes are subarachnoid hemorrhage and intracerebral hemorrhage. Stroke risk factor arises from the particular characteristic that increases the likelihood of having a stroke. Stroke is associated with several risk factors. Some of them are irreversible, and some are modifiable.

A blood biomarker or biological biomarker is an indicator of several conditions such as biological states, conditions, pathological processes, risk factors, or pharmacological responses (Makris et al., 2018a). In stroke patients, a biomarker needs to provide enough information to various clinical, epidemiological, and predict the risk of stroke, diagnose or predict the outcome (Califf, 2018a; Ng et al., 2017a).

Inflammation is a secondary injury mechanism following ischemia (Pawluk et al., 2020a), which elevated serum cytokine secretion such as pro-inflammatory cytokine Interleukin-6 (IL-6). Elevated IL-6 levels correlate with severe deficits, brain damage, and negative clinical outcomes (Vila et al., 2000a). The post-stroke disabilities are caused by the stroke damaged or injured muscles leads to the release of intracellular muscles components or enzymes, including myoglobin, creatine kinase (CK), directly into the extracellular space and bloodstream (Cabral et al., 2020a; Huerta-Alardín et al., 2005).

Markers such as CK identify muscle damage and indicate the muscle cell membranes (Torres et al., n.d.; Wang et al., 2011a). However, Troponin T (cTnT) is more sensitive and specific compared to CK to reveal the minor myocardial injury. Meanwhile, high sensitive cTnT marker is a specific and efficient marker for cardiac damage after stroke. cTnT elevation is observed in patients with stroke in general (Ay et al., 2002a).

One-third of stroke patients with movement impairments achieve a low functional outcome five years after the stroke onset (Dobkin, 2005) . The management of acute stroke has increased, and it has significant progress, mainly to minimise the dependence of post-stroke survivors on rehabilitation. After acute brain injury, the eventual aim of neurorehabilitation is to reach optimal functional recovery. Rehabilitation aims to reduce the neurological deficit and its complications after stroke to help individuals obtain as much improvement in their quality of life and facilitate social reintegration (Marwaa et al., 2020).

Conventional rehabilitation therapy offers treatment and examination of the physical and motor problems caused by the neurological condition and needs of stroke patients. The physiotherapy treatment focuses on the condition and symptoms presented by patients. The physiotherapy rehabilitation will help in several ways, to retrain standard movement patterns, to improve fine and gross motor skills, and to reduce the risk of falling etc. **(**Hatem et al., 2016, Coelho Junior et al., 2016)

Physiotherapy rehabilitation has been suggested to be a helpful intervention to treat stroke patients since it helps regulate most of the conditions, which are the consequences of the stroke on the body (Hatem et al., 2016). Various studies focused on the effects of physiotherapy on cognitive, upper, and lower limb motor function, cardiovascular performance, muscles resistance, balance, gait and mobility, and it was used as an anti-inflammatory therapy for chronic diseases (Coelho Junior et al., 2016). Furthermore, more recently, stroke risk and poststroke recovery are thought to be related to the state of endothelial functions **(**Hatem et al., 2016, Coelho Junior et al., 2016).

Poststroke physiotherapy rehabilitation, which mainly includes physical exercises and training, initiates the release of mediators called myokine. These mediators play a prominent role in the field of biomarkers, which are related to the role of serum, plasma or blood biomarkers in stroke (Gandolfi et al., 2017).

1.2 Problem statement

Rehabilitation began early after a stroke to reduce post-stroke functional disabilities, which results in a more outstanding quality of life for stroke patients and a decrease in potentially expensive long-term care costs (Brewer et al., 2013; Duncan et al., 2005a; Whitehead & Baalbergen, 2019a). The traditional therapeutic disciplines involved conventional rehabilitation therapy (physiotherapy and occupational therapy), speech and language therapy for all stroke survivors (Booth & Hewison, 2002; Whitehead & Baalbergen, 2019a) is an important phase after stroke, and this area is growing with more new interventions such as robotic and brain stimulation.

There is growing evidence that post-stroke rehabilitation is effective, but it tends to rely more on functional outcomes. There is limited research into the specificities of post-stroke rehabilitation on the neurophysiological changes (Duncan et al., 2002). On the other hand, neurorehabilitation research has progressively improved in recent years. However, traditional rehabilitation procedures may have limited efficacy in most patients with common neurological diseases, but it is the main therapy in most hospitals (Tamburin et al., 2019).

