ASSOCIATION OF ABO BLOOD GROUP AND LEPTIN LEVEL WITH MODERATE-HIGH FRAMINGHAM RISK SCORE

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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The eleven-month study investigated the association between cardiovascular risk factors and cardiovascular diseases (CVD), which remains the leading cause of global mortality. Blood group antigens have been linked to an increased susceptibility to CVD. Leptin, a marker of adipose function, has been proposed to enhance the predictive ability for CVD risk. Elevated leptin levels have shown correlations with specific ABO blood groups. The Framingham Risk Score (FRS) remains the standard tool for assessing 10-year CVD risk. This study aimed to determine the association of ABO blood and leptin level with FRS in the determining CVD risk. A cross-sectional study was carried out among 333 patients aged from 30 to 75 years old without CVD and had follow-ups in the Family Medicine Specialist Clinic in Hospital Sultan Abdul Aziz Shah (HSAAS). Collected blood samples from consented patients were subjected to the ABO blood group by antigen-antibody agglutination, Magnetic Luminex by functional sandwich immunoassay was used to determine plasma leptin level.

Potential confounding factors, including conventional FRS risk factors; age, gender, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), systolic blood pressure (SBP), hypertension medication use, smoking status, diabetes, and history of CVD were evaluated and categorized into low, moderate-high risk. Kruskal-Wallis and Mann-Whitney tests were used to assess the association between ABO blood group and leptin levels due to the non-parametric nature of the data. Linear regression was employed to evaluate the correlation between body mass index (BMI) and leptin levels, ensuring assumptions of linearity and normality of residuals were checked. To adjust for potential confounders in the association with FRS, multiple logistic regression was performed. Variables with a p-value <0.25 in univariate analyses were included in the model. Patients' median (IQR) age was 55 (19) years old were 61.6% females and 88% Malays. The O blood group and Rh-positive were dominant with 39.9% and 99.4% respectively. Non-O blood group (64.5%) and AB blood group (71.4%) had the highest percentage in the moderate-high FRS. Median (IQR) ng/ml of leptin levels showed the highest in non-O blood group with 17.98 (19.6), and B blood group with 20.61 (24.78). Significant correlation was observed for leptin and BMI (p<0.001). FRS were found significant in status of use of anti-dyslipidaemia agent (p<0.001) and fasting blood sugar (FBS) level (p: 0.006). The study highlighted the potential influence of blood group, BMI, and leptin level on cardiovascular risk. Additionally, both low-risk FRS exhibited elevated leptin level, underscoring the complex relationship between obesity, leptin, and cardiovascular health. Study findings underscored the importance of integrating use of anti-dyslipidaemia agent and FBS into cardiovascular risk assessment for

management to improve prevention strategies.

Keywords: Cardiovascular diseases, Framingham risk score, Risk assessment, ABO blood group, Leptin.

SDG: GOAL 3: Good health and well being

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

HUBUNG KAIT KUMPULAN DARAH ABO DAN TAHAP LEPTIN DENGAN SKOR RISIKO FRAMINGHAM SEDERHANA-TINGGI

Oleh

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Kajian selama sebelas bulan ini menyiasat hubungan antara faktor risiko kardiovaskular dan penyakit kardiovaskular (CVD), yang kekal sebagai penyebab utama kematian di seluruh dunia. Antigen kumpulan darah telah dikaitkan dengan peningkatan kerentanan terhadap CVD. Leptin, yang merupakan penanda fungsi adipos, dicadangkan untuk meningkatkan keupayaan ramalan bagi risiko CVD. Peningkatan tahap leptin telah menunjukkan hubungan dengan kumpulan darah ABO tertentu. Skor Risiko Framingham (FRS) kekal sebagai alat piawaian untuk menilai risiko CVD dalam tempoh 10 tahun. Kajian ini bertujuan menentukan hubungan antara kumpulan darah ABO dan tahap leptin dengan FRS dalam penentuan risiko CVD. Satu kajian keratan rentas dijalankan melibatkan 333 pesakit berumur antara 30 hingga 75 tahun tanpa CVD dan yang mempunyai rawatan susulan di Klinik Pakar Perubatan Keluarga di Hospital Sultan Abdul Aziz Shah (HSAAS). Sampel darah yang dikumpul daripada pesakit yang bersetuju telah

diuji untuk kumpulan darah ABO melalui aglutinasi antigen-antibodi. Magnetic Luminex menggunakan kaedah immunoassay sandwich berfungsi digunakan untuk menentukan tahap leptin plasma. Faktor pengeliruan berpotensi, termasuk faktor risiko konvensional FRS seperti umur, jantina, kolesterol total (TC), kolesterol lipoprotein berketumpatan tinggi (HDL-C), tekanan darah sistolik (SBP), penggunaan ubat hipertensi, status merokok, diabetes, dan sejarah CVD telah dinilai dan dikategorikan kepada risiko rendah dan sederhana-tinggi. Ujian Kruskal-Wallis dan Mann-Whitney digunakan untuk menilai kaitan antara kumpulan darah ABO dan tahap leptin berdasarkan sifat data yang tidak parametrik. Regresi linear digunakan untuk menilai hubungan antara indeks jisim badan (BMI) dan tahap leptin, dengan memastikan andaian lineariti dan normaliti baki diperiksa. Untuk menyesuaikan faktor pengeliruan yang berpotensi dalam hubungan dengan FRS, regresi logistik berganda dilakukan. Pemboleh ubah dengan nilai p <0.25 dalam analisis univariat dimasukkan ke dalam model. Median umur pesakit (IQR) adalah 55 (19) tahun, dengan 61.6% daripadanya adalah perempuan dan 88% adalah Melayu. Kumpulan darah O dan Rh-positif adalah dominan dengan 39.9% dan 99.4% masing-masing. Kumpulan darah bukan O (64.5%) dan kumpulan darah AB (71.4%) mencatatkan peratusan tertinggi dalam FRS sederhana-tinggi. Median (IQR) tahap leptin dalam ng/ml adalah tertinggi dalam kumpulan darah bukan O dengan 17.98 (19.6), dan kumpulan darah B dengan 20.61 (24.78). Hubungan yang signifikan diperhatikan antara leptin dan BMI ($p<0.001$). FRS didapati signifikan dalam status penggunaan agen anti-dislipidemia ($p<0.001$) dan paras gula dalam darah yang puasa (FBS) (p: 0.006). Kajian ini menyerlahkan potensi pengaruh kumpulan darah, BMI, dan

tahap leptin terhadap risiko kardiovaskular. Tambahan pula, FRS risiko rendah menunjukkan tahap leptin yang tinggi, menonjolkan hubungan kompleks antara obesiti, leptin, dan kesihatan kardiovaskular. Penemuan kajian ini menekankan pentingnya mengintegrasikan penggunaan agen anti-dislipidemia dan FBS dalam penilaian risiko kardiovaskular untuk meningkatkan strategi pencegahan.

Kata Kunci: Penyakit kardiovaskular, Skor risiko Framingham, Penilaian risiko, Kumpulan darah ABO, Leptin.

SDG: MATLAMAT 3: Kesihatan dan kesejahteraan yang baik

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ABO	Blood group A, B, AB and O
AGE	Advanced glycation end products
ASCVD	Atherosclerotic Cardiovascular disease
BBB	Blood brain barrier
BMI	Body mass index
CAD	Coronary artery disease
CHD	Coronary heart disease
CI	Confidence intervals
COR	Crude odd ratio
CPG	Clinical practice guideline
CVD	Cardiovascular diseases
DBP	Diastolic blood pressure
DVT	Deep vein thrombosis
EAT	Epicardial adipose tissue
EC	Endothelial cells
ED	Endothelial dysfunction
FBS	Fasting blood sugar
FLP	Fasting lipid profile
FRS	Framingham Risk Score
GWAS	Genome-wide association studies
HDD	Hypertensive heart disease
HDL-C	High-density lipoprotein cholesterol
HIC	High income country
HPT	Hypertension
IHD	Ischemic heart disease

IS	Ischmic stroke
IQR	Interquatile range
LDL-C	Low-density lipoprotein cholesterol
LMIC	Low-Middle income country
MOH	Ministry of Health Malaysia
NCD	Non-communicable disease
NET	Net-like extracellular structures
NHYA	New York Heart Association
OGTT	Oral glucose tolerance test
PAD	Peripheral arterial disease
PE	Pulmonary embolism
PVD	Peripheral vessel disease
RBC	Red blood cells
RHD	Rheumatic heart disease
ROC	Receiver operating curve
SBP	Systolic blood pressure
SD	Standard deviation
SEA	South-East Asia
SNP	Single nucleotide polymorphisms
TC	Total cholesterol
TG	Triglycerides
VTE	Venous thromboembolism
vWF	Von Willebrand factor
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background study

Cardiovascular diseases (CVD) refer to diseases that affect the circulatory system and heart. Frąk et al. (2022) described the pathophysiological ramifications of CVD and had an emphasis on coronary artery disease (CAD) as well as atherosclerosis. A prospective cohort study in 21 countries including Malaysia by Dagenais et al. (2020) found CVD contributed the highest percentage of mortality. Haneen Hussein Farhood et al. (2023) reported the prevalence of the most common CVD arises from long-term processes involving complex interactions between modifiable and non-modifiable risk factors. The identified CVD non-modifiable factors include age, gender, and genetics, meanwhile, modifiable factors are diabetes, hypertension, dyslipidaemia, obesity, and smoking status Katna et al. (2020) and Wang and Khalil (2018). Musunuru and Kathiresan (2019) highlighted that common CVD is a complex condition shaped by both genetic predisposition and environmental factors.

The ABO Blood group system has a complex relationship with CVD as reviewed by Li and Schooling (2020). Studies have reported a significant relationship between the ABO blood group and CVD risk. The ABO(H) carbohydrate structures are present on both N- and O-linked glycans of von Willebrand factor (VWF). Individuals with non-O blood groups have 25% higher plasma levels of VWF and Factor VIII (FVIII) than those with the O blood group

as simplified by Ward et al. (2020). Thus, Neshat et al. (2024) concluded that non-O blood groups are associated with a higher risk and severity of CAD and acute coronary syndromes, driven by increased VWF in elevated plasma cholesterol levels causing endothelial dysfunction and inflammation. In the blood group O individuals, the VWF is significantly cleared from circulation more rapidly than in the non-O blood groups. Jang et al. (2022) and Mukhtar and Abdullahi (2022) found the non-O blood groups had a higher risk of CVD.

Leptin has a dual role, acting as a hormone and a cytokine. As a hormone, it manages energy balance, including energy homeostasis. It also stimulates inflammatory responses as summarised by La Cava, A. (2017). Leptin exerts proinflammatory cytokines causing chronic inflammation. It can disrupt the body's response leading to leptin resistance, which in turn may contribute to obesity as simplified by Pérez-Pérez et al. (2020). In addition, Obradovic et al. (2021) showed that leptin is a product of the obese (ob) gene and a peptide hormone that regulates food intake and body weight. Li and He (2021) reviewed that leptin level depend on body mass index (BMI). Hyperleptinemia is associated with obesity as discovered by Karpouzas et al. (2023). The physiological level of leptin is vital for maintaining healthy cardiovascular function as Vilariño-García et al. (2024) stated that leptin not only contributes to weight gain but can also result in heightened cardiac inflammation, increased fibrosis, hypertension, and disruption of cardiac metabolism. Leptin is a key biomarker for predicting cardiovascular outcomes, highlighting the critical role of this hormone in obesity-related CVD which was simplified by Zhao et al. (2021). Finelli and Sasso (2024) have demonstrated a relationship

between leptin and arterial stiffness and other adipokines in obese patients. In a previous study, Rama Krishna Reddy et al. (2015) discovered the sensitivity and specificity of leptin as one of the atherosclerotic biomarkers, including for subclinical atherosclerosis.

