THE ASSOCIATION OF LEUCINE-RICH-GLYCOPROTEIN-1 (LRG-1) LEVEL WITH MYOCARDIAL PERFUSION SCAN IN PATIENTS WITH ISCHAEMIC HEART DISEASE

BY

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DECLARATION

I hereby declare that this research was sent to Universiti Sains Malaysia (USM) for the degree of Master of Medicine in Nuclear Medicine. It has not been sent to other universities. With that, this research can be used for consultation and photocopied as reference.

Sincerely,

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATION

ABSI	Ankle Brachial Systolic Index
ACCF	American College of Cardiology Foundation
ACR	American College of Radiology
ACS	Acute Coronary Syndrome
AHA	American Heart association
AMI	Acute Myocardial Infarction
ASE	American Society of Echocardiography
ASNC	American Society of Nuclear Cardiology
BMI	Body mass index
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CCF	Conngestive Cardiac Failure
CK	Creatine Kinase
CKMB	Creatine Kinase muscle/ brain
CMR	Cardiac magnetic resonance imaging
EBCT	Electron beam Computed Tomography
ECG	Electrocardiogram
EDTA	Ethylene-Diamine- Tetra-Acetic acid
EIA	Enzyme Immuno-Assay
ELISA	Enzyme Linked Immunosorbent Assay
FHS	Framingham Heart Study
HPP	Hospital Pulau Pinang
IHD	Ischaemic heart disease

IL-6	Interleukin-6
iv	Intravenous
LBBB	Left bundle branch block
LDH	Lactate Dehydrogenase
LRG-1	Leucine Rich Glycoprotein-1
LVEF	Left ventricular ejection fraction
MBq	Mega Becquerel
MI	Myocardial infarction
MDCT	Multi detector Computed Tomography
MOH	Ministry of Health Malay
MPS	Myocardial Perfusion Study
MREC	Medical Research Ethics Committee
MRA	Magnetic resonance angiography
MRI	Magnetic Resonance Imaging
NCD	Non- Communicable Disease
NSTEMI	Non ST Elevation Myocardial Infarction
PCI	Percutaneous coronary intervention
PET	Positron Emission Tomography
RCA	Right coronary artery
RPM	rotation per minute
SDS	Summed Difference score
SDS	Summed difference score
SNMMI	Society of Nuclear Medicine and Molecular Imaging
SPECT	Single photon emission computed tomography
SRS	Summed rest score

SSS	Summed stress score
STEMI ST	ST elevation myocardial infarction
Tc-99m	Technetium-99m
TGF-ß	Transforming Growth Factor -ß
TROP I	Troponin I
TROP T	Troponin T
UA	Unstable Angina
WHO	World Health Organization

ABSTRAK

Sejak beberapa dekad yang lalu, skan perfusi jantung menggunakan radionuklid foton emisi tunggal telah digunakan secara meluas sebagai imejan tidak invasif untuk menilai pesakit yang telah didiagnosa atau disyaki menghidapi penyakit arteri koronari. Walau bagaimanapun, pemberian radiasi kepada pesakit tetap memerlukan justifikasi. Oleh sebab itu, hubungan antara penanda keradangan baru seperti glikoprotein kaya leusina (LRG-1) telah dikaji sebagai adjung tambahan untuk prognostikasi pesakit. **Objektif**: Tujuan kajian ini adalah untuk mengesahkan sama ada tahap glikoprotein kaya leusina adalah berkadar langsung dengan keputusan skan perfusi jantung. Ini boleh mengurangkan radiasi yang tidak diperlukan oleh pesakit dengan mengukur nilai glikoprotein kaya leusina pada pesakit sebelum melakukan ujian skan perfusi jantung. **Kaedah**: Pesakit yang dirujuk untuk skan perfusi jantung direkrut sebagai peserta dan seterusnya skan perfusi jantung dilakukan mengikut protokol 2-hari. Tahap serum glikoprotein kaya leusina diukur menggunakan kit assai LRG (Teknologi IBL) dengan kaedah ELISA. Susulan dan hasil rawatan ditentukan pada enam bulan dan satu tahun selepas skan. Data klinikal dianalisis dengan menggunakan perisian IBM SPSS Versi 24 dan Stata SE Versi 14. **Hasil dan Perbincangan**: Sebanyak 120 orang peserta, kebanyakannya lelaki (63 pesakit) dengan usia purata 63 tahun telah direkrut, di mana majoriti peserta terdiri daripada etnik Cina (44.2%). Purata tahap glikoprotein kaya leusina ialah 50.51 ng / ml. Walau bagaimanapun, analisis menunjukkan tiada hubungan signifikan antara tahap LRG-1 dan keputusan MPS (p = 0.739). Etnik Cina mempunyai kemungkinan 73% lebih rendah dan mempunyai tahap LRG-1 yang lebih rendah berbanding etnik Melayu (OR = 0.27, 95% CI: 0.07, 0.97). Selain itu, didapati ada hubungan signifikan antara keputusan skan perfusi jantung dengan keadaan pesakit pada

6 bulan dan satu tahun selepas skan, ditunjukkan dengan p <0.001. Hasil kajian ini juga menunjukkan bahawa etnik Cina mempunyai kemungkinan 60% lebih rendah untuk mengalami keputusan skan perfusi jantung yang tidak normal berbanding etnik Melayu (OR = 0.40, 95% CI: 0.16, 1.00). **Kesimpulan**: Kajian menunjukkan tiada hubungan signifikan antara tahap glikoprotein kaya leusina dan skan perfusi jantung pada pesakit dengan penyakit arteri koronari. Walau bagaimanapun, kajian kawalan kes pemerhatian perlu dipertimbangkan pada masa hadapan. Keputusan skan perfusi jantung boleh memberi prognostikasi pesakit pada 6 bulan dan setahun selepas skan. Selain daripada itu, etnik dan jantina perempuan didapati adalah faktor protektif yang menyumbang kepada kurangnya keputusan MPS yang tidak normal.