This study addressed the effects of conventional rehabilitation on changes in biomarkers level, i.e., CK, cTnT, and IL-6, which are related to the severity and damage by the stroke. This study aimed to evaluate the effects of conventional rehabilitation on these blood biomarkers for two main reasons, i.e., to help healthcare professionals to reduce secondary complications by improving protocols. Next, to facilitate the new neurorehabilitation research, which focuses on these biomarkers in stroke patients undergoing physiotherapy rehabilitation.

1.3 Significance of the study and Rationale

This research was intended to evaluate the effects of conventional rehabilitation therapy on changes in blood markers serum CK, cTnT and IL-6. Conventional physiotherapy therapy includes functional, strengthening, balance therapy exercises and gait and mobility. There was no pain or need-pain-relieving method during the period of the study. The design of this study did not require the patient to be hospitalised. The results obtained will help the health professionals improve the physiotherapy procedure applied to stroke patients for better and optimal results. The musculoskeletal biomarkers creatine kinase and cardiac troponin t, and inflammatory biomarker interleukin-6 have shown a significant prediction of post-stroke muscle damage and stroke severity, respectively.

This study can direct future researchers to conduct more research on different neurorehabilitation approaches with patients under physiotherapy rehabilitation, which involves studying physiological changes of blood biomarkers after stroke. Serum CK, cTnT, and IL-6 are modifiable markers that indicate the severity of a stroke. Studies related to this field might help improve the well-being of stroke patients and improve functional outcomes in patients of other neurological diseases other neurorehabilitation regimes.

1.4 Research Hypothesis

1.4.1 General Hypothesis

There are changes in biomarkers levels in Creatine Kinase enzyme, cardiac Troponin T, Interleukin-6 and Modified Barthel Index scores after six weeks of the conventional rehabilitation in first time ischemic and hemorrhagic stroke patients

1.4.2 Alternative Hypothesis:

1. There is a significant decrease in serum CK level after six weeks of conventional rehabilitation in first time ischemic and hemorrhagic stroke patients.
2. There is a significant decrease in cTnT level after six weeks of the conventional rehabilitation in first time ischemic and hemorrhagic stroke patients.
3. There is a significant decrease in the IL-6 level after six weeks of the conventional rehabilitation in first time ischemic and hemorrhagic stroke patients.
4. There is a significant increase in MBI scores after six weeks of conventional rehabilitation in first time ischemic and hemorrhagic stroke patients.
5. The is a significant difference in changes in plasma Serum CK, cTnT, IL-6 levels and MBI scores after six weeks of the conventional rehabilitation between first time ischemic and hemorrhagic stroke patients.

1.4.3 Null Hypothesis:

There are no significant changes in biomarkers levels in serum CK, cTnT, IL-6 levels and MBI scores after six weeks of the conventional rehabilitation in first time ischemic and hemorrhagic stroke patients.

1.5 Research objectives

1.5.1 General Objective:

To evaluate the changes in biomarkers levels, serum CK, cTnT, IL-6 and MBI scores after six weeks of the conventional rehabilitation therapy in first time ischemic and hemorrhagic stroke patients.

1.5.2 Specific Objectives:

1. To determine the changes in CK level after six weeks of conventional rehabilitation therapy in first time ischemic and hemorrhagic stroke patients, separately.

2. To measure the changes in cTnT level after six weeks of the conventional rehabilitation in first time ischemic and hemorrhagic stroke patients, respectively.

3. To establish the changes in IL-6 level after six weeks of the conventional rehabilitation in first time ischemic and hemorrhagic stroke patients, respectively.

4. To compare changes in quality of life with changes in the biomarkers after six weeks of conventional rehabilitation in first time ischemic and hemorrhagic stroke patients.

5. To compare changes in serum CK, cTnT, IL-6 levels and MBI scores after six weeks of the conventional rehabilitation between first time ischemic and hemorrhagic stroke patients.

1.6 Research questions

1.6.1 General questions:

Does the 6 weeks of the conventional rehabilitation have a significant influence in serum creatine Kinase biomarkers, cardiac Troponin T, Interleukin-6 and Modified Barthel Index scores in first time ischemic and hemorrhagic stroke scores patients?

1.6.2 Specific questions:

1. Does the six weeks of conventional rehabilitation significantly influence the changes in creatine kinase in first time ischemic and haemorrhagic stroke patients?

2. Does the six weeks of conventional rehabilitation significantly influence cardiac troponin level in first time ischemic and haemorrhagic stroke patients.

3. Does the six weeks of conventional rehabilitation significantly influence interleukin-6 level in first time ischemic and haemorrhagic stroke patients.