On the other hand, Karagoz et al. (2022) stated that serum leptin level vary with the type of blood group and are related to stimulation or suppression of appetite to regulate energy production which peptide hormone involved in the energy regulation and they found non-O blood group had higher leptin level. As they mentioned that variations in peptide hormone levels across ABO blood groups suggest that obesity may have underlying genetic and immunological components. Moreover, the ABO blood group has been linked to leptin level, with higher leptin concentrations found to correlate with elevated Factor VIII (FVIII) levels, as reported by Buis et al. (2020). This observation aligns with findings by Ward et al. (2020), which show that individuals with non-O blood groups have higher plasma levels of both von Willebrand factor (VWF) and FVIII compared to those with the O blood group, suggesting a possible interplay between leptin, FVIII, and ABO blood type in determining cardiovascular risk.

The FRS was used in determining the CVD risk and it is one of the most commonly utilised tools for CVD risk stratification as stated by Baharudin et al. (2022). The FRS estimates the 10-year risk of developing CAD in individuals aged 30 and above with no prior history of the disease by assigning points to key risk factors such as age, total cholesterol (TC), high-density lipoproteins

cholesterol (HDL-C), systolic blood pressure, diabetes, and smoking status, as described by Haneen Hussein Farhood et al. (2023). Sepehrinia et al. (2024) summarised that FRS, the first widely recognized CVD risk assessment tool, has been extensively validated across various populations and ethnic groups, gaining prominence in primary prevention guidelines and evolving over the years, with a notable update in 2008 to predict global CVD risk. However, FRS does not include biomarkers or genetic factors as a parameter for the risk factors in its assessment. A broader perspective has revealed common prevalent CVDs, such as CAD, atrial fibrillation, HF, hypertension, and stroke, are multifaceted conditions influenced by both genetic predisposition and environmental factors as highlighted by Wojtasińska et al. (2023), Musunuru and Kathiresan (2019). Besides that, a FRS study done in Malaysia by Liew et al. (2018) concluded that many doctor-patient consultations rely on inaccurate cardiovascular risk assessments, often due to underestimating the patient's risk.

1.2 Problem statement

The Ministry of Health Malaysia (MOH, 2020) reported that non-communicable diseases (NCD) contributed to the highest cause of mortality. In Malaysia, CVD mortality was increasing by 120% as Hasani et al. (2023) presented the CVD mortality in 2010 was 18842 and increased to 41484 in 2021. The MOH (2019), National Health and Morbidity Survey (NHMS, 2019) reported that CVD has consistently been the leading cause of death from the 1980s to the present. The CVD burden in terms of mortality and morbidity in Malaysia has been steadily increasing over the past three decades, as viewed by Firus Khan et

al. (2022). Besides that, the global rise in obesity rates is alarming, and Malaysia is no exception, as highlighted by the MOH (2023). According to the Institute for Public Health (IPH, 2024), the prevalence of overweight and obesity rose from 44.5% to 54.4% between 2011 and 2023, as detailed in the Technical Report of NHMS (2023).

Zhang et al. (2022) have reviewed that FRS is widely used in many Asian countries and has been validated in Malaysia by Chia et al. (2015). The FRS study done in Malaysia by Liew et al. (2018) concluded that most consultations between doctors and patients are based on inaccurate cardiovascular risk assessment due to underestimating patient's cardiovascular risk. A similar study by Barton et al. (2021) also found an underestimation of FRS. Kasim et al. (2023) found that FRS demonstrated strong discrimination in predicting CVD risk among Malaysians but showed poor calibration as its predicted risk levels did not align well with observed outcomes. A literature search shows there is no study incorporating the genetic and biomarker parameters together with FRS calculation. Musunuru and Kathiresan (2019) broadly review genetics and biomarkers that play vital roles in CVD development. Previous research has discovered ABO blood group, leptin, obesity, and FRS variables on CVD but limited studies discovered an association of ABO blood groups with leptin and both of them on FRS.

ABO blood group and leptin do contribute in CVD pathogenesis. ABO blood group system has been linked to thrombosis as reviewed by Chen et al. (2016), Parente et al. (2020) and Neshat et al. (2024). Mickelsson et al. (2024) found

that non-O blood groups had lower odds of carotid plaques, indicating a nuanced effect on subclinical atherosclerosis. On the other hand, Vilariño-García et al. (2024) stated that elevated leptin level and cardiovascular issues have indicated a robust positive link between them. An obese individual is known to have high leptin hormones Karagoz et al. (2022). There could be a link between obesity and ABO blood groups, though there is limited literature on the association of novel obesity screening tools with ABO blood types by Siddiqui et al. (2019). Most of the FRS studies focus on risk factors of CVD assessment where its' scoring included key metabolic syndrome-related risk factors as summarised by Adil et al. (2023). Therefore, exploring genetic factors and biomarkers could be a valuable tool for accurately identifying high-risk individuals, facilitating prompt diagnosis, and improving disease prognosis. It is crucial to consider genetic and biomarkers variables in CVD risk assessment which is probably helpful to prevent disease progression and may develop more effective healthcare management.

1.3 Justification for the study

High mortality rates secondary to CVD-related concerns may gradually reduce human resources and thus add more burdens to the global financial system. Roth et al. (2020) reported that CVD is increasing worldwide where it continues to be the foremost contributor to the global disease burden and Malaysia is also not exempt from it. Despite numerous efforts to prevent and control CVD, it still stands as the primary cause of illness and death. Santulli (2021) summarised that, according to the latest epidemiological research, CVD remains a major cause of death, despite advances in patient care, and the

occurrence of cardiovascular issues is on the rise. Thus, translational studies are the most promising way to find fresh treatment options, lower mortality rates, and enhance quality of life.

The non-O blood groups had lower odds of carotid plaques as found by Mickelsson et al. (2024) but Neshat et al. (2024) summarised that numerous studies associate non-O blood groups with a higher risk and severity of CAD and acute coronary syndromes, underscoring the need for vigilant cardiovascular risk management in these individuals. Yaghooti-Khorasani et al. (2020) suggested an additional investigation is needed to elucidate the mechanisms through which the ABO blood group contributed as a risk factor for CVD. Katna et al. (2020) have discovered an association of ABO with cardiovascular risk factors and the same goes for most of the studies. This could evaluate whether specific blood types are linked to an increased cardiovascular risk as estimated by the FRS. However, none of the studies evaluate the ABO blood group as a parameter in the FRS assessment tool. Highlighted by Lyngbakken et al. (2019), research on biomarkers and their clinical applications has grown exponentially over the past few decades. Additionally, as Ruparelia and Choudhury (2020) observed, systemic biomarkers have provided crucial insights into the pathophysiology of atherosclerosis and have contributed to the development of new therapeutic strategies. Consequently, numerous inflammation-related factors have the potential to serve as biomarkers for the early prediction of CVD.

In this context, the current study considers ABO antigens and leptin as genetic and biomarker factors in association with the FRS. This was because the ABO blood group system has been widely investigated for its connections to a range of diseases, including cardiovascular conditions, infections, and metabolic disorders as summarized by Abegaz (2021). Besides that, Bruun-Rasmussen et al. (2023) stated that leptin, a hormone regulating energy balance and metabolism, has been associated with cardiovascular health and metabolic syndrome. This could determine whether leptin level influence the cardiovascular risk linked to various blood types. Moreover, Vilariño-García et al. (2024) reviewed a lot of studies done on leptin and obesity on CVD. But, none of them covers the association of leptin with FRS, and very limited studies investigating the relationship of ABO with leptin levels worldwide. This study may reveal specific patterns of leptin levels across different ABO blood groups, potentially indicating a metabolic pathway influenced by blood group antigens. Thus, this could provide insight into how various blood groups may affect leptin levels, potentially impacting metabolic health.

Identifying blood types with higher or lower cardiovascular risk could help in tailoring preventive strategies based on genetic predisposition. Understanding how leptin modifies cardiovascular risk in different ABO blood groups could lead to the development of personalized risk assessment tools. Thus, this study endeavours to investigate the association between the ABO blood group and leptin level concerning the FRS, to elucidate their combined impact on determining CVD risk. There is an urgent need to focus on adapting existing cost-effective policies and interventions to achieve a reduction in mortality due

to NCD. By exploring these relationships, it may be possible to identify novel risk factors or protective mechanisms that could inform personalized medicine approaches in cardiovascular health.

1.4 Research hypothesis

There is an association between the ABO blood group and plasma leptin level with FRS.

1.5 Objectives of the study

1.5.1 General objective

To determine the association of the ABO blood group, leptin level with moderate to high FRS.

1.5.2 Specific objectives

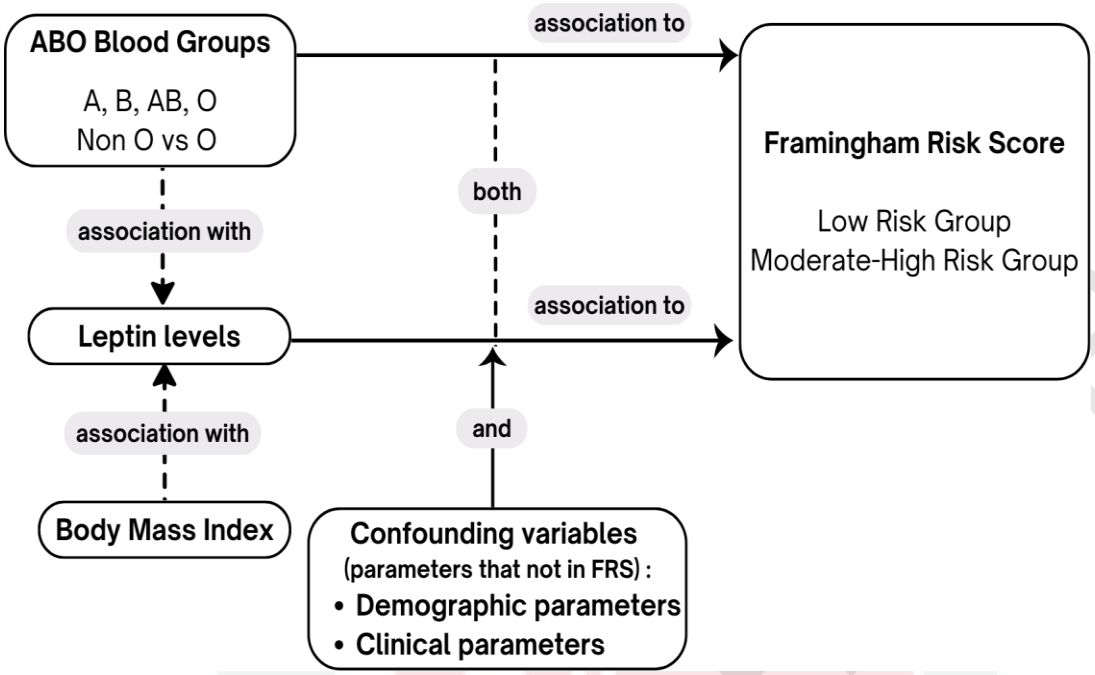
1. To determine the demographic and clinical background of patients according to FRS score.

2. To determine the association between the ABO blood group and FRS among population.

3. To determine the distribution and association of ABO blood group, BMI and leptin level among randomly selected patients.

4. To determine the association of the demographic, and clinical background of patients and FRS among randomly selected patients.

1.6 Conceptual Framework



The conceptual framework for this study aims to understand the complex relationships between several key variables which are the ABO blood group, plasma leptin level, BMI, and the FRS. The framework is built around four specific objectives. The first objective focuses on establishing the baseline characteristics of the study population by determining the prevalence of various demographic and clinical backgrounds on FRS status. The information collected will provide a foundation for analyzing the associations and interactions among these variables in subsequent objectives. The second objective aims to the potential direct association between ABO blood groups and FRS in the whole population. The purpose is to identify whether specific blood groups are associated with a lower or moderate-high cardiovascular risk, as determined by the FRS. This objective allows for an analysis of whether genetic factors (represented by ABO blood groups) have a significant influence on the overall

cardiovascular risk profile. The third objective explores how the distribution of the ABO blood group and BMI relates to plasma leptin level. In this framework, BMI is considered both an independent variable and a confounding factor that could influence leptin level. Similarly, the ABO blood groups may have a direct association with leptin level. This step involves determining if specific ABO blood groups or BMI are associated with leptin level in the 150 patients. The final objective examines the combined effect of multiple variables, including demographic factors, clinical factors, ABO blood groups, and leptin level on FRS. This objective aims to assess how these variables interact with each other and to identify potential confounding effects. Understanding these interactions could help explain the underlying mechanisms that contribute to cardiovascular risk.

