ALZHEIMER DISEASE BIOMARKER BASED ON CAROTID ARTERY REACTIVITY

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Specially dedicated to my beloved mom and dad,
HjhSitiMeriam Bt. Hj Sam and HjJamlos Bin Baba,
my siblings and family, for their encouragement and support;
as well as my lovely wife, KhairunnisaBinti Ahmad and all my friends who always
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ABSTRACT

Alzheimer disease (AD) is a progressive neurodegenerative disorder associated with the disruption of neuronal function. Carotid Artery Reactivity (CAR) is a new biomarker method for AD detection which provides various advantages as compared to existing detection method. Current developed methods have either radiation risk (positron emission tomography [PET] and computed tomography [CT] scanning), high cost and long scanning duration (magnetic resonance imaging [MRI]) or lack accuracy (electroencephalography [EEG]). New AD detection method could be implemented using ultrasound machine by assessing the carotid artery condition since the impairment of this artery leads to brain hypoperfusion, a clinical feature of AD. CAR allows normal functioning artery to dilate in order to permit more bloods flow into the brain. The three different variables utilized to study the CAR were the carotid artery blood flow velocity, its diameter and cross sectional area. Healthy people and Alzheimer patient are believed to have different CAR value. Hence, this study emphasized on finding the normal reactivity value belonging to healthy people and Alzheimer patient. This CAR value could be used to differentiate between healthy people and Alzheimer patient as the new method of detection. The studied subject consisted of 40 healthy people and 20 Alzheimer patients. All subjects had been scanned with ultrasound machine using Doppler and 3D technique before and after performed exercise to achieve 85% of their Maximal Heart Rate (MHR). Readings of each reactivity variables before exercise (rest) and after exercise (stimulated) were recorded to be analyzed to compare its percentage increment value (reactivity). Based on the results, Alzheimer patient recorded very low reactivity value which were 21% (blood flow velocity), 8.1% (diameter changes) and 16.67% (area changes) while normal reactivity recorded high reactivity value which were 109%(blood flow velocity), 22.2% (diameter changes) and 49.59% (area changes).

ABSTRAK

Penyakit Alzheimer merupakan gangguan neurodegenerative progresif yang dikaitkan dengan gangguan fungsi neuron. Kereaktifan karotid arteri sebagai kaedah ‘biomarker’yang baru untuk pengesanan penyakit Alzheimer memberikan pelbagai kelebihan berbandingdengan kaedah-kaedah pengesanan pada masa kini. Kaedah pengesanan terkini berisiko tinggi (tomografi pelepasan positron dan imbasan tomografi berkomputer), kos yang tinggi dan tempoh pengimbasan panjang (pengimejan magnetik resonan) atau kurang ketepatan (elektroencephalografi). Pengesanan baru Alzheimer boleh dilakukan menggunakan mesin ultrasound melalui penilaian keadaan carotid arteri kerana kerosakan arteri ini membawa kepada hipoperfusi oksigen dalam otak, satu ciri klinikal Alzheimer. Kereaktifan karotid arteri membenarkan arteri yang berfungsi secara normal untuk mengembang bagi membenarkan lebih banyak darah mengalir ke dalam otak. Tiga ciri yang berbeza digunakan untuk mengkaji kereaktifan ini iaitu halaju darah carotid arteri, diameter dan luas keratan rentas. Orang yang sihat dan pesakit Alzheimer dipercayai mempunyai kereaktifan karotid arteri yang berbeza. Oleh itu, kajian ini memberi penekanan kepada penilaian kereaktifan dimiliki oleh orang sihat dan pesakit Alzheimer. Nilai ini boleh digunakan untuk membezakan antara orang yang sihat dan pesakit Alzheimer sebagai kaedah baru pengesanan. Subjek kajian ini terdiri daripada 40 orang yang sihat dan 20 pesakit Alzheimer. Kesemua subjek telah diimbas dengan mesin ultrasound yang menggunakan teknik ‘Doppler’ dan tiga dimensi sebelum dan selepas senaman untuk mencapai 85% Kadar Jantung Maksimum. Bacaan setiap ciri kereaktifan sebelum senaman (rehat) dan selepas senaman (dirangsang) diambil untuk dianalisis untuk dibandingkan nilai peratusan kenaikan (kereaktifan). Berdasarkan keputusan, pesakit Alzheimer mencatatkan kereaktifan nilai yang sangat rendah di mana 21% (halaju aliran darah), 8.1% (perubahan diameter) dan 16.67% (perubahan luas) manakala kereaktifan normal mencatatkan nilai kereaktifan tinggi di mana 109% (halaju aliran darah), 22.2% (perubahan diameter) dan 49,59% (perubahan luas).