4. Does the six weeks of the conventional rehabilitation significantly influence Modified Barthel Index scores in first time ischemic and haemorrhagic stroke patients.

5. Does the six weeks of the conventional rehabilitation significantly influence changes in serum CK, cTnT, IL-6 levels and MBI scores after 6 weeks of the conventional rehabilitation between first time ischemic and hemorrhagic stroke patients?

CHAPTER 2

LITERATURE REVIEW

Stroke is defined as a heterogeneous disease with multiple causes and risk factors (Amarenco et al., 2009b). It is one of the major global health problems and the leading cause of death worldwide. Stroke is an acute cerebral vascular disease due to disturbance in the blood supply to a brain region, resulting in death or permanent neurological deficits (Ackerley & Stinear, 2010; Moro et al., 2004). Stroke is mainly classified into ischaemic and hemorrhagic stroke (Campbell et al., 2019). Ischemic stroke is the infarction of the brain, spinal cord or retina, which occurs when cerebral blood flow is interrupted. Ischemic stroke represents 85% of all strokes globally (Ren & Yang, 2018). However, hemorrhagic stroke is due to intracerebral hemorrhage and subarachnoid hemorrhage (Campbell et al., 2019)

2.1 Stroke classification

Stroke subtype classification is an indispensable part of patient evaluation not only for clinical study and treatment but also for therapeutic decision-making in daily practice (Amarenco et al., 2009c; Han et al., 2007). The classification should be based on synthesising the patient's medical history, physical examination, imaging data, and diagnostic tests. Stroke classifications distinguish between two major categories, i.e., ischemic stroke and hemorrhagic stroke (Amarenco et al., 2009c; Garg et al., 2019a). Ischemic stroke (85%) is defined as the interruption of the blood flow to the brain; however, hemorrhagic stroke (15%) results from the rupture of the blood supply to the brain leading to permanent neuroglial deficits (Khoshnam et al., 2017; Tozlu et al., 2019), and these two main categories are divided into subtypes (Figure 2.1).

Ischemic stroke subtypes are large artery thrombosis (atherosclerotic disease, 20%), small penetrating artery thrombosis (25%), cardiogenic embolic (20%), cryptogenic (30%), and other represents 5%. On the other hand, hemorrhagic stroke subtypes are subarachnoid hemorrhage with 7% and intracerebral hemorrhage with 8% of all strokes. This classification is according to Trail of Org 10172 in Acute Stroke Treatment (TOAST) classification (Hauer et al., 2017), which is the most helpful system to categories stroke subtypes (Garg et al., 2019b) based on the mechanism of infarction (Rovira et al., 2005a).

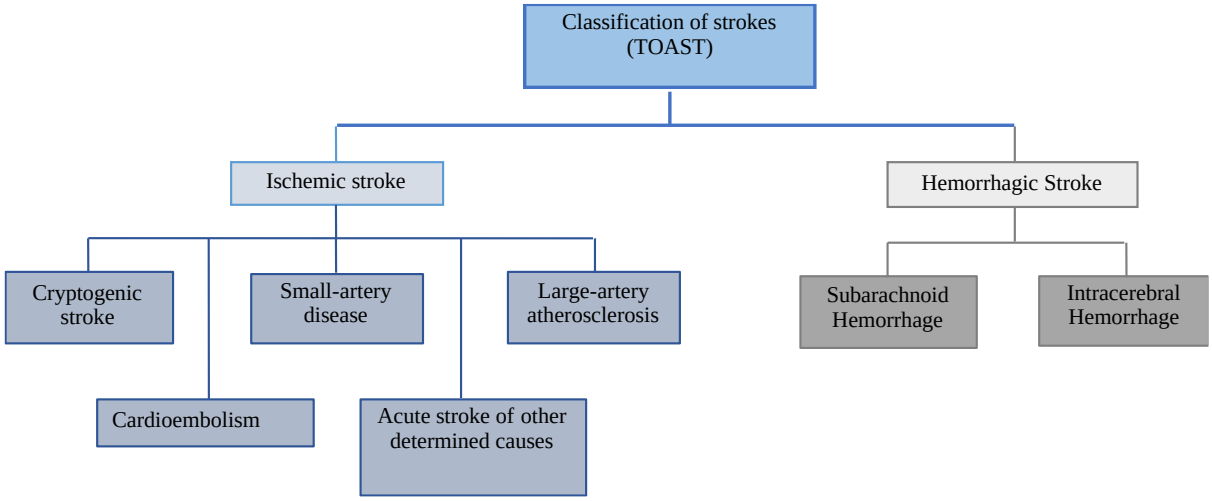

Figure 2.1 : Classification of Stroke

2.1.1 Ischemic stroke subtypes:

The classification system of ischemic stroke subtypes mainly used on the causes and the mechanism leading to the blockage of a blood vessel (Rovira et al., 2005a); the TOAST classification system includes five categories (Figure 2.1):