CHAPTER 2

LITERATURE REVIEW

2.1 Cardiovascular diseases

World Health Organization (WHO, 2021), estimates that 17.9 million deaths worldwide in 2019 were attributable to CVD which is a NCD, representing 32% of all casualties and more than 75% of CVD fatalities occur in low- and middle-income countries (LMICs). Prabhakaran et al. (2023) reported that CVD stands as the primary global cause of mortality and stated that there was considerable diversity in the prevalence of CVD between high-income countries (HICs) and LMICs. Meanwhile, Mensah et al. (2019) viewed that mortality was disproportionately higher in middle-income nations as compared to both high-income and low-income countries. Besides that, Prabhakaran et al. (2018) stated that for both HICs and LMICs, there was a lack of capability to identify this silent disease and lack of early intervention which led to the swift onset of severe complications and premature mortality. The findings from the prospective cohort study conducted by Dagenais et al. (2020) in 21 countries, including Malaysia, were consistent with the global impact of CVD, which is indeed a major cause of mortality and a significant public health concern in many regions around the world.

Both developing and developed countries have witnessed rising mortality rates from CVD over the past decades as reviewed by Nawsherwan et al. (2022). Francula-Zaninovic and Nola (2018) summarized that projections suggested that by 2030, approximately 23.6 million individuals will succumb to CVD

annually due to it being the foremost reason for premature deaths worldwide. The World Bank (2021) reported that Malaysia is an upper middle-income country and the MOH (2020) reported that Malaysia was significantly affected by the global NCD crisis. As one of Asia's developing nations, Malaysia is dealing with an escalation in cardiovascular mortality and morbidity. The CVD severity among Malaysians is supported through data sourced from the Statistics on cause of death in Malaysia (2023), which reveals that the leading cause of mortality was CVD accounted for 16.1% of deaths in 2022 and there was a growing trend in the occurrence of this cardiovascular issue since 1991.

On top of that, the high prevalence of NCD in Malaysia raises concerns for the MOH (2020) regarding the substantial economic burden it may impose as it not only affects health directly but also has a significant impact on society and the economy. People who were suffering from CVD had to lose productivity due to absenteeism, presenteeism, and mortality based on the report on the impact of NCD from MOH (2020) that significantly influences economic outcomes. In conjunction with the crisis, Ndubuisi (2021) stated that the productivity loss and high costs of long-term treatment for NCD can create a vicious cycle, driving families and societies into poverty. Thus, MOH (2020) concluded that this crisis could ultimately burden countries due to slowed economic growth and increased expenditure on social welfare programs. Nonetheless, despite significant advancements in cardiovascular medicine over the past 50 years, the world possesses the tools and knowledge to improve heart health, yet these resources often fail to reach the communities most in need for diagnosing, preventing, and treating cardiovascular diseases

as summarised by Cesare et al. (2023) in World Heart Report 2023.

2.1.1 Cardiovascular diseases definition and pathophysiology

WHO (2021) defined CVD as a range of conditions affecting the heart and blood vessels which included coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease (PAD), rheumatic heart disease (RHD), congenital heart disease, deep vein thrombosis (DVT) and pulmonary embolism (PE). In addition, Parente et al. (2020) define CVD events as including ischemic heart disease (IHD), ischemic stroke (IS), and PAD. These conditions collectively constitute a set of heterogeneous diseases, whose primary underlying cause of development is often atherosclerosis as simplified by Wong et al. (2022). Besides that, Thiriet (2018) simplified that CVD encompasses various conditions that comprise the blood circulation system, involving both the heart and blood vessels, which transport blood throughout the body. He also simplified that atherosclerosis is a primary contributor to CVD as CVD includes CAD, which is a subtype of atherosclerosis. Moreover, atherosclerosis, an inflammatory disease of the large arteries, is the leading cause of CVD and stroke, with CAD being its most prevalent form as summarised by Björkegren and Lusis (2022). In advanced stages of CAD, the rupture of plaque might trigger atherothrombosis, resulting from fibrin formation and platelet activation which may lead to the blockage of blood vessels, causing damage to organs such as heart attack, stroke, limb ischemia, and even death as summarized by Bauersachs and Zannad (2018). On top of that, Francula-Zaninovic and Nola (2018) stated that CVD is a chronic condition that progresses slowly over a person's lifetime and often remains asymptomatic for an extended period.

2.1.2 Atherosclerosis and coronary artery disease

Poznyak et al. (2022) reviewed that atherosclerosis is the primary cause of CVD, affecting the inner layer of medium and large arteries, particularly at branching points which can lead to heart problems by stenosis and atherothrombosis due to reduced blood flow. Atherosclerosis stated by Mitchell and Powell (2020), involves the thickening and stiffening of artery walls, is often associated with aging, and has significant adverse effects on the cardiovascular system as well as various other diseases. Atherosclerosis, marked by the build-up of lipids, fibrous components, and calcification in major arteries, starts with endothelium activation, setting off a series of events that narrow vessels and activate inflammatory pathways, forming atheroma plaques which lead to cardiovascular complications, persisting as the primary global cause of mortality as explained by Jebari-Benslaiman et al. (2022). Poznyak et al. (2022) reviewed that atherosclerosis is a precursor to CVD and frequently results in significant morbidity and mortality. Advanced atherosclerosis is characterized by lesions in the arterial wall's inner layer, accompanied by plaque accumulation, and the subsequent rupture or erosion of these plaques can lead to potentially fatal thrombotic events as summarised by Poznyak et al. (2020).

Simplified by Medina-leyte et al. (2021) CAD was the most prevalent type, involving the build-up of lipids and immune cells in the subendothelial space of the coronary arteries, leading to atherosclerosis. Shao et al. (2020) stated that the leading factor behind CAD is the development of atherosclerosis within the coronary arteries. Simplified by Frąk et al. (2022), CAD is a prevalent heart condition characterized by the narrowing or obstruction of primary blood

vessels, specifically the coronary arteries, primarily due to the accumulation of plaque, in which the plaque refers to the build-up of fatty material within the inner lining of blood vessels, often accompanied by significant inflammation, especially if chronic inflammation. The chronic situation may lead to arterial stiffness or arteriosclerosis as it develops due to an increased production or more extensive involvement of rigid load-bearing components within the arterial wall as explained by Mitchell and Powell (2020). Frąk et al. (2022) summarized from their review that atherosclerosis and CAD are linked by dysfunction of the endothelium, along with inflammation.

2.1.2.1 Endothelial dysfunction

Endothelial cells (EC), as described by Marchio et al. (2019), create a semi-permeable layer resembling a thin sheet, separating the arterial wall from substances in the bloodstream. This layer, according to the findings of Medina-leyte et al. (2021), regulates blood vessel constriction and relaxation, prevents blood platelet aggregation, and maintains fluid balance in the body or fluid homeostasis. The vascular endothelium served as a semipermeable barrier between vascular smooth muscle cells and the vascular lumen, while also providing a nonthrombotic lining for the cardiovascular system as stated by Abdelsalam et al. (2019). Biswas and A. Khan (2020) emphasized that endothelial function plays a critical role, engaging with almost every bodily system and selectively providing nutrients and growth factors to every individual organ. Besides that, Chistiakov et al. (2015) explained that EC forms a crucial barrier and exhibits high metabolic activity, while in conditions like atherosclerosis, disturbances in normal endothelial function, termed endothelial

dysfunction (ED), may progress to irreversible loss of EC functionality, contributing to overall vascular dysfunction and promoting the development of multiple cardiovascular disorders including CAD. This was briefly explained by Poznyak et al. (2020) that the pathological chain occurrences triggering the development of atherosclerosis are thought to commence with localized ED. The ED has been linked to health issues that affect the endothelium's ability to prevent blood clotting, maintain its anti-inflammatory role, regulate blood vessel growth, and manage the restructuring of blood vessels as supported by Biswas and A. Khan (2020). The ED stands out as one of the initial and pivotal factors contributing to the progression of CAD and atherosclerosis as highlighted by Medina-leyte et al. (2021) and it was considered a hallmark of various human cardiovascular diseases, including atherosclerosis as simplified by Xu et al. (2021). Thus, in the context of atherogenesis, the dysfunction of endothelial cells primarily manifests as a breakdown in intima anatomical integrity as described by Biswas and A. Khan (2020).

2.1.2.2 Inflammation

Endothelial dysfunction and inflammation are closely related to each other and this was supported by Frąk et al. (2022), who mentioned that in the progression of endothelial dysfunction, inflammatory elements significantly contribute to its development. This was because in normal balance endothelial cells impede blood clotting, platelet activation, and leukocyte adhesion as simplified by Mussbacher et al. (2022), but these substances attract monocytes and neutrophils, which adhere to the activated endothelium, infiltrate the arterial wall, and triggered inflammation in response to the injury as explained by Chistiakov

et al. (2018). Malech et al. (2020) further explained that neutrophils release net-like extracellular structures (NETs) in response to infections and inflammatory stimuli like cholesterol crystals, oxidized low-density lipoprotein cholesterol (LDL-C), oxysterols, platelets, and chemokines. In addition, Hidalgo et al. (2022) stated that excessive NETs production during inflammation can result in vascular blockages, tissue injury, and prolonged inflammation, exacerbating various pathological conditions like atherosclerosis. Inflammation significantly influences the pathogenesis of endothelial dysfunction, as observed from its underlying mechanisms as highlighted by Wojtasińska et al. (2023). This was because atherosclerosis involves the accumulation of lipids and inflammatory cells within the injured intima as simplified by Bäck et al. (2019). Thus, Frąk et al. (2022) concluded that a healthy endothelium is a key indicator of cardiovascular well-being, while its dysfunction serves as an early sign of atherosclerosis and CAD. In conclusion, Ghazizadeh et al. (2020) stated that inflammation and oxidative stress are key contributors to the pathophysiology of various diseases, including CVD.

2.1.3 Cardiovascular diseases risk factors

Wojtasińska et al. (2023) stated that the symptoms of CVD greatly affect the quality of life, with atherosclerosis often remaining latent and symptom-free despite its varied consequences. Thus, it is vital to rule out CVD risk factors. Kopp (2019) categorized CVD falls into the category of lifestyle-related conditions known as “civilization diseases” as throughout 6 to 8 generations, particularly in the most recent 2 to 3 generations, there has been a surge in obesity and non-communicable degenerative illnesses. Poznyak et al. (2022)

are concerned that various risk factors for atherosclerosis have been identified, yet, many are broad and don't distinctly explain how atherosclerosis starts. Therefore, it is important to clearly identify the broad risk factor of CVD. In addition, Kong et al. (2022) summarized that atherosclerosis is a chronic inflammation in arteries caused by both modifiable and nonmodifiable risk factors, leading to the build-up of plaques in the vessel walls. Parallel with Gallucci et al. (2020) who stated that CVD can result from various factors, including those that are nonmodifiable like age, gender, and genetics, and others that can be modified through lifestyle changes such as quitting smoking, managing blood pressure, addressing diabetes, controlling cholesterol level, and managing obesity. Furthermore, Poznyak et al. (2022) highlighted some of the CVD risk factors, like age, gender, and genetics are non-modifiable, while others, such as high blood pressure, diabetes, dyslipidaemia, obesity, and smoking are modifiable and all of these factors should be considered as those are the primary factors that contribute to atherosclerosis and consequently lead to CVD. As simplified by Thiriet (2018) CVD has multiple causes, including clinical factors and as well as behavioural factors, or also can be understood by modifiable and non-modifiable risk factors. The common cardiovascular risk factors, including hypertension, diabetes, dyslipidaemia, smoking, and overweight or obesity have been steadily increasing in prevalence as reported by Mohd Nor et al. (2022), IPH (2024).