Kata kunci: Penyakit arteri koronari, penanda radang, Perubatan Nuklear, Pengimejan Radionuklid, Skan Perfusi Jantung, glikoprotein kaya leusina-1.

ABSTRACT

Over the last several decades, radionuclide myocardial perfusion imaging (MPI) with single photon emission tomography has been a mainstay for non-invasive imaging for the evaluation of patients with known or suspected coronary artery disease (CAD). However, subjecting the patient to radiation still needs justification. Hence, association with novel inflammatory markers like leucine rich glycoprotein (LRG-1) was studied as an adjunct to assist in decision making. **Objective**: Aim is of this study was to validate whether the level of LRG-1 was directly proportional with myocardial perfusion scan findings. This may reduce unnecessary radiation to patient by measuring the level of LRG-1 prior subjecting patient for myocardial perfusion test, and subsequently become an adjunct to prognosticate the patients. **Methods**: Patients referred for myocardial perfusion scan were recruited as participants. Myocardial perfusion scan was done following 2-days protocol. Serum LRG-1 level was measured with Human LRG Assay kit (IBL Technology) with ELISA method. Follow-up and the outcome of the treatment was determined at six months and one year. Clinical data was analysed using IBM SPSS software Version 24 and Stata SE Version 14. **Results and Discussion**: A total of 120 participants, predominantly male (63 patients) with the mean age of 63 years were recruited, where the majority of the participants consisted of Chinese (44.2%) ethnicity. The mean of LRG-1 level was 50.51 ng/ml. However, analysis indicated that there was no significant association between LRG-1 level and MPS results (p=0.739). Chinese ethnicity had 73% lower odds and presented with lower LRG-1 level compared to Malay ethnicity (OR=0.27, 95% CI: 0.07, 0.97). Apart from that, significant association between MPS results and outcome at 6 months and one year were demonstrated with p<0.001 for both occasions. Findings also indicated that the Chinese ethnicity had 60% lower odds to

experience abnormal MPS results compared to Malay ethnicity (OR=0.40, 95% CI: 0.16, 1.00). **Conclusion**: Study showed there was no significant association between LRG-1 and MPS results in patient with CAD. However, observational case control study should be considered in future study. MPS results could prognosticate the outcome of patients at 6 months and a year. Besides that, ethnicity and female gender are the factors that contributed to lower abnormal MPS results.

Keywords: Coronary artery disease, inflammatory marker, Nuclear Medicine, Radionuclide imaging, Myocardial Perfusion Imaging, Leucine Rich Glycoprotein-1.

CHAPTER ONE

INTRODUCTION

1.1 Research Background

Around the world, coronary artery disease (CAD) which also known as ischemic heart disease (IHD) remains a persistent public health burden. CAD is the primary cause of mortality in non-communicable disease (NCD). In 2016, there were total of 17.9 million death caused by CVD , accounting for 44% of all NCD deaths (WHO, 2018). Of these death, around 7.3 million were contributed by CAD and 6.2 million contributed by stroke. Coronary artery disease is the only largest contributor to global death and is expected to continue to lead mortality trends in the future (Lu *et al.*, 2016).

Moreover, the burden of coronary artery disease also consists of years of disability lived with three non-fatal CAD sequelae, comprising of nonfatal acute myocardial infarction (AMI), angina pectoris, and congestive cardiac failure (CCF) (Moran, 2014). Similar alarming statistics pertaining to cardiovascular disease scenario is also witnessed in Malaysia (MOH, 2016). CAD has been recorded as the leading killer for the past 30 years (Abdullah *et al.*, 2017). In 2016, report conducted by Ministry of Health of Malaysia showed that CAD was placed first among the ten principal causes of death in 22.7% of government hospitals and 27.7% of private hospitals in Malaysia (MOH, 2016).

Effective treatments can reduce the likelihood of subsequent adverse events. Hence, early diagnosis, identifications of individuals at high risk of future cardiac events in whom medical therapy or revascularisation may enhance survival is important.

Currently, the gold standard for diagnosing obstructive coronary disease is invasive coronary angiography (Montalescot, 2013). Nevertheless, this procedure is not without risks and complications. Complications do occur even in proficient hands. Some of the adverse reactions are access site bleeding, deteriorating renal function, cerebral embolism and allergic reactions (Jolly *et al.*, 2009).

Because of the complications stated above, non-invasive methods such as stress echocardiography, myocardial perfusion scan (MPS), cardiac magnetic resonance (CMR) and Multi Slice Computed Tomography are more desirable. MPS is generally used for diagnosing CAD. In patients with suspected and known CAD, its use has seen a remarkable progression during the last decade and has become the most commonly used non-invasive imaging tool for risk stratification (Taqueti and Di Carli, 2015). Due to improvement in socio-economic and technology advancement leading to longer life expectancy of the population and changes in cardiovascular disease epidemiology, the number of stress single-photon emission computed tomography (SPECT) studies performed annually is likely to increase further (Hachamovitch *et al.*, 1996).

The search of ideal biomarker for myocardial injury is still in progress because none of well- known biochemical markers for myocardial damage such creatine kinase (CK), creatine kinase iso enzyme MB (CKMB), lactate dehydrogenase (LDH) and cardiac specific proteins like troponin T(cTnT) and troponin I (cTnI) possess all the criteria required (Nigam, 2007). At present, cardiac troponins are considered to be 'gold standard' classis biomarkers, however it cannot distinguish the etiologies of myocardial injury.