1) **Large-artery atherosclerosis,** which refers to the hardening of the arteries that supply the brain (Yaghi et al., 2019), occurs due to the formation of the lipid-laden atherosclerotic plaques (Derdeyn, 2007; Rovira et al., 2005b) in the inner layer of the wall of the extracranial or intracranial arteries. It can originate from different sources such as cardiac embolism, atheroembolism, local cerebral arterial thrombosis, venous embolism, embolisation from other sources (e.g., neo-plasms, vessel walls fragments, detachment of devices, biological valves) (Bacigaluppi et al., 2019).

2) **Cardioembolism** occurs as a result of a blood clot created by functional or structural abnormalities of the heart entering the circulation, causing occlusion of the cerebral artery (Derdeyn, 2007; Rovira et al., 2005b). The thrombus formed by the cardioembolism is red (formed by interlocking red blood cells). The thrombus results from intracardiac stasis of blood or adhering to a thrombogenic lesion.

3) **Small-artery disease** refers to the variety of the abnormalities involving microcirculation of the brain, these abnormalities affect deep areas of the hemisphere white matter (Rovira et al., 2005b), and a major leading cause of functional loss, disability (motor symptoms face and arm or arm and leg), cognitive impairment in the elderly which called vascular cognitive impairment (Q. Li et al., 2018).

4) **Cryptogenic stroke** is defined by TOAST as a stroke of undetermined etiologic despite an extensive evaluation (Amarenco et al., 2009d; Love & Bendixen, 1993), and a diagnosis of exclusion as a result of two or more causes being identified, including negative or incomplete evaluation. This type of stroke results in fewer neurologic deficits, fewer long-term disabilities, and lower mortality than other strokes of determined origin (Love & Bendixen, 1993).

5) **Acute stroke of other determined causes** according to TOAST classification system, patients in this category have noncommon causes of stroke, nonatherosclerotic vasculopathies, hypercoagulable states, or hematologic disorders (Amarenco et al., 2009d; Love & Bendixen, 1993).

2.1.2 Hemorrhagic stroke subtypes

Hemorrhagic stroke is when the bleeding occurs directly into the brain by the rapture of weak blood vessels, which accumulates and compress the surrounding brain tissue (Love & Bendixen, 1993; Mehta & Aka, 2020a). The leaking blood presses on the surrounding brain tissues, which damages them, and the damaged area becomes unable to function correctly, which leads to neurological deficits. Hemorrhage stroke is further subdivided into two main categories, i.e., intracerebral hemorrhage and subarachnoid hemorrhage; both are devastating diseases with a significant proportion of stroke mortality, morbidity and or survival with severe long-term disability (Love & Bendixen, 1993; Mehta & Aka, 2020a). In an intracerebral heemorrhage, the bleeding occurs in the brain parenchyma; however, in subarachnoid hemorrhage, the bleeding is into the subarachnoid space (Mehta & Aka, 2020a).

1) **Intracerebral hemorrhage** is defined as bleeding in the brain which results from the rupture of the small artery, which has been weakened and damaged by deep perforator arteriopathy (hypertensive arteriopathy) or cerebral amyloid angiopathy (Hostettler et al., 2019). Intracerebral hemorrhage is also defined by its location (Aguilar & Brott, 2011) within the parenchyma of the brain; intracerebral hemorrhage mainly occur in a few different areas of the brain but mainly occur in the basal ganglia, cerebral lobes, cerebellum, or pons (Hostettler et al., 2019).

2) **Subarachnoid hemorrhage** is the second primary subtype of hemorrhagic stroke based on the TOASR classification system. Subarachnoid hemorrhage is defined as bleeding between the arachnoid membrane and the pia membrane. Subarachnoid hemorrhage is mainly due to an intracranial aneurysm's rapture in most cases, vascular malformations, and vasculitis (Aguilar & Brott, 2011; Mehta & Aka, 2020b).