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LIST OF SYMBOLS

Bt/m	-	Beat per minute
CI	-	Confidence Interval
Cm/s	-	Centimeter per second
DR	-	Dynamic Range
Hz	-	Hertz
MHz	-	Mega Hertz
Mm	-	Millimeter
Mm^2	-	Millimeter per square
Mm Hg	-	Substrate thickness.
p	-	Significant value
r	-	Correlation

LIST OF ABBREVIATIONS

Aβ	-	β-Amyloid
AD	-	Alzheimer Disease
AGD	-	Argyrophilic Grain Disease
ALZM	-	Alzheimer
APOE	-	Apolipoprotein E
APP	-	Amyloid Precursor Protein
ASL	-	Arterial Spin Labeling
BOLD	-	Blood Oxygenated Level Dependent
CAD	-	Coronary Artery Disease
CANTAB	-	Cambridge Neuropsychological Test Automated Battery
CAS	-	Carotid Artery Structure
CAR	-	Carotid Artery Reactivity
CBD	-	Corticobasal Degeneration
CBF	-	Cerebral Blood Flow
CCA	-	Common Carotid Artery
CO	-	Cardiac Output
CO_2	-	Carbon Dioxide
CSF	-	Cerebrospinal Fluid
CT	-	Computed Tomography
CVR	-	Cerebral Vessel Reactivity
DTI	-	Diffusion Tensor Imaging
DWI	-	Diffusion Weighted Imaging
EEG	-	Electroencephalography

EF	-	Ejection Fraction
ERPS	-	Event-related Potentials
FDG	-	Fluorodeoxyglucose
fMRI	-	Functional Magnetic Resonance Imaging
HR	-	Heart Rate
HRmax	-	Maximum Heart Rate
MAP	-	Mean Arterial Pressure
MCA	-	Middle Cerebral Artery
MCI	-	Mild Cognitive Impairment
MRI	-	Magnetic Resonance Imaging
MTL	-	Medial Temporal Lobe
NFT	-	Neurofibrillary Tangles
NMDA	-	N-Methyl-D-Asparatic Acid
NOF	-	Normal Old Female
NOM	-	Normal Old Male
NSAID	-	Nonsteroidal Anti-Inflammatories
NYF	-	Normal Young Female
NYM	-	Normal Young Male
PAL	-	Paired Associative Learning
PET	-	Positron Emission Tomography
PSP	-	Progressive Supranuclear Palsy
QEEG	-	Quantitative Electroencephalography
RAVLT	-	Rey Auditory-Verbal Learning Test
SMA	-	Smooth Muscle Alpha Actin
SPECT	-	Single Photon Emission Tomography
SPSS	-	Statistics Package For Social Science
TCD	-	Transcranial Doppler
TICS-m	-	Telephone Interview for Cognitive Status-Modified
WMHI	-	White Matter Hyperintensity
WMS	-	Wechsler Memory Scale
3D	-	Three Dimensions
7MS	-	7-minute Screen

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CHAPTER 1

INTRODUCTION

1.1 Introduction

This thesis proposes the novel design of experiment and new specific formula for Alzheimer Disease (AD) biomarker. This work involves new formula to evaluate carotid artery structure (CAS) of healthy people and Alzheimer patient before and after having adequate exercise in order to reach 85% of maximum heart rate to come out with specific value to distinguish the people with and without AD. Ultrasound machine with Doppler and three dimensions (3D) technique applied on human carotid artery within this new method is proven safe, cheap, fast and accurate compared to current methods to detect AD.