2.1.3.1 Modifiable risk factors

Modifiable risk factors significantly contribute to the development of CVD. In Malaysia NCVD-ACS Registry (2018-2019) by Wan Ahmad (Ed) (2022) reported that the majority of patients (93.5%) presented with at least one established cardiovascular risk factor, with hypertension (61.9%), diabetes (44.2%) or dyslipidaemia (36.7%) being the most common. Roth et al. (2020) classified high blood pressure, high glucose level, high cholesterol, high body weight, and smoking as modifiable risk factors for CVD. The metabolic disorder or syndrome encompasses a constellation of critical risk factors for heart attack, including elevated fasting plasma glucose and diabetes, abdominal obesity, high cholesterol levels, and hypertension as simplified by IDF (2022). Gradinaru et al. (2015) further explained that the onset of the atherogenic process begins with damage to the intimal endothelial lining triggered by irregular blood flow patterns like hypertension-induced abnormal blood flow, high blood sugar, oxidized cholesterol, high lipid or cholesterol level, or cigarette smoke. This was supported by data from National Health and Morbidity Survey (NHMS) in 2019 by IPH (2020) showed that 1.7 million Malaysians currently have the three primary cardiovascular risks which are hypertension, diabetes, and dyslipidaemia, while 3.4 million Malaysians have two of the three primary risk factors of CVD as updated by IPH (2020). Besides high blood pressure, diabetes, and high cholesterol level, Tsao et al. (2023) also added body weight and smoking cigarettes as several recognized risk factors that contribute to atherosclerosis. All these risk factors by their impact on populations were largely consistent across studies, even though there were significant regional differences as reviewed by Hankey (2020).

2.1.3.1.1 Hypertension

The World Health Organization (WHO, 2023) reported that in 2019, Malaysia recorded 185,000 mortalities among over 6.1 million individuals aged 30-79 years who had been diagnosed with hypertension. Malaysian Society of Hypertension (MSH, 2018) classified hypertension (HPT) as a consistent increase in SBP of 140 mmHg or higher and/or diastolic blood pressure (DBP) of 90 mmHg or higher and individuals at risk when SBP of 130-139 and DBP of 85-89. Poznyak et al. (2022) emphasized that HPT is a powerful risk factor for atherosclerosis, leading to subsequent CVD and cardiac events in epidemiological studies, HPT emerged as the most crucial modifiable cardiovascular risk factor, contributing to 48% of strokes and 18% of coronary events. In the same vein, Stewart et al. (2017) stated that among those risk factors, HPT is highlighted as having the most compelling evidence for a direct causal relationship with atherosclerosis and is widespread among the population. The hypothesis by Ogata et al. (2019) that not only hypertension but also suboptimal blood pressure is a risk factor for the advancement of atherosclerosis. On top of that, Roth et al. (2020) said that hypertension can lead to hypertensive heart disease (HHD).

2.1.3.1.2 Diabetes

International Diabetes Federation (IDF, 2022) reported that in 2021, 537 million people had diabetes worldwide, while South-East Asia (SEA), including Malaysia, estimated 6.7 million individuals succumbed to diabetes over only 90 million who aged 20-79 years had diabetes. IDF (2022) defined diabetes as a

serious and chronic condition that occurs when elevated glucose level in the blood result from insufficient insulin production or ineffective use of the produced insulin, leading to the body's inability to regulate blood sugar level adequately. Insulin is a crucial hormone produced in the pancreas, facilitates the entry of glucose into cells for energy conversion, and it is essential for protein and fat metabolism. Insufficient insulin or impaired cellular response results in elevated blood glucose level and thus that indicates diabetes. Diabetes is identified by Wojtasińska et al. (2023) as a catalyst for atherosclerosis due to dyslipidaemia, hyperglycaemia, oxidative stress, and chronic inflammation, closely intertwining the pathogenesis of atherosclerosis and diabetes. On top of that, IDF (2022) establishes diagnostic criteria for diabetes mellitus, considering fasting plasma glucose ≥7.0 mmol/L, 2-hour post 75-gram oral glucose load in the oral glucose tolerance test (OGTT), HbA1c ≥48 mmol/mol, or random plasma glucose ≥11.1 mmol/L and the diagnosis is confirmed if one or more of these criteria are met. Škrha Jan (2021) emphasized that atherosclerotic cardiovascular disease (ASCVD) arises from accelerated atherosclerosis through various metabolic pathways associated with diabetes.

2.1.3.1.3 Dyslipidemia

As reported by IPH (2020) was 38.1% prevalence of hypercholesterolemia was observed. Dyslipidaemia cut-off values, outlined in the National Heart Association of Malaysia (NHAM, 2023) in Clinical Practice Guideline (CPG) Management of Dyslipidaemia, consist of TC > 5.2 mmol/l, HDL-C < 1.0 mmol/l for males and < 1.2 mmol/l for females, TG > 1.7 mmol/l, and LDL-C level determined based on the patient's cardiovascular risk by clinical judgement after

an 8-12 week therapeutic trial of Therapeutic Lifestyle Changes (TLC), with a target of <3.0 mmol/L considered as low CVD risk. The early stage of atherosclerotic lesion development, known as "fatty streak," involves intracellular lipid accumulation in foam cells within vascular smooth muscle cells (VSMCs) and T lymphocytes, with potential progression to atherosclerotic lesions contingent upon persistent chronic endothelial injury as explained by Poznyak et al. (2020). Over decades, studies have clarified that the development of atherosclerosis is marked by chronic inflammation and lipid buildup in the arteries, which narrows blood flow as viewed by Libby et al. (2019). This was because atherosclerosis is traditionally linked to modification in lipid metabolism and elevated cholesterol level as simplified by Miname and Santos (2019). However, Taleb (2016) reviewed that the pathogenesis of the disease seems to be more intricate than a modification in lipid metabolism and encompasses various factors with inflammation being the most prominent. Nevertheless, modified lipids in the intima trigger inflammation, leading to the formation of atherosclerotic plaques through a cascade involving various cells and molecules in which by the production of chemokines and cytokines, thereby initiating activation of leukocytes, endothelial cells, and adhesion molecules, the endothelial surface as highlighted by Frąk et al. (2022). Briefly, Kong et al. (2022) mentioned that traditionally, atherosclerosis was seen as a condition where cholesterol, especially LDL-C, builds up in the walls of arteries. In the same vein, Bäck et al. (2019) stated that the formation of inflammatory atherosclerotic lesions in atherogenesis is primarily caused by the accumulation of lipids in the arterial intima and the resolution of inflammation can be made if this proinflammatory source can be reduced.

2.1.3.1.4 Obesity

According to IPH (2020), over 50.1% of Malaysian adults were overweight or obese with 30.4% being overweight and 19.7% obese. The MOH (2023) in its CPG 2023 in Management of Obesity defined obesity as a chronic and complex health issue that occurs when the body stores too much fat, leading to various health problems and a higher risk of early illness and death. Aligned with Roth et al. (2020) highlighted that obesity has become a global epidemic and exacerbates several risk factors for CVD affecting blood pressure, blood sugar, lipids, inflammation, and cardiac structure and function. Parameter of BMI stands as a globally recognized metric for gauging obesity, endorsed by the World Health Organization (WHO) and it is calculated by dividing an individual's weight in kilograms (kg) by the square of their height (height2) in meters (m), serving as a universal standard for assessing weight status [BMI = Weight (kg) / height2 (m2)]. MOH (2023) agreed with WHO Expert Consultation (2004) that every adult should undergo an annual screening using the BMI measurement, with a cut-off point of ≥23 kg/m2 and as triggering additional assessment for overweight with BMI ≥23 kg/m2 and obesity with BMI ≥ 27.5 kg/m2. Battineni et al. (2021) simplified that overweight and obesity are linked to a higher risk of CVD, along with other health conditions like diabetes, hypertension, and insulin resistance. This was because obesity is recognized as an independent predictor of CHD, indirectly influencing it through associated comorbidities like dyslipidaemia, hypertension, glucose intolerance, endothelial dysfunction, and inflammation as simplified by Vilariño-García et al. (2024). It was found by Luo et al. (2024) that obesity elevates the risk of CVD even in individuals without conventional CVD risk factors.

2.1.3.1.5 Smoking

Smoking is the first CVD risk that can easily and effectively be prevented. Lee and Lee (2023) observed in their review that unlike hypertension, diabetes, or dyslipidaemia, which become more prevalent with age, smoking disproportionately affects young adults, making it a distinct and significant risk factor for CVD. Bouabdallaoui et al. (2021) in their study revealed that active smoking continues to be a significant factor influencing both overall mortality and cardiovascular-related death. Gallucci et al. (2020) summarized that smoking escalates mortality across all causes and plays a pivotal role in atherosclerotic cardiovascular disease (ASCVD). It triggers oxidative processes that detrimentally impact platelet function, fibrinolysis, inflammation, and vasomotor function. These proatherogenic effects collectively double the 10-year risk of fatal events in smokers compared to non-smokers. In the same vein, nicotine's adrenergic effects trigger a series of physiological changes such as increased heart rate, enhanced myocardial contractility, higher coronary vascular resistance, and reduced insulin sensitivity that elevate the risk of cardiovascular disease by summarization of Benowitz and Burbank (2016). This can be concluded that smoking not only poses a risk for chronic CVD but also triggers acute atherothrombotic events like stroke or myocardial infarction, and influences glucose tolerance and HDL-C level.

2.1.3.2 Non-modifiable risk factors

Roth et al. (2020) highlighted that the epidemiology of CVD may be influenced by factors such as age and gender. Furthermore, Wojtasińska et al. (2023) in

their review, concluded that atherosclerosis is thought to arise from various factors, encompassing both genetic and environmental influences. Thus, those studies concluded that non-modifiable risk factors like age, gender, and genetics would be risk factors for CVD.

2.1.3.2.1 Age

Reviews done by Yandrapalli et al. (2019) demonstrated that NCD have risen steadily over the years and indicated a potential age-dependent association between modifiable risk factors and the risk of developing CVD. In the same vein, Kaneko et al. (2022) stated that CVD mainly affects middle-aged and older individuals and their findings align with previous studies of Huo et al. (2018) and Rawshani et al. (2018), indicating a potential age-dependent association between modifiable risk factors and the risk of developing CVD. This view was supported by Nedkoff et al. (2023) who concluded in their review that the rising absolute number of CVD deaths and incidence worldwide is largely driven by population growth and aging. Donato et al. (2018) have explored the mechanisms driving age-related large elastic artery stiffening and endothelial dysfunction at the tissue level through inflammation, with these two arterial changes serving as independent predictors of future CVD and likely contributing to its development in older adults. Thus, this arterial stiffness is regarded as a hallmark of cardiovascular aging and an independent risk factor for cardiovascular disease as simplified by Galiuto and Locorotondo (2017). When people age, they become more susceptible to various health conditions involving CVD which drives a rise in CVD cases. Thus, age-specific trend monitoring is crucial, given the potential rise in premature CVD burden in certain

settings.

2.1.3.2.2 Gender

Suman et al. (2023) stated that the ongoing debate about improving scientific validity and reproducibility increasingly emphasizes the importance of including gender information in order to enhance the accuracy of research findings. This was because gender differs in epidemiology, pathophysiology, and therapeutic considerations which arise from variations in gene expression influenced by sex chromosomes and hormonal differences as reviewed by Hägg and Jylhävä (2021). Borén et al. (2020) stated that as per the Progression of Early Subclinical Atherosclerosis study, 71% of middle-aged men and 43% of women experience subclinical atherosclerosis. Similarly, Roth et al. (2020) found that hypertensive heart disease (HHD) is overrepresented in men in younger ages. However, Suman et al. (2023) mentioned that women are protected from atherosclerosis due to the beneficial effects of estrogen on the cardiovascular system through genomic and non-genomic mechanisms during fertile years but after menopause, estrogen deficiency results in a significant rise in cardiovascular risk due to various structural and functional changes such as visceral obesity, endothelial dysfunction, autonomic imbalance with increased adrenergic activity, and heightened systemic inflammation. Thus, it highlights that not only men, women also experience more hospitalizations for heart failure and stroke compared to men.