2.2 Stroke risk factors

Stroke risk factors are certain conditions that give a high chance of developing stroke. Stroke risk factors are either modifiable or irreversible risk factors (which can be measured and changed) and non-modifiable risk factors (which mean cannot be changed) (Choudhury et al., 2015; Rovira et al., 2005c). Stroke is associated with several risk factors, and these factors are similar for hemorrhagic and ischemic stroke; however, there are few significant differences; furthermore, the etiologic categories of ischemic stroke are associated with different factors (Boehme et al., 2017b; Choudhury et al., 2015).

Stroke risk factors can be classified as either modifiable or non-modifiable risk factors. Age, gender, and race or ethnicity are the non-modifiable or irreversible risk factors for both hemorrhagic and ischemic strokes. However, modifiable risk factors are more essential and manipulating these factors is aimed to reduce the risk of stroke. Meanwhile, early identification of modifiable risk factors can help predict and prevent the occurrence of stroke (Boehme et al., 2017b; Choudhury et al., 2015). Modifiable risk factors can be further classified into medical such hypertension, diabetes mellitus, hyperlipidemia, and behavioural risk factors such as smoking, physical inactivity, and alcohol consumption (Table 2.1, Figure 2.1, Figure 2.2) (Boehme et al., 2017b; Choudhury et al., 2015).

Table 2.1: Ischemic and hemorrhagic strokes risk factors (Boehme et al., 2017b)

Type of stroke / **Risk Factors**	**Ischemic Stroke**	**Hemorrhagic Stroke**
Modifiable risk factors	Hypertension Hyperlipidaemia Cardiac disease Diabetes mellitus Smoking Alcohol Consumption Diet Physical inactivity	Hypertension Smoking Alcohol Consumption Diet
Nonmodifiable risk factors	Age Gender Race or Ethnicity Heredity or Genetics	

2.2.1 Non-modifiable risk factor

1) Age: Stroke is a disease of ageing; it is the most critical marker of stroke risk factors that can be modified. The incidence of stroke increases with age, and this incidence approximately doubles for each successive 10 years after age 55 in both men and women (Boehme et al., 2017b; Choudhury et al., 2015).

2) Gender: The relationship between gender and stroke risk is associated with age and other factors as well. Gender is considered as a marker and non-modifiable stroke risk factor. The stroke incidence rate is higher in males than females. However, females have a higher lifetime risk for stroke (Boehme et al., 2017b; Samai & Martin-Schild, 2015), and they are older at the time they have their first stroke. Research has also demonstrated that women have more strokes during their lifetime, which is related to the longer lifespan, and they are more likely to have severe strokes than men (Choudhury et al., 2015).

3) Race or Ethnicity: The concept of racial or ethnic disparities are complex and difficult challenges, but the definition is based on the physical, biological characteristics, social characteristics and their interaction (Cruz-Flores et al., 2011). Racial disparities in health care is a growing concern, prevention interventions of stroke mortality, morbidity and disability are required to highlight the racial-ethnic differences. Based on previous studies, differences are significant in stroke prevalence between races in the United States of America (Samai & Martin-Schild, 2015). The burden of stroke risk factors is different among ethnic groups.

4) Heredity/Genetics: Heredity is identified as a non-modifiable marker of stroke risk factors. The presence of these factors helps to identify those at high risk. Multiple studies also showed that genetic risk factors for stroke are related to parental and family history. The genetic risk factors vary by age, gender, and race (Boehme et al., 2017b; Cruz-Flores et al., 2011).

2.2.2 Modifiable Risk Factors

1) Hypertension: Hypertension is one of the most important modifiable risk factors for stroke, with the significant, direct and continuous association between blood pressure and stroke incidence (Boehme et al., 2017b). The incidence of stroke due to hypertension is defined as symbolic blood pressure 160 mm Hg and or diastolic blood pressure 90-95 mm Hg; blood pressure has more signification on the hemorrhagic stroke than ischemic stroke. Hypertension is a modifiable risk factor which means that it is associated with other risk factors; patients with a high risk of hypertension are encouraged to change a few behavioural lifestyles such as diet and physical activities to reduce the impact of hypertension (Choudhury et al., 2015).

2) Cardiac disease: Numerous heart-related diseases have been shown to increase stroke risk. Cardioembolic infraction resulted from atrial fibrillation (AF) and atrial flutter. Atrial most modifiable risk factors associated with cardioembolic ischemic stroke subtype, which is the most severe type of stroke with the high disability and mortality (Arboix, 2015; Choudhury et al., 2015; Jx et al., 2020). The presence, incidence and prevalence of AF increase with age, representing 20-25% (1 in 4 person was due to AF) of stroke patients 80 years and older (Arboix, 2015; Choudhury et al., 2015).