Overall, this thesis describes a new method to detect AD including the literature review on AD, experimental set up until the carotid artery reactivity measurement process. In first chapter, brief background of the project is discussed, providing problem statements, objectives, methodology and scopes of work in conducting research including project's possible outcomes and contributions as well as thesis organization.

1.2 Study Background

AD is a progressive neurodegenerative disorder associated with disruption of neuronal function [1]. It reduces the capability of brain to perform its regular activity including daily routine such as bathing, eating, drinking and many more. AD becomes worse as it progresses and eventually able in leading to death. The common symptoms include disturbances in memory, attention, orientation, changes in personality, language difficulties and walking problem as well as movement limitation. AD usually begins after age of 60 and the risk increases with age. Due to the expectation of increasing in life span particularly in developed countries' citizens, more people will have higher risk and potential to get AD [2]. AD has affected 24.3 million people worldwide in 2010 with increment around 4.6 million yearly [3]. Based on the statistic produced by Health Ministry of Malaysia, it is estimated around 63,000 people having AD and expected to reach 127,000 in the next 10 year [4].

Mild cognitive impairment (MCI) is among the earliest sign and symptom of AD [5]. MCI occurred due to brain hypoperfusion where the amount of blood flown toward brain is insufficient or inadequate with the need of brain cell in performing cognitive activities. According to Torre J. C. et al, performances of cognitive tasks require the delivery of adequate oxygen and glucose toward specific regions of the brain. Any insufficiency of oxygenated blood occurred especially in the older brain resulted in cognitive dysfunction. Brain hypoperfusion could be realized from variety pathology within carotid artery such as atherosclerosis, wall hardening and stenosis that lead to carotid artery impairment [6]. Hence, it is important to evaluate carotid artery condition accurately and efficiently to ensure the artery functions normally and free from any pathology.

Previously, in vitro studies found that one of the best methods to evaluate vessel function is on its contractility through measurement of cerebral vessel reactivity (CVR). Thus, a lot of new techniques have been explored to study vascular function based on its reactivity including diffusion weighted imaging (DWI), diffusion tensor imaging (DTI), arterial spin labeling (ASL) and blood oxygenated level dependent (BOLD) [7]. However, Doppler imaging technique using ultrasound

machine is the most suitable one compared to other methods since this modality has been used safely, accurately, cost effectively and quickly in evaluating carotid artery structure.

1.3 Problem Statement

New biomarker method for AD detection is very essential in treating AD where treatment in the early stage is very efficient especially before any clinical symptoms shown [8]. Ideally, with the early detection of AD, it should be possible to diagnose AD earlier or at a stage at which neurons are not irreversibly impaired by the disease process yet and have the potential to be treated [9]. A lot of imaging modalities with different techniques have been explored to detect AD biomarker. However, each of the techniques have its own weaknesses where high risk (positron emission tomography [PET] and computed tomography [CT] scanning), high cost and long scanning duration (magnetic resonance imaging [MRI]) or not accurate enough (electroencephalography [EEG]) [3]. However, the ultimate goal of using new method for diagnosing AD is not to replace other techniques but to add to the consistency and reliability of established indicators across a variety of tests [1].

Apart from that, vascular abnormalities has great potential to lead vascular dysfunction which can stimulate synaptoxic B-amyloid *(Ab)* accumulation in the brain. This is considered as the central process for AD. Previous studies which applied measurement of resting cerebral blood flow (CBF) or CBF changes during active condition are not an accurate indicator to assess vascular function. This method however is more sensitive in determining neural activity rather than evaluating vessel properties. Hence, it is highly recommended to investigate on contractility of the cerebral vessel or CVR [7]. Hence, in this research, carotid artery reactivity is applied in evaluating its vascular function since Kolb B. et al found that carotid artery blood flow could replace the cerebral blood flow in evaluating cerebral vessel reactivity [10].