2.1.3.2.3 Genetic

A broader perspective has been revealed by Musunuru and Kathiresan (2019) who viewed those common prevalent CVD, such as CAD, atrial fibrillation, heart failure, hypertension, and stroke, are multifaceted conditions influenced by both genetic predisposition and environmental factors. Khera et al. (2016) reviewed genome-wide association studies have uncovered over 50 independent genetic loci linked to coronary artery disease, offering deeper insight into its genetic architecture, when combined into a polygenic risk score, these factors can quantify an individual's genetic susceptibility and help identify those at higher risk of developing the disease. Besides that, Klarin et al. (2017) have identified the genes shared among most of the over 150 CVD sites discovered so far reveal several main risk factors, LDL-C, TG-rich lipoproteins, insulin resistance, blood clotting, inflammation, cell adhesion, cell movement through blood vessel walls, cell growth, changes in blood vessel structure, and nitric oxide signalling. In conjunction with the cholesterol parameter, obesity also can result from genetic influences as simplified by MOH (2023). Björkegren and Lusis (2022) suggested that "genetics is to biology what mathematics is to physics," and while genetic studies are crucial for understanding biological processes, identifying non-additive interactions in human studies remains challenging.

2.2 ABO blood group

Karl Landsteiner's discovery of the ABO blood group system in 1901 laid the groundwork for modern immunology and safe transfusion practices as reviewed by Mohd Noor and Siti Asmaa (2024). The blood group system

consists of inherited antigens encoded by a single gene or a cluster of closely linked homologous genes as stated by Li and Guo (2022). ABO blood group is defined by the presence or absence of A and B antigens on RBC and their corresponding antibodies, anti-A and anti-B, in the serum as simplified by Lei et al. (2020). ABO blood group was suggested by Mohammed et al. (2024) in potential as epidemiological markers for targeting populations vulnerable to specific diseases and identified non-O blood groups as being at higher disease risk. This was related to blood antigens have been associated with either increasing the susceptibility of individuals to certain diseases like cancer, diabetes, infectious diseases, and heart illnesses as reviewed by Vasan et al. (2016), Rummel and Ellsworth (2016) or by providing protection against certain diseases such as diabetes as according to Zhang et al. (2015).

2.2.1 ABO blood group biochemistry

The ABO(H) blood group is carbohydrate based stated by Jajosky et al. (2023). Blood is classified into four groups, A, B, AB, and O blood groups based on the presence or absence of A and B antigens on RBC as simplified by Goldman and Schmalstieg (2019), with a reciprocal relationship between these antigens and the antibodies in the plasma, as described by Landsteiner's Law in Branch (2015) review. Paquette et al. (2018) explained that the ABO gene, located on chromosome 9 (9q34.2), encodes glycosyltransferase enzymes responsible for synthesising ABO(H) blood group antigens, with A and B alleles producing specific enzymes, while the O allele lacks glycosyltransferase activity and A or B antigens. The inheritance of enzymes that enable specific molecular modifications determines the expression of corresponding blood

group antigens, while these enzymes also play a critical role in driving biochemical reactions essential for human health and disease understanding as summarised by Jajosky et al. (2023).

2.2.2 Population distribution of ABO blood groups

The distribution of the blood group was different among people of different geographical areas and ethics. Blood group distributions vary across regions. In Western Asia, AlShamlan et al. (2021) reported that blood group A was more common than B in Saudi Arabia, similar to findings in Europe by Browne et al. (2021) in Ireland. Conversely, in Southeast Asia, Hajar et al. (2020) observed that blood group B was more prevalent than blood group A in countries like Malaysia and Thailand, aligning with findings in South Asia by V Vaghela and V Shah (2022) in India. In addition, in the same country of Pakistan showed different distribution in different cities as a study done by Sabir et al. (2021), demonstrated that Safdarabad city had the B blood group the highest, while Faisalabad city had the O blood group the highest followed by the B blood group. However, most of the country demonstrated O blood group is the highest compared to the non-O blood group as this can be seen in studies done by V Vaghela and V Shah (2022), Browne et al. (2021), Sabir et al. (2021), Hajar et al. (2020), Tiruneh et al. (2020), Canizalez-Román et al. (2018), Jahanpour et al. (2017). Therefore, Canizalez-Román et al. (2018) highlighted that ABO blood group distribution showed regional differences, potentially reflecting genetic differentiation, as analysed using ABO loci as genetic markers.

2.2.3 Determination of ABO blood group

Karl Landsteiner was the first to demonstrate that agglutination occurs because human blood is not uniform, leading to his discovery of distinct blood groups as reviewed by Cowan (2017). In 1901, Karl Landsteiner discovered that agglutination, caused by interactions between antigens on RBC and antibodies in blood serum, follows a predictable pattern, leading to his identification of the A, B, and O blood groups as summarized by Mohd Noor and Siti Asmaa (2024). A blood group system is identified serologically through specific antibodies and serological testing was the standard method for blood group typing, relying on hemagglutination reactions between RBC antigens and their corresponding antibodies as simplified by Li and Guo (2022). Fundamental practices in an average blood group serology laboratory remain largely unchanged in day-to-day operations. This view is supported by Das et al. (2023), who used the same principle of blood grouping in their research study and concluded that ABO blood group identification is crucial for identifying individuals in mass disasters and holds significance in clinical medicine and human genetics. This was because, since discovery of the ABO blood group system by Karl in 1900 until the write-up of I Lai (Ed.). (2017) in her book applied the same principle like the agglutination of antigens and antibodies. Despite advancements in knowledge and technology in ABO blood grouping by slide, tube, microplate agglutination, novel paper-based, microcolumn gel, dye-assisted paper-based,microfluidic, waveguide-mode sensor, erythrocyte-magnetized technology, protein chip, surface plasmon resonance, flow cytometry and genotyping that reviewed by Li and Guo (2022) summarized that tube method was cost-effective and efficient compared to

another method.

2.2.4 ABO blood groups on cardiovascular diseases

Human ABO blood groups have been known to have an impact on CVD risk factors for a long time as stated by Parente et al. (2020). Chen et al. (2016) summarized that ABO blood groups have been linked to CVD risk in numerous studies which influenced the prognosis of CAD and acute coronary syndrome, potentially impacting complication rates and mortality emphasized by Neshat et al. (2024). In addition, Paquette et al. (2018) found that ABO blood groups have been linked to an elevated cardiovascular risk in individuals with familial hypercholesterolemia. Parente et al. (2020) concluded that the blood group associated with the highest risk varies across different populations, although the O blood group was generally considered the reference group with the lowest risk of ischemic CVD. Meanwhile, BA et al. (2017) research found that non-O blood groups are associated with a higher risk of venous and arterial thrombosis, as well as an increased risk of CVD and related complications, compared to blood group O individuals. Capuzzo et al. (2016) also found that non-O blood groups were associated with a nearly threefold increased risk of subclinical or clinical cardiovascular events compared to individuals with blood group O.

2.2.5 Potential Mechanism between ABO blood group and cardiovascular diseases

Human ABO blood group antigens, with genetically derived glycoconjugate structures on red cell surfaces, significantly influence both cellular physiology

and pathology as reviewed by Abegaz (2021). The ABO antigens, expressed not only on red blood cells (RBC) but also on platelets, endothelial, and epithelial cells, extend this association to cellular dysfunctions underlying cardiovascular pathologies as summarized by Capuzzo et al. (2016). Furthermore, ABO histocompatibility influences the expression of vWF and FVIII on N-linked sugars, affecting thrombosis risk beyond these factors, as mechanisms independent of vWF and FVIII have been proposed as reviewed by Cheung et al. (2018). These antigens also affect plasma levels of vWF and coagulation FVIII, key mediators of hemostasis. Individuals with blood group O have plasma vWF levels approximately 20–30% lower than those with non-O groups, resulting in reduced thrombotic risk as emphasised by Ward et al. (2020). Conversely, non-O blood groups are associated with higher levels of vWF and FVIII, with concentrations being lowest in blood group O, followed by A, then B, and highest in AB, reflecting a graded impact on thrombosis risk as reviewed by Cheung et al. (2018). These differences, attributed to ABO glycosyltransferase activity and its influence on vWF clearance, underpin the link between blood groups and thromboembolic diseases highlighted by Parente et al. (2020). Notably, the non-O blood group is also tied to reduce vWF clearance, which raises its plasma concentration and elevates the risk of thrombogenic events and CVD as reviewed by Cheung et al. (2018). Elevated vWF and FVIII levels have been consistently linked to an increased risk of venous thromboembolism as found by Rietveld et al. (2019) and ischemic diseases as summarized by BA et al. (2017). Interestingly, heightened susceptibility to ADAMTS13 proteolysis and enhanced clearance of vWF in blood group O individuals represent distinct and independent processes

summarized by Ward et al. (2020). Together, these findings highlight the integral role of ABO blood groups in modulating hemostatic balance and CVD risk.

2.3 Leptin

Leptin, derived from the Greek word leptos meaning "thin," is the product of the obese (ob) gene as described by Fan et al. (2023). Hence, adipose tissue, particularly visceral fat, shows a strong correlation with leptin concentrations as simplified by Krupa-Kotara and Dakowska (2021). In a clear picture, leptin is primarily produced by fat cells (adipocytes) and its secretion depends on the amount of fat stored in the body. On top of that, receiver operating characteristic (ROC) curve analysis was conducted by Rama Krishna Reddy et al. (2015) to assess the sensitivity and specificity of leptin, revealing a larger area under the curve alongside favourable sensitivity and specificity values, thus this elucidating the utility of these biomarkers in subclinical atherosclerosis. Similarly stated by Fan et al. (2023) that the specificity of leptin is evident in its role across various diseases and abnormalities in leptin or its receptors widely associated with numerous diseases.

2.3.1 Leptin Definition and Pathophysiology

Leptin is a protein categorized as an adipokine and is acknowledged as a hormone composed of 167 amino acids and boasting a molecular weight of 16 kD as defined by Vilariño-García et al. (2024). The identification of leptin, a hormone derived from fat tissue, has sparked significant interest in

understanding how peripheral signals interact with brain targets to regulate feeding and energy balance. This can be understood by Vilariño-García et al. (2024) which leptin travels through the BBB to affect the central nervous system, where it interacts with receptors in different regions of the hypothalamus, such as the arcuate, paraventricular, and ventromedial nuclei. Its main action occurs in the arcuate nucleus of hypothalamus, which contains two types of neurons: one releasing neuropeptide Y, promoting appetite (orexigenic pathway), and the other releasing proopiomelanocortin, inducing satiety (anorexigenic pathway). Simplified by Obradovic et al. (2021) that a crucial aspect involving leptin's need is to cross the blood-brain barrier (BBB) to access the hypothalamus and perform its appetite-suppressing functions. However, disruptions in leptin signaling, such as leptin deficiency or leptin resistance which can impair this regulatory mechanism as explained by Wang et al. (2023), Obradovic et al. (2021), Raman and Khanal (2021) that leptin deficiency is associated with increased risks of obesity and CVD, and leptin resistance is a complex phenomenon that is influenced by genetic mutations, autoregulation, and impaired cellular signaling. This condition has significant implications for heightened cardiac inflammation, fibrosis, hypertension, and impaired cardiac metabolism as simplified by Pérez-Pérez et al. (2020). According to Krupa-Kotara and Dakowska (2021), leptin exhibited dual effects, regulates appetite by suppressing it at high levels and stimulating it at low levels, with leptin deficiency leading to increased hunger and obesity risk. However, high leptin level produced by adipose tissue in obesity is expected to suppress appetite and increase energy expenditure to facilitate weight loss as explained by Rangareddy et al. (2023) and hyperleptinemia in obesity often

contributes to leptin resistance as further explained by Krupa-Kotara and Dakowska (2021). This was because leptin resistance implied that cerebrums no longer respond to the hormone leptin as simplified by Battineni et al. (2021). Rangareddy et al. (2023) summarized that, leptin resistance in obesity was thought to result from a combination of factors, including persistent inflammation, insulin resistance, and alterations in the hypothalamus and other brain regions responsible for regulating appetite and energy balance.