3) Diabetes mellitus: Diabetes is a stroke risk factor following hypertension, and it has been recognised independent factor for stroke. Diabetic patients have a 2-fold risk of stroke (Arboix, 2015), with a high proportion of ischemic stroke compared to hemorrhagic stroke (Gaede et al., 2008). The increase in diabetes explains the increase in the incidence of stroke in the younger population (Boehme et al., 2017b). Diabetes mellitus is a modifiable stroke risk factor, the use of behavioural and medical therapy combinations has remarkable effectiveness in reducing the risk of stroke in diabetic patients (Gaede et al., 2008).

4) Hyperlipidaemia: Hyperlipidaemia is an important risk factor for cardiac diseases, and the relationship to stroke is still complex and unclear (Jx et al., 2020). The stroke incidence and hyperlipidaemia relationship is complicated and vary depending on stroke type, cholesterol level, and lipid parameter (Gainey et al., 2018). The risk of ischemic stroke increases with the increased total cholesterol. However, total cholesterol is inversely related to the risk of intracerebral hemorrhage. The benefits of using statins appear to reduce the risk of ischemic stroke and its mortality without increasing the risk of intracerebral hemorrhage stroke (Boehme et al., 2017b; Jx et al., 2020).

5) Smoking: Cigarettes smoking doubles the risk of stroke with clear dose-response relation. Smoking cessation reduces the risk rapidly and nearly disappear within 2 to 4 years after stopping (Choudhury et al., 2015; Jx et al., 2020).

6) Alcohol consumption: Heavy alcohol consumption increases the risk of any type of stroke; light to moderate (<4 units/day) consumption is associated with a lower risk of ischemic stroke. However, alcohol consumption has a linear relationship with intracerebral hemorrhage stroke risk (Choudhury et al., 2015; Jx et al., 2020).

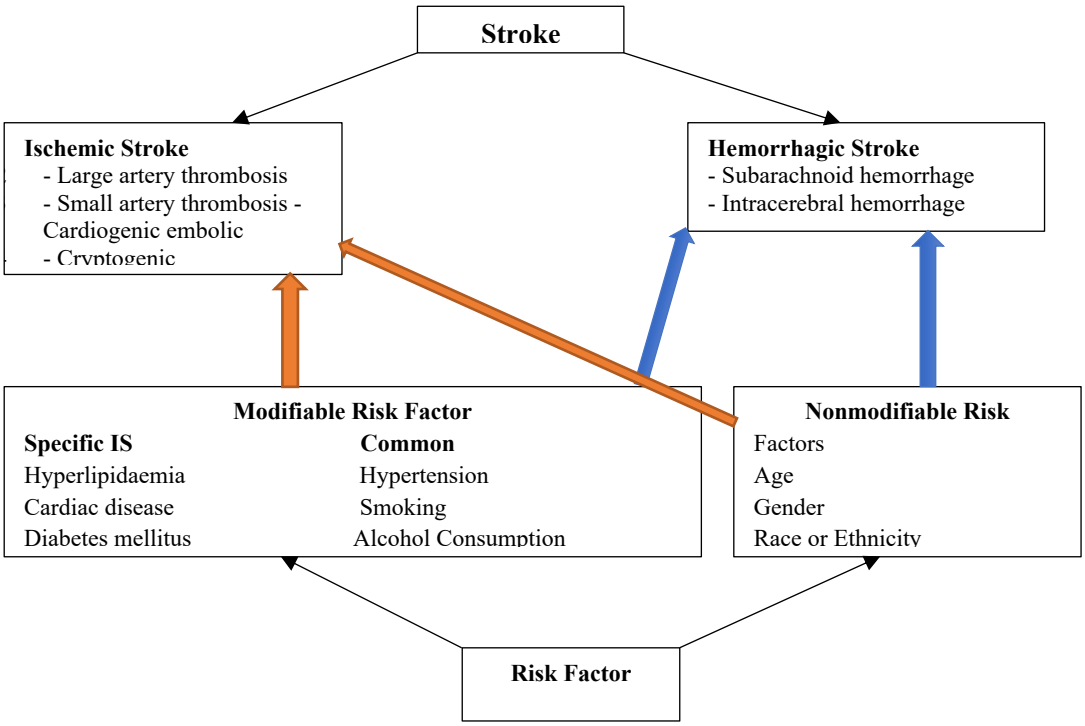

Figure 2.2: Summary of Stroke Classification and Risk Factors

2.3 Biomarkers

A blood biomarker or biological markers are objectively measured biological indicators of biological states and addition, normal or pathologic pathological processes, risk factors, or pharmacological responses to therapeutic interventions (Makris et al., 2018b; Ng et al., 2017b). An ideal biomarker should be highly sensitive and specific for the outcome, accurate, reproducible, have good positive and negative predictive values, assist decision-making, easy to interpret, and cost-effective (Ng et al., 2017b).

