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EFFECTS OF PCSK9 INHIBITORS ON ATHEROGENESIS BIOMARKERS IN STIMULATED HUMAN CORONARY ARTERY ENDOTHELIAL CELLS, CORRELATION BETWEEN PCSK9 AND LIPOPROTEIN (A), AND AS INDEPENDENT PREDICTORS FOR CORONARY ARTERY DISEASE IN PREMATURE CAD PATIENTS

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ABSTRACT

Coronary artery disease (CAD) is the leading cause of death worldwide and has a strong link to familial hypercholesterolemia (FH). FH is an autosomal dominant lipoprotein metabolic condition that causes an increase in LDL-c levels and an elevated risk of premature CAD (pCAD). Elevation of proprotein convertase subtilisin/kexin type 9 (PCSK9) level in pCAD patients may contribute to the initiation of atherogenesis by an increase in inflammation. One of the gene mutations present in FH involves a gain-of-function point mutation in PCSK9. Individuals with FH are more likely to have high Lp(a) levels than the general population. Despite extensive research, the underlying mechanism by which Lp(a) mediates atherogenesis and inflammation is still incompletely understood. The release of PCSK9 during inflammation in atherosclerosis suggests that, in addition to the elevation of Lp(a), a potential correlation could exist between Lp(a) and PCSK9. Targeting Lp(a) and PCSK9 might provide an additive benefit beyond LDL-c lowering. The anti-atherogenic properties of PCSK9 inhibitors on atherogenesis biomarkers via PCSK9 reduction have yet to be established. Hence, this study aimed to (1) investigate the enhancement of PCSK9 production by LPS and Lp(a)-stimulation in human coronary artery endothelial cells (HCAEC), (2) determine the pleiotropic effects of PCSK9 inhibitors in LPS and (3) Lp(a)-stimulated HCAEC on atherogenesis biomarkers, and monocyte endothelial cell binding capacity, (4) explore the correlation and association between Lp (a) and PCSK9, and (5) determine the role of Lp(a) and PCSK9 as independent predictors for CAD in pCAD patients. This study was divided into two parts, *in vitro* and human studies. For the *in vitro* study, the stimulation effects on PCSK9 production, followed by the determination of pleiotropic effects of PCSK9 inhibitors on atherogenesis biomarkers were determined using ELISA (protein), QuantiGene Plex 96-well (gene), and monocyte binding capacity. For human studies, pCAD (with and without clinically diagnosed FH) and normal controls (NC) were subjected to blood samples to analyse Lp(a) and PCSK9 concentrations. The pleiotropic effects of PCSK9 inhibitors beyond cholesterol-lowering were mediated by PCSK9 inhibition and attenuation of early atherogenesis biomarkers that possibly triggered foam cell formation, which marked the initiation of atherosclerotic lesions. Up to 24 hours incubation, biomarkers expression suggests that overall, Evolocumab possesses early, stabilised beneficial effects on protein and gene expression in both *in vitro* stimulation models. Even though Alirocumab was postulated to have lag effects in reducing the atherogenesis biomarkers, the potency of Alirocumab in preventing monocyte binding is greater than Evolocumab. The Lp(a) and PCSK9 were significantly higher in pCAD compared to the NC group. A significant correlation was found in all pCAD and NC groups, however, no association was found across all groups. Lp(a) and PCSK9 were regarded as significant predictors for CAD ($p<0.05$). However, after correcting for the confounding factors, only Lp(a) remained significant as independent predictor for CAD in pCAD patients. The exclusion of PCSK9 as independent predictors in the final model may be attributed by LDL-c lowering medication (statins), which was unavoidable in CAD patients which causes reduction in hepatic intracellular cholesterol, resulting in an increase of LDLR as well as PCSK9. Alternatively, the role of Lp(a) and PCSK9 as independent predictors for CAD may be established by measuring distinct forms of PCSK9 (mature-PCSK9 and furin cleaved-PCSK9) separately, as they exhibit different atherogenic activity towards LDLR degradation.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Worldwide, prevalent cases of total cardiovascular diseases (CVD) nearly doubled from 271 million in 1990 to 523 million in 2019, representing a 93% rise. CVD deaths steadily increased from 12.1 million in 1990 to 18.6 million in 2019 (Roth et al., 2020). National Health and Morbidity Surveys (NHMS) 2019 have shown a growing increase in the prevalence of common risk factors for CVD; 1.7 million people in Malaysia currently live with three major risk factors (diabetes, hypertension, and cholesterol), while 3.4 million people live with two major risk factors (hypertension and high cholesterol) (Institute for Public Health, 2020). According to the latest World Health Organization (WHO) data published in 2020, coronary artery disease (CAD) accounted for 136.21 per 100,000 population in Malaysia (21.86 % of all deaths). Department of Statistics Malaysia (2021) reported CAD as the most significant cause of death, with 36,729 fatalities or about one-fifth of all deaths. It is the most common cause of death in Malaysia and globally.

National Cardiovascular Disease-Acute Coronary Syndrome (NCVD-ACS) Registry indicated that from 2011 to 2019, Malaysians developed Acute Coronary Syndrome (ACS) at a younger age than those seen in neighbouring countries. The mean age was 58.5 years with peak incidence between 50 to 59 years (Wan Ahmad, 2022; Lee et al., 2021). The age group is younger than noted in Thailand (63.5 to 65.5 years) and Singapore (69.5 to 70.5 years) (Srimahachota et al., 2012; Singapore Myocardial Infarction Registry of Disease Office Ministry of Health, 2020). Atherosclerotic cardiovascular disease (ASCVD) in young adults represents a specific and growing challenge. The burden of ASCVD in young adults is an important public health issue because of the potential loss of lifetime productivity and increased lifetime healthcare use. According to NCEP (National Cholesterol Education Program) ATP III guidelines, premature coronary artery disease (pCAD) age is onset of <55 in men and <65 years in women. However, there is no universally accepted cut-off age defining premature coronary artery disease (pCAD). Several other groups of researchers used the pCAD cut-off age that has been utilised in the Dutch Lipid Clinic Criteria (DLCC), where it