2.3.2 Leptin role

Leptin, a key adipocyte-derived hormone, plays a vital role in regulating appetite, cellular metabolism, energy balance, and inflammatory processes as simplified by Rangareddy et al. (2023). However, Mohanraj et al. (2022) suggested that leptin therapy may help prevent individuals from developing severe obesity as they age. This was because the leptin signaling pathway plays a vital role in regulating lipid metabolism by promoting lipolysis, inhibiting fatty acid synthesis, stimulating fatty acid oxidation, and maintaining overall energy balance and metabolic regulation as explained by Rangareddy et al. (2023). Besides that, it also maintained cardiovascular system equilibrium through its interaction with the obese receptor (ObR) to influence hypothalamic neurons and manage the body's energy, as reviewed by Krupa-Kotara and Dakowska (2021). Furthermore, leptin plays a critical role in immunometabolism, underscoring its importance in both metabolic and cardiovascular health as simplified by Pérez-Pérez et al. (2017). Similarly by Rangareddy et al. (2023) stated that leptin plays a role in exhibiting anti-inflammatory and cardiovascular protective effects, offering potential

therapeutic value for managing obesity-related conditions. In summary, leptin, which is an adipocyte-derived hormone, plays a crucial role in managing cellular metabolism, balancing energy, regulating inflammatory processes, and upholding cardiovascular system equilibrium.

2.3.3 Leptin and ABO blood group

Based on my research, there has been a very limited study conducted investigating the relationship between leptin and ABO blood group. Similarly, Karagoz et al. (2022) found it a novel aspect of investigation in the exploration of the relationship between anorexigenic hormones such as leptin and ABO blood groups. A study of over a decade ago among the Malaysian population by Qunq Y A, and Abdel Hamid A Z. (2012) observed high leptin level with a range of more than 20ng/ml was observed in B and AB blood groups and obesity seen the highest in B blood group. Besides that, Karagoz et al. (2022) found higher leptin in the A blood group among the Turkey population compared to other blood groups.

2.3.4 Leptin on cardiovascular diseases

Epicardial adipose tissue (EAT), a source of free fatty acids, secreted leptin promoting atherosclerosis through paracrine and endocrine pathways, with its volume linked to the presence and severity of coronary artery calcium (CAC), a marker of atherosclerosis as according to D'Marco et al. (2020). The crucial role of EAT in cardiac physiology and pathology, including cardiac remodeling and CVD, where elevated leptin production in obesity contributes to cardiac

remodeling through mechanisms such as fibrosis, apoptosis, and adipocyte hypertrophy, emphasizing the importance of understanding leptin's molecular effects on cardiac fibroblasts, cardiomyocytes, and EAT adipocytes as explained by Zou et al. (2024). Besides that, Vilariño-García et al. (2024) simplified that leptin acts as a proinflammatory adipokine, adding to the "low-grade inflammatory state" commonly observed in overweight and obese individuals. This means that a signalling molecule produced by adipose tissue (fat cells) promotes inflammation in the body. In the context of obesity, excess fat tissue can produce higher levels of proinflammatory adipokines, contributing to chronic low-grade inflammation, which is associated with various health issues, including insulin resistance and CVD. This was because elevated leptin secretion from EAT has been linked to diastolic dysfunction, cardiac hypertrophy, and myocardial abnormalities, with its release correlating to oxidative injury, inflammation, and mitochondrial dysfunction and promoted inflammatory gene expression as explained by Chen et al. (2023). That's highlighted adipokines, which were cytokines produced by adipose tissue, such as leptin, had the potential to impact inflammatory responses systemically. Thus, Vilariño-García et al. (2024) indicated robust positive links between elevated leptin level and cardiovascular issues such as hypertension, diabetes, CHD, and stroke across various populations. This was due to increased blood volume in obese individuals prompts elevated cardiac output, inducing stress and structural remodeling, which may subsequently result in cardiac hypertrophy as further explained by Vilariño-García et al. (2024). Therefore, despite a crucial role of leptin in regulating appetite, metabolism, energy balance, and inflammation as highlighted by Rangareddy et al. (2023),

leptin would have impact in CVD.

2.4 Framingham risk score

As highlighted by Chou et al. (2024), the “general CVD” algorithm, developed by D’Agostino et al. (2008) calculates the FRS using sex-specific parameters derived from the Framingham Heart Study and the Framingham Offspring Study, with outcomes including coronary heart disease, stroke, peripheral artery disease, and heart failure. Since, morbidity and mortality from CVD rose, the Framingham study began to explore factors and processes related to CVD, providing a way to categorize the likelihood of a heart-related event over the next decade as viewed by Poznyak et al. (2022). Karmali et al. (2017) said predictive risk models can contribute to CVD prevention and control by identifying individuals with a high risk of CVD who would benefit most from preventive interventions. Therefore, Azahar et al. (2022) pointed out that assessing a single cardiovascular risk factor was insufficient for predicting future CVD risk, thus, tools like FRS provided a practical and comprehensive estimation of an individual's 10-year likelihood of developing cardiovascular events. The Framingham Risk Score (FRS, 2008) was developed for an online calculator which included age, gender, SBP, TC, HDL-C, anti-hypertensive agent status, diabetes status, smoking status, and history of known vascular disease. In conjunction with smoking status, validation study by Chia et al. (2015) emphasized that smokers were defined if individuals were still smoking, meanwhile non-smokers were those who never smoked or have stopped smoking.

In addition of Cox models by D'Agostino et al. (2008), covariates encompassed gender, age, TC, HDL-C, SBP, anti-hypertensive agent status, current smoking, and diabetes status, while variables like DBP, BMI, and triglycerides (TG) were also evaluated but found to lack statistical significance while the incorporation of LDL-C did not enhance model fit or performance. On top of that, based on the calculated risk score, individuals will be categorized into low risk with less than 10% risk score, moderate with 10% to 20% risk score, and high-risk group with more than 20% risk score in developing CVD for 10-years CVD risk. Consistent with Poznyak et al. (2022) who simplified that, the risk assessment helps in evaluating the risk of CVD over a decade using factors like SBP, TC, HDL-C, smoking status, and anti-hypertensive agent status. However, Poznyak et al. (2020) simplified that charts and their digital versions facilitate risk assessment and management, but their interpretation should rely on the clinician's expertise and familiarity.

2.4.1 Framingham risk score and Factors associated

The FRS, a widely utilized tool for estimating the 10-year risk of developing CVD as it incorporated a combination of clinical and demographic factors to predict future cardiovascular events. While its predictive factors have been well established, recent researches suggested that both conventional risk factors that were evaluated in the FRS calculation and non-conventional risk factors not explicitly part of the FRS may influence its outcomes. These can be seen by studies done among Malaysians without history of CVD by Azahar et al. (2022), Razman et al. (2022) and Baharudin et al. (2022) were included all conventional FRS factors like age, gender, smoking status, diabetes status,

use of anti-hypertensive agent SBP reading, TC and HDL-C levels and non-conventional FRS factors like ethnicity, locality, education level, occupation status, hypertension status, DBP reading, medication adherence, use of anti-dyslipidemia agent, waist circumference (WC), BMI, FBS, LDL-C and TG levels in risk association. Similarly, Jahangiry et al. (2017) study among Iranians without history of CVD evaluated on association of metabolic syndrome components with additional indirect factors like DBP, WC, FBS and TG level. Besides those common indirect factors to evaluate the risk of association in FRS, Adil et al. (2023) also included areca nut used and chew tobacco used.

2.4.2 Framingham risk score calibration and validation

Calibration and validation of FRS has been done by Selvarajah et al. (2014) in the Asian population and Chia et al. (2015) in the Malaysian population. Validation done among Malaysians by Chia et al. (2015) with consideration of direct and indirect factors on FRS stated that the FRS CVD risk prediction chart serves as a practical and reliable tool for cardiovascular risk assessment in multiethnic populations within primary care settings. In line with Damen et al. (2019) and Wallisch et al. (2019) that FRS was among the most globally recognized and extensively validated cardiovascular risk assessment tools, with proven reliability across diverse populations and ethnic groups. As supported by Sepehrinia et al. (2024), developed from the landmark Framingham study, the FRS was the first cardiovascular risk assessment tool, quickly gaining prominence and being incorporated into primary prevention guidelines like Adult Treatment Panel (ATP) III, with iterative refinements

enhancing its utility over the years.

2.4.3 Framingham risk score limitation

Despites of many CVD risk tool assessments, CVD is still the leading cause of death worldwide. Supported by Cesare et al. (2023), highlighted in World Heart Report 2023 that despite significant advancements in cardiovascular medicine over the past 50 years, the tools and knowledge to mitigate cardiovascular health risks often fail to reach the communities that need them most. Thus, Rama Krishna Reddy et al. (2015) suggested that biomarkers offer insights into diverse CVD pathologies, aiding in the prompt and accurate diagnosis of subclinical such as atherosclerosis in at-risk individuals, thereby facilitating effective prognostication and lifestyle interventions to prevent disease progression. Aligned with that, Lyngbakken et al. (2019) stated that, over the past decades, research on biomarkers and their clinical application has experienced exponential growth. Besides that, systemic biomarkers have offered invaluable insights into the pathophysiology of atherosclerosis and the creation of new treatment approaches as viewed by Ruparelia and Choudhury (2020). Hence, numerous inflammation-related factors may be considered as biomarkers for the early prediction of CVD. And a broader perspective highlighted by Wojtasińska et al. (2023) and Musunuru and Kathiresan (2019) that common CVD, including CAD, atrial fibrillation, heart failure, hypertension, and stroke, are complex conditions shaped by an interplay of genetic predisposition and environmental influences. While the FRS incorporates conventional cardiovascular risk factors, emerging evidence suggests that novel biomarkers and genetic factors, such as leptin and ABO blood group,

may influence FRS predictions indirectly through their impact on components like lipid levels, inflammation, and obesity. By exploring these associations, this study seeks to contribute to a more comprehensive understanding of cardiovascular risk, beyond the factors explicitly included in FRS calculations.

However, Katsiki et al. (2018) have reviewed that FRS incorporates established risk factors such as age, gender, lipid profile parameters, diabetes, and hypertension but does not account for genetic factors or biomarkers, which are increasingly recognized as contributors to CVD susceptibility. That showed large-scale studies directly investigating the link between ABO blood types and leptin as biomarkers with FRS are limited. As most research focuses on broader cardiovascular risk factors rather than the FRS specifically. Nonetheless, in addition to examining the significance and discrimination of the FRS for conventional risk factors, Chou et al. (2024) also explored non-conventional risk factors, however, their study did not incorporate genetic or biomarker-based factors. This was consistent with Battineni et al. (2021), Pérez-Pérez et al. (2020), and Krupa-Kotara and Dakowska (2021), studies on leptin which primarily produced by white adipocytes but also synthesized in cardiovascular tissues, has been implicated in the pathophysiology of CVD through mechanisms such as promoting cardiac hypertrophy, fibrosis, mitochondrial dysfunction, oxidative stress, and inflammation. And studies by Neshat et al. (2024), Parente et al. (2020), Paquette et al. (2018), BA et al. (2017), Chen et al. (2016) and Capuzzo et al. (2016) found that ABO blood groups have been associated with CVD risk which non-O blood groups linked to higher risks of venous and arterial thrombosis, ischemic CVD, and

cardiovascular complications. Although the association between ABO blood groups and CVD has been recognized for decades which could indirectly impact FRS. However, these findings are often inconsistent across different populations and study designs. As there were inconsistencies found by Amrani et al. (2024), Katna et al. (2020) and that ABO blood groups showed no significant differences in association with cardiovascular risk factors. And Yaghooti-Khorasani et al. (2020) concluded that the variation among ABO blood groups appears to be a consequence rather than a cause. In a summary, lipid profile levels in FRS did not differentiate whether the individual is on medication of an anti-dyslipidaemia agent or not. Thus, the hypercholesterolemia status only reflects on the level only as this had led to perceived CVD risk as being low due to the treatment availability options and behavioural lifestyle modifications as stated by Liew et al. (2018). As supported by Grand et al. (2022), Barton et al. (2021) and Hermansson and Kahan (2017), FRS has been found to underestimate cardiovascular risk, as well as in Malaysian populations, where similar findings highlighted its tendency to misjudge risk levels which was found by Liew et al. (2018).

CHAPTER 3

METHODOLOGY

3.1 Study design

This study applied a cross-sectional study involving prospective recruitment of patients who fulfilled inclusion and exclusion criteria from March 2022 to February 2023.