In addition to that, the comprehension on how CAD developed has undergone an incredible evolution throughout the years. Main culprit of CAD which is atherosclerosis, used

to be considered as an effect of cholesterol storage disease, in recent times is perceived as an inflammatory disorder (Libby and Theroux, 2005).

In view of this, there is a need in researching novel inflammatory markers for myocardial injury and predictor of coronary artery disease (Pai *et al.*, 2004).

Leucine-rich ∝ 2-glycoprotein 1 (LRG-1) was first isolated from the human serum (Haupt and Baudner, 1977) . Structurally, out of 312 amino acid residues, 66 of it are leucines (leucine-rich repeat). LRG-1 is involved in interaction between proteins, signalling and cell adhesion (Kobe and Kajava, 2001). It promotes angiogenesis by modulating endothelial tumour growth factor ß (TGF-ß) signalling towards the pro-angiogenic pathway (Wang *et al.*, 2013). Additionally, it was previously reported that arterial stiffness, endothelial function and peripheral arterial disease is a significant predictor for higher LRG-1 level (Pek *et al.*, 2015).

To the best of our knowledge, no studies to date have assessed the association of LRG-1 levels and patients with IHD. Therefore, the goals of this study was to study the association of LRG-1 levels and MPS findings in patients with ischaemic heart disease so that it could be used as prognostic indicator to the IHD patients.

1.2 Literature Review

1.2.1 Coronary Artery Disease.

1.2.1.1 Definition of Coronary Artery Disease

Coronary artery disease, sometimes termed as ischemic heart disease, comprises of a broad clinical spectrum extending from silent atherosclerosis to painful stable angina, acute coronary syndrome (ACS) and congestive heart failure (Ashley and Niebauer, 2004).

With angiogram, most studies have defined CAD to be significant when the stenosis involves >70% diameter narrowing of more than one major epicardial artery segment or stenosis >50% diameter narrowing of the left main coronary artery (Levine *et al.*, 2011).

Epidemiologic relations of cigarette smoking, blood pressure, and cholesterol levels with the incidence of CAD was established in Framingham heart study (FHS). (Mahmood et al., 2014)

1.2.1.2 Pathophysiology of Coronary Artery Disease

Coronary artery disease which interchangeably known as ischemic heart disease is most of times caused by atheromatous narrowing and subsequent occlusion of the vessel. Onset of formation of early atheroma began from early adulthood onwards. Composition of a mature atheromatous plaque is made out of two elements, each linked with a particular cell population. The lipid core is mainly released from necrotic foam cells (monocyte derived macrophages), which migrate into the intima and ingest lipids. In contrary, matrix of connective tissue was derived from smooth muscle cells and this involves migration process from the media into the intima. Subsequently, the connective tissues multiply and change their phenotype to form a fibrous capsule around the lipid core (Grech, 2003). Impaired oxygen supply to myocardium resulted from the fibrous plaque which leads to progressive narrowing (Libby and Theroux, 2005).

When a plaque produces > 50% diameter stenosis (or > 75% reduction in cross sectional area), reduced blood flow through the coronary artery during exertion may lead to angina. Another factor leading to acute coronary event is thrombus formation following rupture of a plaque and endothelial erosion of a plague (Hansson, 2005). Plaque rupture detectable in 60 to

70% of cases is threatening because it exposes pro thrombotic material from the core of the plaque such as phospholipids, tissue factor and platelet-adhesive matrix molecules to the blood (van der Wal *et al.*, 1994). Ruptures mostly occur where the fibrous cap is thin and partly destroyed. At these sites, abundant immune cells are activated and subsequently produce numerous inflammatory molecules and proteolytic enzymes that can weaken the cap and activate cells in the core. This will then transform the stable plaque into a vulnerable structure that can rupture, induce a thrombus and elicit an acute coronary syndrome (Hansson, 2005).

In acute myocardial infarction, occlusion is more complete than in unstable angina, where arterial occlusion is usually subtotal. Downstream embolism of thrombus may also produce micro infarcts. (Grech, 2003)

1.2.1.3 Clinical Manifestation of Coronary Artery Disease

CAD commonly manifests as angina, a pressure-type chest pain that typically occurs at rest or with minimal exertion lasting >10 minutes (Zipes *et al.*, 2018). The pain frequently starts in the retrosternal area and can radiate to either one or both arms, the neck, or the jaw (Gibbons *et al.*, 2003). Angina pain is sometimes associated with diaphoresis, dyspnoea, nausea, abdominal pain or syncope and typically relieved by nitroglycerin (Amsterdam *et al.*, 2014). Classification of chest pain is provided in Table 1.1

Table 1.1: Classification of Chest Pain

Type of Chest Pain	Characteristic
Typical angina (definite)	1) Substernal chest discomfort with a characteristic quality and duration that is 2) provoked by exertion or emotional stress and 3) relieved by rest or nitroglycerin
Atypical angina (probable)	Meets 2 of the above characteristics
Noncardiac chest pain	Meets 1 or none of the typical anginal characteristics

The patient presenting with angina must be categorized as having stable angina or unstable angina (UA). UA is defined as new onset, increasing (in frequency, intensity, or duration), or occurring at rest. The categorization is important for evaluation, treatment and short term prognosis of the patients. Three principal presentations of UA are explained in Table 1.2.