Biomarkers include molecular, histologic, radiographic, or physiologic characteristics. Biomarkers are divided into subtypes according to their applications. Any biomarker can have multiple criteria for different uses and clear distinguishing features that specify uses. Thus, blood markers can be classified mainly into subcategories (Califf, 2018b): Diagnostic

biomarkers which detect or confirm the presence of a disease or condition of interest or identify a subtype of the disease (Figure 2).

Monitoring biomarkers are used to assess medical conditions or problems, the status of problems, or the impact of external products or agents. Predictive biomarkers are used to identify individuals who experience a favourable or unfavourable effect from exposure to a medical product or an environmental agent that similar individuals without the biomarker experience. Prognostic biomarkers determine the likelihood of a clinical event, recurrence or disease progression in patients with a disease or disease of interest. Risk biomarkers are used to determine the occurrence of a disease or medical condition in people who do not have a disease or medical condition; the possibility of disease or no health is currently clinically identifiable (Califf, 2018b). The use of appropriate biomarkers for specific purposes is directly related to the quality of research.

In stroke patients, a useful biomarker is a biomarker that is able to provide enough information to various clinical, epidemiological, and different research situations, e.g., to predict the risk of stroke, to diagnose stroke and its subtypes (stroke from mimics and ischemic from hemorrhagic), to predict outcome and to monitor complications, to follow-up patients, selection and guidance of therapy, and clinical research and drug development (Califf, 2018b; Ng et al., 2017b; Pullagurla et al., 2015). Moreover, stroke heterogeneity, the complexity of its pathophysiology, and the presence of the blood-brain barrier (BBB) limit the access of biomarkers in sufficient quantities to the vasculature makes biomarker isolation difficult (Pullagurla et al., 2015).

On the other hand, many blood biomarkers associated with stroke are not stroke-specific. They are related to different acute brain injuries such as ischemic stroke, hemorrhage stroke (intracerebral and subarachnoid stroke) and traumatic brain injury (Jickling & Sharp, 2015). Due to the complexity and heterogeneity of the pathogenesis of stroke, the use of a single biomarker may not be sufficient to identify different aspects of the pathogenesis of stroke; Instead, multiple biomarkers may be required to assess the importance of various molecular changes associated with stroke (Jickling & Sharp, 2015; Ng et al., 2017b). Multiple biomarkers strategy is used to assess the significance of multiple stroke-related molecules, which reflect different pathophysiological processes of disease, dynamic processes, inflammation, oxidative stress, cardiac function and endothelial injury (Califf, 2018b; Jickling & Sharp, 2015).

Following cerebral ischemia, ischemia-associated excitotoxicity and neurodegenerative insults, mitochondria increase the calcium, which leads to increased production of reactive oxygen species (ROS) have a vital role in neuroinflammatory reactions (Popa-Wagner et al., 2013). The ROS triggers the activation of downstream inflammatory mechanisms and multiple immune cells recruitment such as macrophages, neutrophils, and T cells that release the pro-inflammatory cytokines such as interleukin-6 (IL-6), which can cross the blood-brain barrier into the circulation (Ng et al., 2017b).

IL6 level in patients with hemorrhagic stroke may have prognostic significance (Oto et al. 2008). IL-6 is an important pro-inflammatory cytokine after stroke (Manolescu et al., 2011), and IL-6 concentration increased after hemorrhagic and ischemic stroke in cerebrospinal fluid and blood of stroke patients (Acalovschi et al., 2003; Manolescu et al., 2011). According to Manolescu et al. (2011), Aref et al. (2020), Shamseddin Hejazi et al. (2014), IL-6 is associated with stroke outcome, and it contributes to the determination of clinical outcome, prediction of recurrence the severity and prognosis.

2.3.1 Proinflammatory Cytokine interleukin-6 (IL-6)

The pathophysiology of stroke is a complex process that includes the excitotoxicity mechanisms and inflammatory pathways, which constitute the key mechanisms involved in neuronal damage (Pawluk et al., 2020b). Inflammation is implicated as a secondary injury mechanism following stroke (Feigin et al., 2017). The inflammatory response is a well-known and widely studied phenomenon. There is increasing evidence that the inflammatory response plays an important role in the potentiation of the central nervous system (CNS) (Danton & Dalton Dietrich, 2003).