3.2 Study Location

This study was conducted in the Family Medicine Specialist Clinic (FMSC) in Hospital Sultan Abdul Aziz Shah (HSAAS), a suburban community in the district of Serdang in Selangor, a state of West Malaysia. This clinic covers some aspects of medicine, including disease prevention, treatment for acute and chronic illness, and counselling on healthy lifestyle. Most FMSC patients have regular follow-ups for chronic NCD like diabetes, hypertension and dyslipidaemia.

3.3 Study Population

The study population consisted of patients attending care at the Family Medicine Specialist Clinic (FMSC) in Hospital Sultan Abdul Aziz Shah (HSAAS) during the study period. Selected patients in this study were patients who fulfilled the study's inclusion and exclusion criteria. The inclusion criteria include: fasting patients for at least 8 hours, those aged between 30 to 75 years old, patients not known to have any cardiovascular event with documented blood pressure,

and anthropometry reading. Meanwhile, patients who were pregnant, lactating mothers, patients with active diseases like cancer, liver disease, autoimmune disease, and thyroid disease were excluded from the study population.

3.4 Sample Size Calculation and Response Rate

The sample size was calculated based on objectives and the biggest number of sample size estimations was chosen and applied in this study. It was calculated by using OpenEpi (www.openepi.com) by Dean et al. (2013). The reference of population (P) of blood group O from a previous study by Musa et al. (2012) was taken in applying the formula in Open Epi as blood group O was the highest percentage in the study (36.7%). The manual formula is shown below with 95% confidence level and 5% (0.05) of precision (d). The calculated total sample size for this study was 428, covering two cohorts as required by the grant. However, only 352 participants were recruited due to challenges in obtaining ethical approval for the second cohort and time constraints of the study. And, due to budget constraints, 150 patients were randomly selected using an electronic calculator for random selection by CalculatorSoup (2023) for leptin immunoassay. The selection was made randomly based on the FRS risk group which was 50 patients from low, moderate and high FRS groups.

3.4.1 Sample Size Calculation:

$$n = \frac{Z^2(P)(1-P)}{d^2}$$

n = Sample size

Z = Confidence level of 95% (1.96)

P = Expected prevalence: 36.7 % (0.367) (Musa et al. (2012)

d = Precision of 5% (0.05) as P between 10%-90%

$$n = \frac{3.8416(0.367)(1-0.367)}{0.0025}$$

$$n = 357$$

Dean et al. (2013)

Non-responder rate: 20%

$$n = \frac{20}{100}\ x\ 357$$

$$n = 71$$

Total sample :

n + non-responder rate = 357 + 71 = 428

3.4.2 Response rate

This study initially enrolled 352 patients. However, only 333 patients met the inclusion and exclusion criteria and were included in the final analysis, resulting in a response rate of 94.6% as per calculation below. Patients diagnosed with any heart disease, such as those classified under the New York Heart Association (NYHA) criteria, those with incomplete clinical BMI data, or those with insufficient laboratory samples were excluded from the study.

Response Rate = (Number of Responses / Number of People enrolled) × 100

$$Response\ rate = \frac{333}{352}\ x\ 100$$

$$n = 94.6\%$$

3.5 Materials and Methods

3.5.1 Flowchart of Methodology

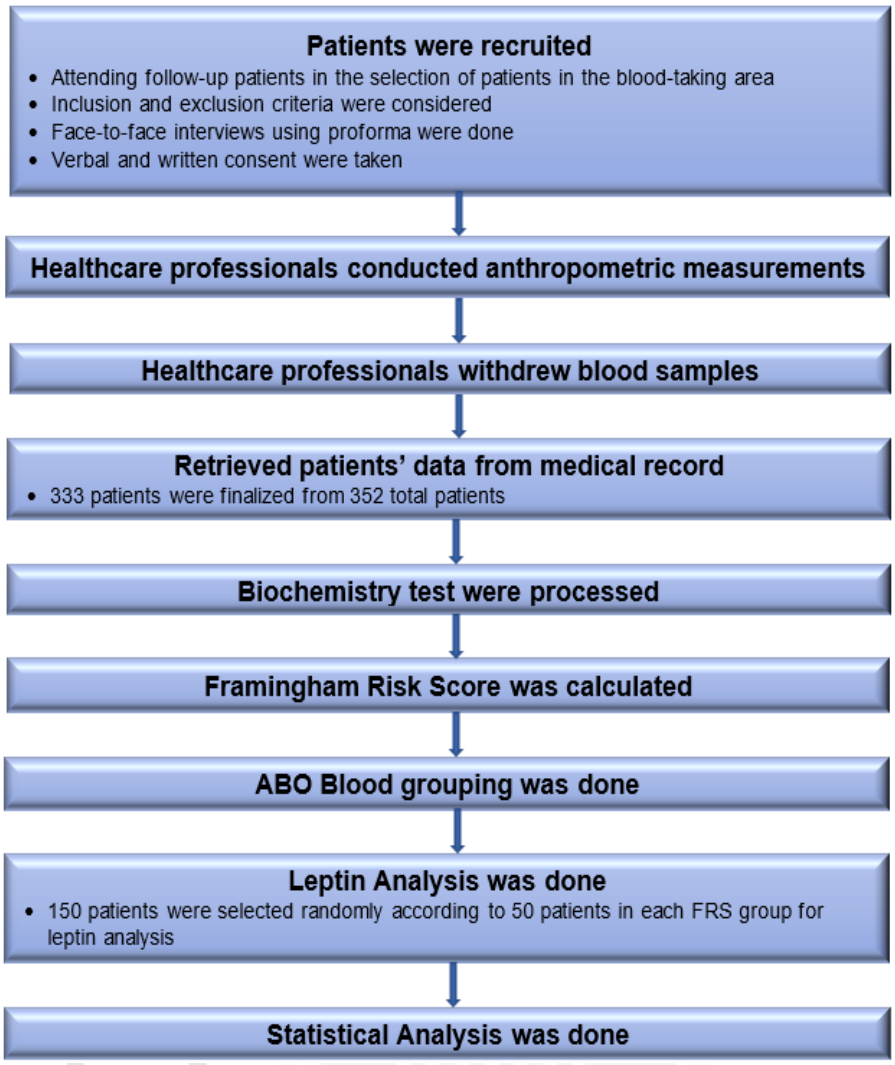


Figure 3.1 Flowchart of methodology. Note: The overall flow of this study began with patient recruitment, followed by anthropometric measurements, blood sample collection, retrieval of patient's medical data, and performance of biochemical tests and ABO blood grouping. The FRS was then calculated, and leptin level was measured using the Luminex assay for randomly selected samples. The statistical analyses were conducted on the collected data.

3.5.2 Recruitment Method

Convenient sampling was applied for follow-up patients who visited the clinic and had planned blood taking. Patients were chosen according to the study eligibility criteria by face-to-face interview using adapted and adopted proforma (clinical report form) from the Cardiovascular Risk assessment sheet (2018) which included information on demographic details, medical history, lifestyle, risk factors for CVD, and clinical measurements. Verbal and written consent were obtained from those who were keen to participate. Consented patients were then proceeded with blood taking. Their collected samples were then transported to the laboratory for further processing.

3.5.3 Anthropometric measurements

The patients' anthropometric measurements such as blood pressure, weight, and height were taken by trained healthcare professionals. Their clinical data were retrieved from the hospital information system. Weight in kilogram (kg) and height in meters (m) were measured using the measuring scale of SECA 286 (Germany) and the BMI kg/m2 was calculated. The patients' blood pressure assessment was measured using a validated and calibrated IntelliVue MX450 Patient Monitor (Philips, Germany).

3.5.4 Blood samples withdrawal

Trained healthcare professionals collected patients' venous blood samples. A total of 20ml blood was collected and separated into three types of blood cointainers, ethylenediaminetetraacetic acid (EDTA) tube for blood grouping

and leptin level, sodium fluoride tube for fasting blood sugar (FBS), and plain/serum separator tube (SST) tube for fasting lipid profile (FLP). The samples were kept in a cool box with ice packs within 2 to 4 hours of collection and were transported to the laboratory for further processing.

3.5.5 Clinical parameter

This study's clinical parameters refer to health-related variables retrieved from the hospital information system. These clinical parameters assess an individual's physiological or pathological status. In this study, clinical parameters include variables such as BP, BMI, blood test, medication status and other cardiovascular-related indicators that are utilized in the calculation of the FRS. These parameters are obtained through standardized clinical data or performed laboratory tests. Patient’s medical records were assessed and their relevant clinical parameters were retrieved with the assistance of the specialist in charge and co-researchers.

3.5.6 Biochemistry Test

The FBS and fasting lipid profile (FLP), TC, TG, LDL-C, and HDL-C were analysed through fully automated Alinity ci-series Integrated Clinical Chemistry and Immunoassay (Abbott, USA). This machine applied Photometric, Potentiometric, and Chemiluminescence methods. FBS analysis was used (M) ALINITY C Glucose Reagent 4000T (Abbott, USA), while TC, LDL-C, and HDL-C were used (M) ALINITY C Cholesterol Reagent 4000T (Abbott, USA) and TG was used Triglyceride Reagent 4000T (Abbott, USA). The machine has been

calibrated and quality control (QC) has been satisfied within acceptable range before using it. The normal range setting of FBS is 3.9-6.1 mmol/L, meanwhile for TC, TG, LDL-C, and HDL-C is 0-5.2mmol/L, 0.08-1.7mmol/L, 0-0 mmol/L and 0-1.2 mmol/L respectively.

3.5.7 ABO Blood Grouping

ABO blood grouping was determined using the tube method. Monoclonal antibodies of anti-A, anti-B, anti-AB, and anti-D antisera (Novaclone, Canada) were used in forward grouping, while reverse blood grouping was performed using known A and B antigen (ID Diacell, Switzerland) as those are established method explained by Malomgré and Neumeister (2009) and I Lai (2017). Forward blood grouping is to determine the presence of A or/and B and D (Rh) antigens in participants' erythrocytes, while reverse blood grouping is to determine the specific antibody of anti-A, anti-B or anti-H antibodies in the patient's plasma for confirming the accuracy of the ABO blood group. These processes rely on an antigen-antibody reaction, with visible agglutination of red cells indicating a positive result, meanwhile, a smooth suspension of post-resuspension indicates a negative result as shown in Figure 3.2. Both forward and reverse blood grouping for determination of the ABO blood group. However, only forward grouping is performed to test for the D antigen on red cells, for Rh classification. Instruments used in blood grouping procedures were calibrated and passed the preventive maintenance service (PMS).

First of all, for forward blood grouping, for each patient, labelled 4 glass tubes with the patient's research identification and each type of antibody reagent on

each glass tube. A drop of the reagents was placed into an individual glass tube according to the label, for example, a drop of anti-A antisera was in the tube labelled as anti-A and the same step was repeated for the other type of antibodies. Then, a drop of the patient's RBC from the EDTA tube was added by using a Pasteur pipette into the same labelled glass tube as in Figure 3.3. Reagents and blood were mixed thoroughly by gently flicking the base of the tube. The tubes were centrifuged briefly using a sero-centrifuge DiaCent-12 (DiaMed, Switzerland) at 500 g for 15 seconds. The tubes were then gently resuspended the cell buttons and were examined for agglutination as in Figure 3.2. In forward blood grouping, the patient was classified A blood group if agglutination happened in the anti-A tube only, the patient was classified B blood group if agglutination happened in the anti-B tube only, patient was classified AB blood group if agglutination happened in both anti-A and anti-B tube and patient was classified O blood group if no agglutination happens in the both anti-A or anti-B tube only. The classification of blood grouping as shown in Figure 3.4 on erythrocytes carried antigens and were agglutinated with antibodies. In addition, the patient was classified Rh positive if there was agglutination in the anti-D tube as shown in Figure 3.5 as on erythrocytes carried Rh factor (RhD) which was agglutinated with the presence of anti-D antibodies.