Table 1.2: Three Principal Presentations of UA

Types of Angina	Presentation
Rest angina	Angina occurring at rest and usually prolonged ≥ 20 min, occurring within 1 week of presentation.
New-onset angina	Angina of at least CCS Class III severity with onset within 2 months of initial presentation.
Increasing angina	Previously diagnosed angina that is distinctly more frequent, longer in duration, or lower in threshold (i.e., increased by ≥ 1 CCS class within 2 months of initial presentation to at least CCS Class III severity)

Canadian Cardiovascular Society (CCS). Source (Braunwald, 1990).

1.2.1.4 Diagnosis of Coronary Artery Disease

Patients with IHD usually present with a range of cardiac events related to the degree of myocardium ischemia, which includes UA, Non ST elevation myocardial infarction (NSTEMI), ST elevation myocardial infarction (STEMI) or sudden cardiac death. The presence of at least two of the following criteria is needed to diagnose ACS based on WHO guideline (Panel, 2007)

a) Typical history of ischaemic type chest pain (Table 1).

b) Serial evolutionary ECG changes.

c) Elevated serum cardiac markers (Panel, 2007)

Hence, good clinical judgement and assessment upon presentation is vital in making the diagnosis for acute cases. Stable cases or chronic CAD is usually detected in outpatient basis or primary healthcare centre. Thus risk assessment of individual patient helps in deciding further management of patients. Various imaging modalities have been introduced over the years to aid in diagnostic accuracy and cardiac risk stratification of CAD, and subsequently guide further therapeutic decisions (Gaemperli *et al.*, 2008). Spectrum of CAD is summarized in Figure 1.1.

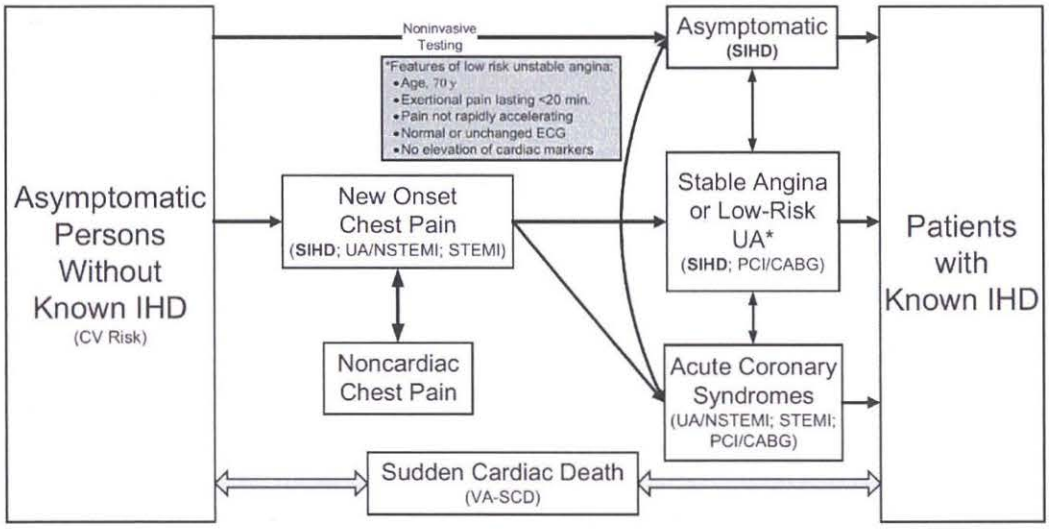

Figure 1.1: Spectrum of CAD
Guidelines relevant to the spectrum of CAD are in parentheses -adapted from (Fihn *et al.*, 2012). CABG indicates coronary artery bypass graft; CV, cardiovascular; ECG, electrocardiogram; IHD, ischemic heart disease; PCI, percutaneous coronary intervention; SCD, sudden cardiac death; SIHD, stable ischemic heart disease; STEMI, ST-elevation myocardial infarction; UA, unstable angina; UA/NSTEMI, unstable angina/non–ST-elevation myocardial infarction; and VA, ventricular arrhythmia.

1.2.1.5 Imaging of Coronary Artery Disease

In this era, patients with known or suspected coronary artery disease is being evaluated and diagnose using multimodality cardiovascular imaging. It can be broadly divided into the categories in the table below (Table 1.3)

Table 1.3: Imaging in coronary artery disease- adapted from (Pakkal *et al.*, 2011)

Technique	Type of procedure	
Invasive techniques	• Invasive coronary angiography • Fractional flow reserve (FFR) • Intravascular ultrasound • Optical coherence tomography	
Non-invasive imaging techniques	Functional	Anatomical
	• Myocardial Perfusion Imaging (MPI) using SPECT or PET • Stress Echocardiography (SE) • Cardiac MRI (CMR)- including stress CMR and delayed enhancement sequences	• Direct visualization of the coronary arteries (CTCA) • Calcium score (CAC) • Electron beam CT (EBCT) Multi detector CT (MDCT) • Magnetic resonance angiography (MRA) of the coronary arteries

Invasive coronary angiography remains as the gold standard for luminal assessment, but carries some risks resulting from its invasive nature (Patel *et al.*, 2014). It is reliable to detect luminal stenosis but delivers limited information on vessel wall or plaques (Pakkal *et al.*, 2011). In addition to that, ample large studies have shown that the majority of patients who have invasive coronary angiography, have no obstructive CAD (Patel *et al.*, 2014). This is leading to unnecessary invasive investigations done to the patients. In view of that, non-invasive imaging techniques have been developed and used extensively over the years (Schuijf *et al.*, 2005).