As mentioned before, brain hypoperfusion could be realized from variety pathologies within the heart and carotid artery that can critically reduce blood flow to the healthy and elderly brain. It is supported by Torre J. C. et al where asymptomatic and symptomatic carotid artery narrowing resulted in cognitive decline due to cerebral perfusion reduction [6]. Hence, it is suggested to do more research on carotid artery at especially on its structure and function. This study therefore emphasized on the characterization of the carotid artery including the blood flow velocity and diameter as well as cross sectional area.

Apart from that, preliminary results have shown that there are varieties of influencing factors for CVR. They are acetazolamide, CO_2 [11] and exercise [12]. All of the study results shown significant increment in cerebral blood flow after being stimulated with influencing factor compared to under normal condition [11, 13]. This is because the stimulators have dilated the vessel enables the blood to flow easily, freely and faster. Most of the current study using acetazolamide and admission of CO_2 which is still considered as high risk and dangerous to the patient or subject. Therefore, this study used the exercise method which is safe and low risk to be applied to the human as the influencing factor to dilate the carotid artery. However, CO_2 admission and injection of acetazolamide still being used in other study only for mice usage.

1.4 Objective

The main objectives of this study are as follows:

i. Investigate correlation of ultrasound carotid blood flow (CBF) and carotid artery structure (CAS) between normal people and Alzheimer patient.
ii. Develop a new biomarker method for AD detection.

1.5 Scope and Limitation of the Study

The main scopes of this study are:

i. Analytics and Statistics Correlation between CBF (Carotid Blood Flow), CAS (Carotid Artery Structure), CAR (Carotid Artery Reactivity) and AD (Alzheimer Diseases).
ii. Sensitivity and resolution of ultrasound for CBF and CAS measurement.
iii. Correlation between AD parameter in human.
iv. Effect of stress test in human CAS and CAR value.

The work scopes are to investigate the correlation among CBF, CAS, CAR and AD. In this research, ultrasound imaging applied to measure carotid artery blood flow, its diameter as well as the cross sectional area in order to develop new AD biomarker. The characterization of carotid artery structure affected in AD among human is done. The characterizations consist of the velocity of carotid artery and resolution of carotid structure. Both results before and after being stimulated by adequate exercise are compared to find differences and ratio that is used to create new formula based on CAR to categorize group of Alzheimer and non Alzheimer.

The limitations of this study are:

i. Getting full cooperation from Alzheimer patient since they tend to forget the instructions given earlier during the experiment process.
ii. There are multiple factors can reduce the blood flow to the brain. However, this research only focuses on carotid artery impairment.
iii. Bigger size of subject especially Alzheimer patient.
iv. This study focused only on evaluating carotid artery using ultrasound machine.

1.6 Organization of the Thesis

This thesis is divided into five chapters that describe all the work done for this study. The first chapter consists of the introduction, study background, problem statement, objectives, scope and limitation of the study. Chapter 2 is the literature review that explains literature about Alzheimer Disease and Carotid Artery Reactivity. Details of Alzheimer Disease introduced and explained including its definition, factors of cause, symptoms, statistics, treatment and precautions measurements. Apart from that, carotid artery structure is also viewed so that the relation of carotid artery and AD is clearly understood. Variety techniques of carotid artery imaging using MRI, CT Scan and ultrasound machine to evaluate carotid artery structure and its functions are described as well. Carotid Artery Reactivity which the proposed method in this research for AD early detection also being explain in chapter 2. Some overview of previous studies is presented too.

Research Methodology which covers experimental design and experimental set up is presented in chapter 3. In this chapter, research flow, design methodology and data collection method is briefly described. The research flow described the characterization of carotid artery reactivity in Alzheimer patient and normal people. This chapter also presents the measurement process of carotid artery reactivity of Alzheimer patient and normal people. The measurement results, analysis and discussion are presented in chapter 4. The results such as carotid artery blood flow,

its diameter and area are clearly presented. The results belong to Alzheimer patient and normal people are analyzed to come up with specific formula to be used as an AD early detection indicator. A discussion of the results including the accuracy, error, and difficulty are completely presented.

Finally, as the last chapter, chapter 5 covered the conclusion part. This chapter concludes the findings of the project, stated some key of contributions and provides recommendations for future work.

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