The inflammatory responses appear to be mediated by interleukins (ILs), i.e., a multifunctional subclass of cytokines (Kochanek & Hallenbeck, 1992). Pro-inflammatory interleukins such as IL1 and IL6 play an important role in the function and synthesis of other cytokines through a complex cytokine network. Interleukins are produced by a variety of immune cells, including microglia, astrocytes, and leukocytes, and seem to regulate CNS cell apoptosis and direct differentiation and spread (Benveniste, 1992; Kochanek & Hallenbeck, 1992).

IL-6 is a multifunctional cytokine produced by various cell types during infection and immunological challenge. It has been shown that cerebral ischemia triggers IL-6 release into blood (Eldaief et al., 2013). In acute stroke patients, elevated serum IL-6 is correlated with a more severe deficit, with the extent of brain damage, and with worse clinical outcomes. In addition, the relationship between serum IL-6 and biomarkers of acute-phase reaction (C-reactive protein, erythrocyte sedimentation rate, body temperature, and cortisol) was found in stroke patients (Dziedzic et al., 2004; Vila et al., 2000b).

In fact, the role of IL6 in the central nervous system is rarely explored. However, the indirect evidence that IL6 is involved in ischemic injury after stroke comes from clinical studies, which show that cerebrospinal fluid (CSF) and IL6 levels can predict patient functional recovery and are related to patient infarction size (Dziedzic et al., 2004). (Figure 2.3). (Dziedzic et al., 2004).

2.3.2 Serum Creatine Kinase

Stroke is the main cause of disability. Hemiplegia is the most common chronic disabling consequence of stroke, leading to significant changes in skeletal muscle structure and metabolism (Hafer-Macko et al., 2008). Disability after stroke leads to relative inactivity and decreased muscle mass and function. Skeletal muscles may be damaged by impaired central nervous system activity, spasticity and overactive of the stretch reflex. Injured or damaged muscles can cause intracellular muscle components of enzymes (including myoglobin, CK, aldolase, and electrolytes) to be released directly into the bloodstream and extracellular space (Cabral et al., 2020b; Torres et al., 2015). These enzymes have been used as biomarkers of the functional status of skeletal muscle. Markers such as the enzyme CK can identify muscle damage and indicate the state of muscle cell membranes (Torres et al., 2015; Wang et al., 2011b). As skeletal muscle membrane permeability is disrupted in stroke, these biomarkers can indirectly examine skeletal muscle damage.

CK enzyme was first documented as a cardiac biomarker in the year 1979 (Mythili & Malathi, 2015a); creatine kinase-MB (CK-MB) activity has been shown to increase in certain patients with ischemic stroke, sub-arachnoid hemorrhage (Ay et al., 2002b). CK is an enzyme present in tissue and energy-demanding cells, such as skeletal and cardiac muscles. It is considered one of the best sensitive and specific biomarkers to detect and monitor skeletal muscle damage and diseases (Nogueira et al., 2019). CK enzyme has three isoenzymes: MM, MB and BB. CK-MM is the skeletal muscle

fraction,CK-MB is the cardiac muscle fraction, and CK-BB is the brain fraction, CK-MB appears in both in cardiac muscle with approximately 20% of total CK, and it represents in normal skeletal muscle (Kiranmayi et al., 2015; Mythili & Malathi, 2015b).

Total serum CK levels are elevated in myocardial damage, and it may also be released after non-cardiac muscle damage, which decreases its specificity (Mythili & Malathi, 2015b; Nogueira et al., 2019). Stroke may bring significant pressure to the patient's heart, a continuing low-grade myocardial necrosis suggestive of myocytolysis, which is a pathological sign of stroke-related heart damage. It is characterised by inflamed muscle cell lesions, and interstitial bleeding, and mononuclear in the vicinity of cardiac nerves has been implicated as the reason for the rise of CK-MB (Kiranmayi et al., 2015).

CK is not complete ultimately specific; as we mentioned, it is also related to other skeletal muscle diseases or injuries and conditions. However, cTnT is more sensitive and specific to the CK enzyme in revealing minor myocardial damage. cTnT levels in a subset of patients with ischemic stroke help to understand the stroke-related cardiac damage in parallel to CK (Ay et al., 2002c). (Figure 2.3) (Ay et al., 2002c)