The two main objectives of non-invasive imaging for diagnosis of CAD is to asses hemodynamic effects of obstructive CAD by means of functional imaging and to visualize the coronary artery tree non-invasively using anatomical imaging (Schuijf *et al.*, 2005). As stated

previously in Table 1.3, MRA and CTCA are forms of anatomical imaging, and it provides direct visualisation of coronary arteries anatomy. On the other hand, the role of functional imaging is to assess the physiological impact of obstructed coronary arteries to the myocardium tissues. Examples of this imaging includes MPS using SPECT, stress echocardiography, MRI (Schuijf *et al.*, 2005) and to lesser extent, MPS using positron emission tomography (PET) (Thygesen *et al.*, 2007).

Hallmark of functional imaging is the detection of IHD by assessing the haemodynamic consequences of IHD rather than by direct visualisation of the coronary arteries. For this purpose, abnormalities of regional perfusion or wall motion are induced (or worsened) during stress, mirroring the presence of stress induced ischaemia. Ischaemia induction is based on the principle that although resting myocardial blood flow in regions supplied by stenotic coronary arteries is preserved, the increased flow demand during stress cannot be met, resulting in a sequence of events referred to as ''the ischaemic cascade''. Initially perfusion abnormalities are induced, followed by diastolic and (at a later stage) systolic dysfunction. Only at the very end of the cascade do ECG changes and angina occur (Figure 1.2). Accordingly, the occurrence of perfusion abnormalities during stress may be more sensitive for the detection of CAD than the induction of systolic dysfunction (wall motion abnormalities) (Schuijf *et al.*, 2005).

Clinical usage of functional imaging depends on local availability and expertise. Presently, it can be performed using (gated) single photon emission computed tomography (SPECT) or positron emission tomography (PET), (contrast) stress echocardiography and MRI. All of these techniques allow integrated assessment of perfusion and function, at rest and after stress.

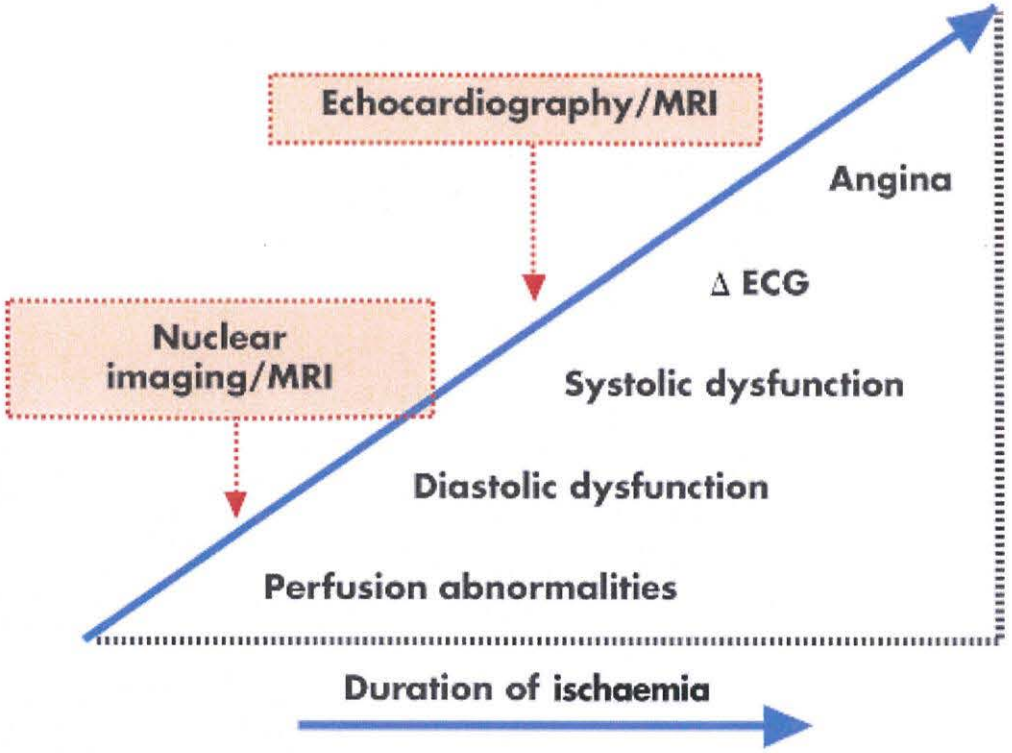

Figure 1.2: The ischemic cascade represents the sequence of pathophysiological events following ischemia.

1.2.2 Appropriate Use Criteria (AUC) for Radionuclide Imaging

Appropriate Use Criteria for Cardiac Radionuclide Imaging was developed by ACCF/ASNC/ACR/AHA/ASE/SCCT/SCMR/SNM in 2009. It serves as an important guide to physicians to ensure that radionuclide cardiovascular imaging is appropriately selected for indicated patients (Hendel *et al.*, 2009)

Probability of CAD is the sole criteria in the development of appropriate use of radionuclide imaging in evaluating patients with symptoms of CAD or ECG changes. The radionuclide imaging hence indicated for patients with the following conditions: - an

intermediate or high probability of CAD, patients with low probability of CAD but are unable to exercise or patients with uninterpretable ECG changes. Resting ST-segment depression, complete left bundle branch block, or paced rhythm, pre excitation (Wolff-Parkinson-White Syndrome) are examples of uninterpretable ECG. In contrast, radionuclide imaging is performed in high risk patients who do not have any symptoms of heart disease (Hendel *et al.*, 2009). Additionally, an expert consensus by Asian Society of Cardiovascular Imaging (ASCI) in 2017 on Multimodality Appropriate Use Criteria for Non-invasive Cardiac Imaging recommends that usage of SPECT MPS is appropriate for all patients with acute chest pain suspected of CAD, regardless of low, intermediate or high global CAD risk (Beck *et al.*, 2017).

1.2.3 Single Photon Emission Computed Tomography (SPECT)

Myocardial perfusion imaging with single photon emission computed tomography (SPECT) is a well-known method for noninvasively evaluating the hemodynamic significance of coronary artery stenosis (Gaemperli *et al.*, 2008).

Moreover, myocardial perfusion SPECT has been shown to have a high negative predictive value to rule out myocardial infarction (99%) or future adverse cardiac events (97%) in patients presented to the emergency department with chest pain, normal initial serum markers and enzymes accompanied with non-diagnostic ECG(Klocke *et al.*, 2003). In view of this, several publications have evaluated MPS to be an effective tool within a chest pain work-up algorithm. (Vesely and Dilsizian, 2008).

In principle, MPS is a non-invasive three dimensional imaging method acquired with gamma camera. Tc-99m Sestamibi or Tetrafosmin which provides superior imaging resolution are used as the radiopharmaceuticals (RPC). The RPC is retained within cardiac tissue in proportion to blood flow thereby providing excellent localisation and determination on

extension of perfusion abnormalities. The stress condition can be induced by either physical exercise or pharmacologically (Verberne *et al.*, 2015). Electrocardiographic (ECG) gated SPECT MPS are created by acquiring images corresponding to different intervals of the cardiac cycle and reconstruction into tomographic images. This allows visual or quantitative assessment of functional parameters such as left ventricular ejection fraction (LVEF), myocardial wall motion of thickening (Dorbala *et al.*, 2013)

Presently, cardiac stress MRI is the best method to assess anatomic imaging and is parallel to the standard of MPS in evaluating the myocardial perfusion (De Carvalho *et al.*, 2017). A study has found that MPS has equivalent value compared to cardiac MRI in terms of sensitivity and specificity for detection of angiographically significant stenosis (Cremer *et al.*, 2014). Other than MPS, cardiac perfusion Positron Emission Tomography/Computed Tomography (PET/CT) and Multi-sliced Computed Tomography (MSCT) is another known modality for perfusion evaluation (Ziessman *et al.*, 2013). Other modalities such as stress echocardiography has similar negative predictive value as SPECT MPS which is around 94-99% (Metz *et al.*, 2007).

1.2.4 Electrocardiogram (ECG)

The 12-lead electrocardiogram (ECG) is an integral part of diagnostic work-up for evaluation of CAD. As early as 1928, ECG was recognised as a tool for detection of CAD with the observation of exercise-induced ST- and T-wave changes in patients with chronic stable angina (Feil and Siegel, 1928) and followed by development of a standardised exercise stress protocol for evaluation of functional capacity. Later, in 1941, it was reported that exercise-induced myocardial ischemia from coronary insufficiency was detected by ECG monitoring. (Mattingly, 1962).

The observation that exercise produces a marker not seen in resting conditions serves

as the foundation of the two component structure of current stress testing modalities—a stressor and a subsequent detectable signal (Vesely and Dilsizian, 2008). To date, accepted ECG criteria for the detection of occlusive CAD is the development of 1-mm horizontal or down sloping ST segment depression in at least 2 contiguous ECG leads (Bailey *et al.*, 1977).

1.2.5 Leucine Rich Glycoprotein-1 (LRG-1)

1.2.5.1 Chemical composition of LRG-1

Leucine-rich glycoprotein was isolated for the first time in 1977 by Haupt and Baudner. Resolution of the primary structure in 1985 indicated that it existed as a single-chain glycoprotein with a molecular weight of approximately 45k Dalton and a carbohydrate content of 22.9% (Watson *et al.*, 2011). The carbohydrate moiety consists of: galactose (4.1%), mannose (3.8%), fructose (0.3%), N-acetylglucosamine (8.1%) and N-acetyl neuraminic acid (6.6%). Thus the molecule contains only N-glycosidically linked oligosaccharide chains. The extremely high leucine content of nearly 17% is a remarkable characteristic of the amino acid composition, where every fifth amino acid is leucine (Takahashi *et al.*, 1985). The average content of LRG-1 in adult serum is 2.1 mg/ml (Haupt and Baudner, 1977)

1.2.5.2 Function of LRG-1 as novel inflammatory markers

Based on mRNA analysis, LRG-1 is predicted to be expressed by liver cells and by neutrophils (O'Donnell *et al.*, 2002). Previous study showed that it was up-regulated in a mouse model of inflammation (Norkina *et al.*, 2004). The precise function of LRG-1 has yet to be defined, but evidence to date suggests that it is associated with inflammatory responses and neutrophilic differentiation (Shirai *et al.*, 2009) as well as cellular responses to the pro fibrotic cytokine transforming growth factor (TGF) (Sun *et al.*, 1995). It is suggested that the formation of leucine-zipper structure was contributed by the repetition of leucine at at every 7^{th} position

in segments of this protein, which consequently lead to protein-DNA and protein-protein interaction.

Overexpression of LRG-1 along with other acute phase proteins noted in patients with severe acute respiratory syndrome (SARS) (Chen *et al.*, 2004). Similarly, its expression is induced synergistically by Interleukin-6 (IL-6) and tumour necrosis factor (TNF) in hepatocytes (Takahashi *et al.*, 1985).

Apart from the above, increased serum LRG was observed in patients with several types of cancer including pancreatic (Kakisaka *et al.*, 2007), liver (Kawakami *et al.*, 2005) and lung cancers (Okano *et al.*, 2006). In the cerebrospinal fluid of patients with idiopathic normal pressure hydrocephalus, the amounts of LRG-1 increased about 3-fold (Nakajima *et al.*, 2011). In addition to extracellular matrix (ECM), LRG-1 is also expressed in cardiac fibroblasts (Ifkovits *et al.*, 2014) and cardiomyocytes (Chen *et al.*, 2004).

Unlike C-reactive protein (CRP), a biomarker widely used to evaluate inflammation, LRG is induced not only by IL-6 but also by other pro-inflammatory cytokines. As the LRG is upregulated not only in liver cells but also in local inflammatory sites (Serada *et al.*, 2010), it is considered a novel inflammatory marker applicable to evaluate the clinical activities of inflammatory diseases (Kumagai *et al.*, 2015).

Inflammation is the modification in vascular dynamics and conscription of innate and adaptive immune cells responding to tissue injury. Chronic systemic inflammation drives initiation, progression and sustainment of atherosclerotic cardiovascular disease (Goyal *et al.*, 2019). Atherosclerosis results from increase in inflammatory factors such as acute phase

proteins, cytokines and subsequent changes in endothelium of coronary arteries (Shrivastava *et al.*, 2015).

CHAPTER TWO

RATIONALE/ BENEFIT OF THE STUDY

This study was conducted to determine the association of the level of leucine rich glycoprotein-1 (LRG-1) with findings in myocardial perfusion scan. By knowing the correlation of level of leucine rich glycoprotein-1 (LRG-1) with findings in myocardial perfusion scan performed on patients with coronary artery disease, level of LRG-1 can be a guide whether myocardial perfusion scan is indicated for a patient with CAD. If the level of LRG-1 is low/ normal, there will be no indication for MPS and subsequently patient will not be subjected to radiation. It is hypothesized higher LRG-1 level is expected in patients with abnormal MPS.

2.1 Aim

This study aims to assess the value of LRG-1 as adjuvant tool in deciding whether the myocardial perfusion scan is indicated in patients suspected or / diagnosed with coronary artery disease.

2.2 Objective

2.2.1 General Objective

The objective of this study is to validate that the level of LRG-1 to be directly proportional with myocardial perfusion scan findings. This may reduce the need for unnecessary radiation for patient by measuring the level of LRG-1 prior subjecting patient for myocardial perfusion test.

2.2.2 Specific objectives

1. To profile the demographic of patients with coronary artery disease referred for MPS.
2. To determine the association between level of LRG-1 with MPS results.
3. To study the association between the stress ECG findings and MPS results.
4. To determine for the association of MPS results with the outcome of the patients.

2.3 Hypothesis statement

- Null hypothesis: Level of LRG-1 has no association with MPS result.
- Alternate hypothesis: Level of LRG-1 is associated with MPS result.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design and Location

A single arm randomised prospective study was carried out in the Department of Nuclear Medicine, Hospital Pulau Pinang and Oncology Lab, Institut Perubatan dan Pergigian Termaju, Pulau Pinang from the period of August 2017 until March 2019.

3.2 Study Sample

This study was performed in all consented patients referred to Department of Nuclear Medicine, Hospital Pulau Pinang for myocardial perfusion imaging for assessment of CAD based on the following inclusion and exclusion criteria.

3.3 Inclusion and Exclusion Criteria

Inclusion criteria

i) Above 18 years old

ii) Referred for myocardial perfusion scan

Exclusion criteria:

i) Pregnant

ii) Patient with prior cardiac related intervention

-angioplasty; coronary artery bypass graft.

iii) Patients with peripheral artery disease

-Ankle brachial systolic index (ABSI) less than 0.9

iv) Patient who are contraindicated for stress test:

-recent (1week) myocardial infarction; unstable angina; uncontrolled hypertension (SBP >220mmHg); systolic blood pressure less than 90mmHg; decompensated heart failure.

3.4 Sample Size Calculation

$$n = (\frac{z}{\Delta})^2 \text{ p(1-p)}$$

$$= (\frac{1.96}{0.01})2 \text{ (0.62) (1-0.62)}$$

$$= \frac{0.9051}{0.01} \quad = 90.5 \text{ (+10\% drop out)}$$

$$= 100$$

n: sample size

z: statistic for a level of confidence= 1.96 (95% confidence interval)

p: expected prevalence or proportion

Δ: precision

3.5 Myocardial Perfusion Study

Myocardial perfusion study was performed based on Standard Operating Procedure Department of Nuclear Medicine, Penang Hospital (Appendix H)

3.5.1 Patient preparation

Preparations of the patient were done as described below:-

i) Prior to the procedure, patients were required to have pre scan preparation. They had to abstain from fatty food, caffeinated beverages or alcohol and discontinuing beta-blockers for three days prior to study. Patient was required to fast for at least 4-6 hours and to wear sports attire on day of scan.

ii) Weight and height of the patient were measured. Patient was then seen by medical officer for history taking and physical examination. Measurement of ankle brachial systolic index (ABSI) which used hand-held Doppler was also included in physical examination. Patient with normal range of ABSI (0.90 to 1.30) was included in the study.

iii) Patients was then subjected to either exercise or pharmacological stress test. Fit patient was assigned for treadmill exercise stress test and those reckoned unsuitable for exercise was subjected to pharmacological stress test.

iv) Medical officer explained to the patients about the stress and scan procedures The patients were informed the possible side effects of the pharmacological agents. Patients who gave written consent were enrolled in this study.

v) Intravenous branula (20-24G) was then inserted by the nurse for radiopharmaceutical injection during the stress test. Two millilitre of blood was

withdrawn simultaneously during intravenous cannulation and was placed into EDTA tube. The blood sample was then centrifuged at 2500 rpm for 10 minutes.

vi) Patient was then subjected to Tc-99m Tetrofosmin gated stress/rest SPECT (Single Photon Emission Computed Tomography) MPS studies two-day protocol following treadmill exercise or pharmacological stress test. The tests procedure was performed complied to the protocol of Department of Nuclear Medicine, Hospital Pulau Pinang.

vii) Both treadmill exercise and pharmacological stress test were performed under the supervision of trained medical assistant and a medical officer.

viii) Prior the stress test, the patients rest electrocardiogram (ECG), blood pressure and heart rate were charted. Stress test was then performed under continuous ECG monitoring. During this step, 12-lead ECG recording, heart rate and blood pressure were obtained in each stage of exercise, at the point of maximal exercise and the recovery phase.

3.5.2 Stress test

Stress test was done either by exercise or pharmacological stress test.

3.5.3

3.5.3.1 Exercise stress test

Fit patient and those who were not contraindicated for exercise stress test underwent this procedure using treadmill exercise (Quiton Q Stress TM55 Made in USA) with standard or modified Bruce Protocol.

Patient had to exercise until maximally tolerated (symptom-limited) or at least achieving 85% of maximal age adjusted predicted heart rate unless early termination is indicated. This includes moderate to severe angina, marked dyspnoea or fatigue, cyanosis, pallor, exercise ST segment depression >2 mm, ST elevation >1 mm, sustained supraventricular or ventricular tachycardia, newly developed LBBB, fall in systolic blood pressure >10 mmHg with co-existent evidence of ischaemia and hypertensive response (systolic >250 mmHg; diastolic BP >115 mmHg).

In the next step, radiopharmaceutical (Tc-99m Tetrofosmin 185-250MBq) was injected at peak of exercise. After injection, patient was asked to have a light fatty meal shortly after injection and images were acquired 60 minutes after radiopharmaceutical administration.

The absolute contraindications to exercise stress test were uncontrolled hypertension (BP >200/110 mmHg), decompensated cardiac failure, symptomatic uncontrolled arrhythmias, severe aortic stenosis, acute pericarditis or myocarditis.

Fall in systolic blood pressure > 10 mmHg with co-existent evidence of ischaemia and hypertensive response (systolic BP >250 mmHg; diastolic >115 mm) and complete left bundle branch block (LBBB) are the relative contraindications of exercise stress test.

3.5.3.2 Pharmacologic stress test

Pharmacological stress test was done on patients who were not fit for exercise stress test. Dipyridamole was infused intravenously at 0.56mg/kg during 4 minutes under continuous electrocardiographic monitoring. It acted as indirect vasodilator by inhibiting the intracellular reuptake and deamination of adenosine, thus leading to an increased cellular levels of

adenosine. Relative flow heterogeneity was induced depending on the severity of coronary stenosis and coronary flow reserve limitation.

Usage of dipyridamole is contraindicated for patients who have had a history of asthma or chronic obstructive pulmonary disease, second- or third-degree atrioventricular block without a pacemaker, or systemic blood pressure <90/60 mm Hg. Patients was instructed to avoid xanthine-containing products for 24 hours before the test.

At the end of the infusion, isometric exercise was performed for 3 minutes followed by injection of Tc-99m Tetrofosmin 185-250 MBq at the 7^{th} minutes and exercise was continued for another 1-2 minutes post-injection.

Administration of intravenous aminophylline 125-250 mg was given to the patients who developed side effects to dipyridamole such as flushing, dizziness, headache, hypotension and ischaemic ECG changes. ECG was monitored continuously along with heart rate and blood pressure measurements every 3 minutes until patient's symptom resolved or ECG changes returned to baseline.

Dobutamine stress test protocol is best reserved for patient contraindicated for dipyridamole. It has direct β adrenergic stimulation leading to increase heart rate, blood pressure and myocardial contractility, and subsequently increases the myocardial blood flow.

A total of 50 ml dobutamine (250 mg in 20 ml) was given intravenously with an infusion rate of 10 μg/kg/min, increasing by an additional dose of 10 μg/kg/min every 3 minutes and finally to a maximum dose of 40 μg/kg/min.

In these patient, the radiopharmaceutical was injected once the target heart rate achieved, and the infusion of dobutamine was continued for another two minutes after radiopharmaceutical injection. Atropine (0.250 mg-0.5 mg) was given intravenously if target heart rate was not achieved. ECG and BP were monitored at baseline and at 3 minutes interval. Patient was also monitored for side effects such as palpitation, chest pain, headache, flushing, supraventricular or ventricular tachycardia. Dobutamine was contraindicated for patients with unstable angina, uncontrolled hypertension (systolic ≥200 mmHg, diastolic >110 mmHg) severe aortic stenosis and history of ventricular tachycardia.

Patient who developed severe side effects or ischaemic changes from either exercise or pharmacological stress test were evaluated and monitored by the medical officer and nuclear medicine specialist.

3.5.3 Rest Scan

Another appointment within one to two weeks was given to patient to perform rest scan in order to complete the myocardial perfusion study. During rest scan, Tc99m-Tetrofosmin (185-250 MBq) was injected and image acquisition was performed one hour after the administration of radiopharmaceutical.

3.6 Imaging Protocol and Image Interpretation

Nuclear medicine technologist performed the Tc-99m Tetrofosmin Gated SPECT imaging with a dual–head gamma camera (Siemens E-CAM). The gamma camera was equipped with a low energy, high-resolution collimator (LEHR) and photo peak at 140±20% keV in a 64x64 matrix. Non attenuation correction system was applied. Images were acquired in 180° orbit with 25s readings every 5.6°.