A similar initial step in reverse blood grouping is labelling tubes. Labelled 2 tubes with the patient's research identification and known red cells of A and B respectively. Whole blood in the EDTA tube was centrifuged by using Centrifuge 5430 (Eppendorf, Germany) at 750 g for 15 minutes to have plasma. Then,

known red cells were placed into an individual glass tube according to the label, for example, a drop of known A red cells was dropped into the tube labelled as A, and the same step was repeated for the other type of known red cells. Then, a drop of the patient's plasma was added by using a Pasteur pipette into the same labelled glass tube as shown in Figure 3.6. Reagents and plasma were mixed thoroughly by gently flicking the base of the tube. The tubes were centrifuged briefly using a sero-centrifuge DiaCent-12 (DiaMed, Switzerland) at 500 g for 15 seconds. The tubes were taken out from the machine and gently resuspended the cell buttons and examined for agglutination as in Figure 3.2. In reverse blood grouping, the patient was classified A blood group if agglutination happened in the B tube only, the patient was classified B blood group if agglutination happened in the A tube only, patient was classified AB blood group if no agglutination happened in both A and B tube and patient was classified O blood group if agglutination happened in the both A and B tube. The classification of blood grouping as shown in Figure 3.7 on plasma contained antibodies and were agglutinated with the presence of specific antigens. The test tube methods were analyzed and conducted by two independent researchers to ensure the reliability and accuracy of the blood grouping results and the results were compared for confirmation. The plasma was kept at -800C for future procedure.

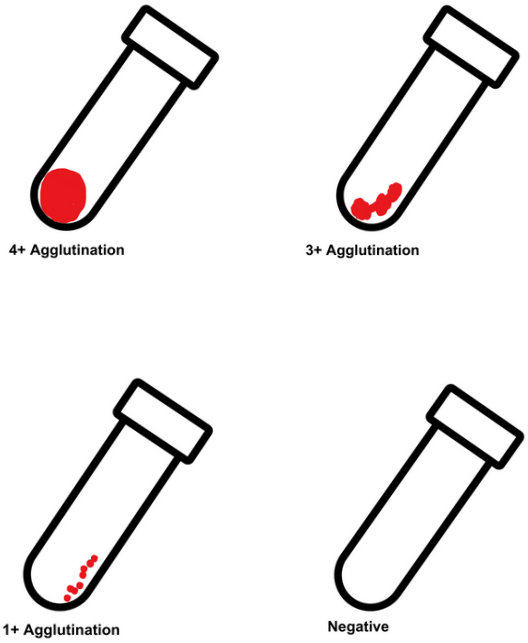


Figure 3.2: Red cell agglutination test scoring

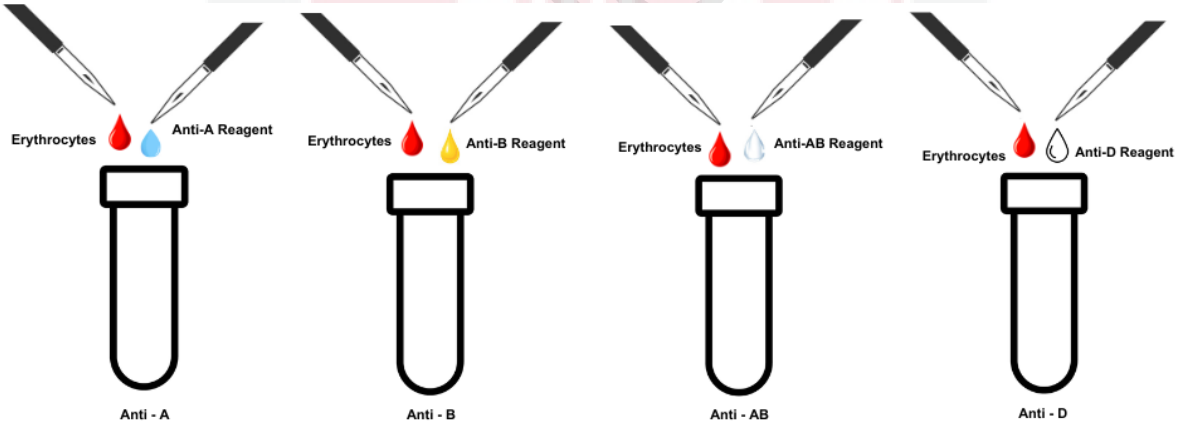


Figure 3.3: Drop in erythrocytes and reagent for forward blood grouping.

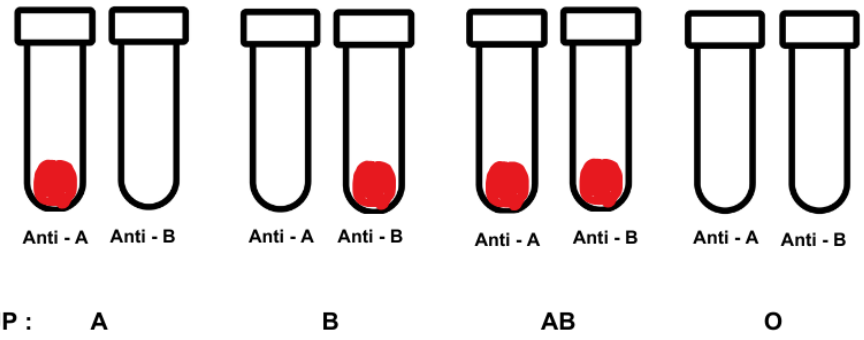


Figure 3.4: Forward grouping: The agglutination indicates the presence of antigens for respective antibodies. Note: Agglutination in anti-A means blood group A, agglutination in anti-B means blood group B, Agglutination in anti-A and anti-B means blood group AB and no agglutination in anti-A and anti-B means blood group O.

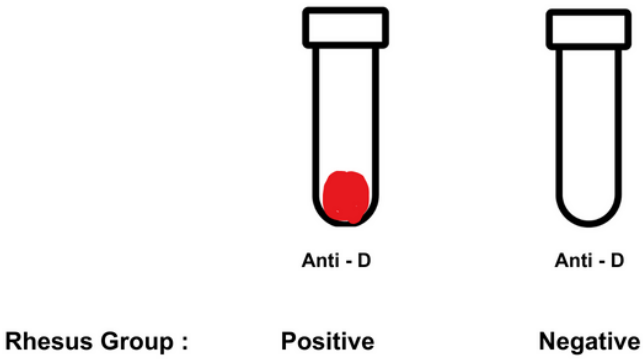


Figure 3.5: The agglutination with anti-D reagents indicates the presence of D (Rh positive). Note: Agglutination in anti-D means Rh positive and no agglutination in anti-D means Rh negative.

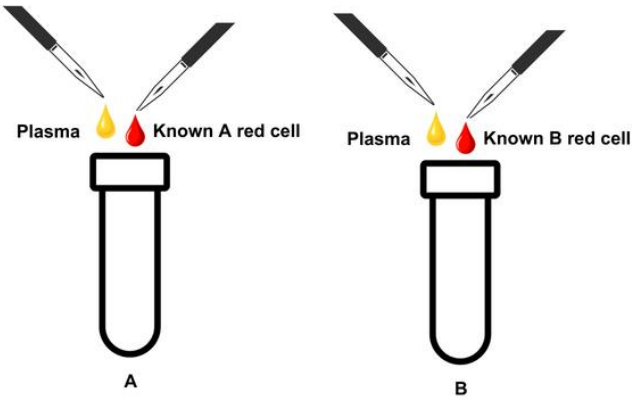


Figure 3.6: Drop in plasma and reagent for reverse blood grouping.

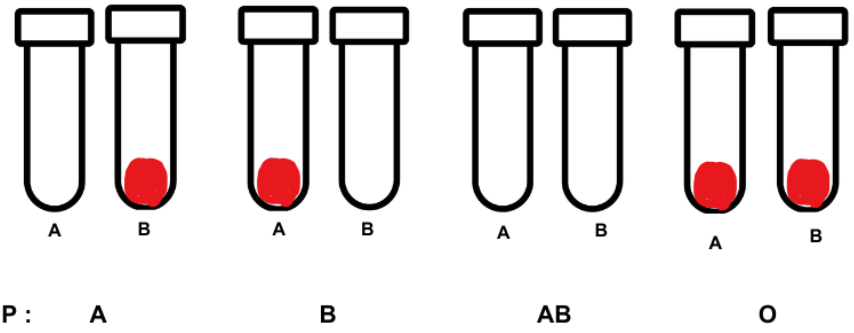


Figure 3.7: Reverse grouping: The agglutination indicates the presence of antibodies for respective antigens. Note: Agglutination in B means blood group A, agglutination in A means blood group B, No agglutination in A and B means blood group AB, and agglutination in both A and B implies blood group O.

3.5.8 Leptin assay

Leptin assay in ng/m lwas measured using the Human Magnetic Luminex® Assays (RandD System, USA). Luminex is applied to xMAP technology principles which is multiplex biological testing (x as biomarker/analyte and MAP as Multi-Analyte Profiling) and used microsphere-based arrays that can detect 5.6 microns biomolecules to give fast liquid phase kinetics, proprietary dyeing

process, unique bead signatures, high surface-to-volume ratio and surface carboxyl groups. These biomolecules involve, antigens, antibodies, modified oligonucleotides, purified proteins or peptides, carbohydrates, and small molecules (cortisol, thyroxine, metabolites). Luminex assays used a bead Protein Capture Sandwich in applying five basic components of the technology that were involved, biological reagents, beads, fluidics, optics, and high-speed digital processing. A liquid suspension array based on flow cytometry with functional sandwich immunoassay as shown in Figures 3.8 and 3.9. A certificate of analysis was provided for the microparticle region which consists of a standard value for leptin.

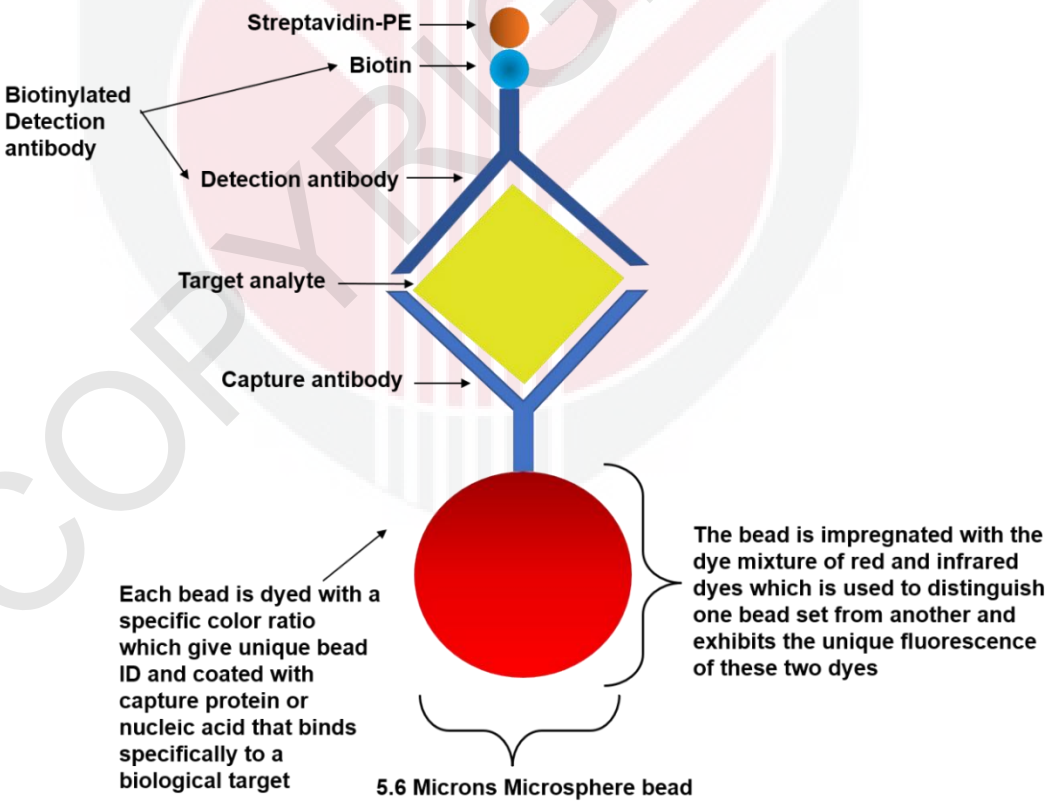


Figure 3.8: Luminex assay was done with functional sandwich immunoassays.

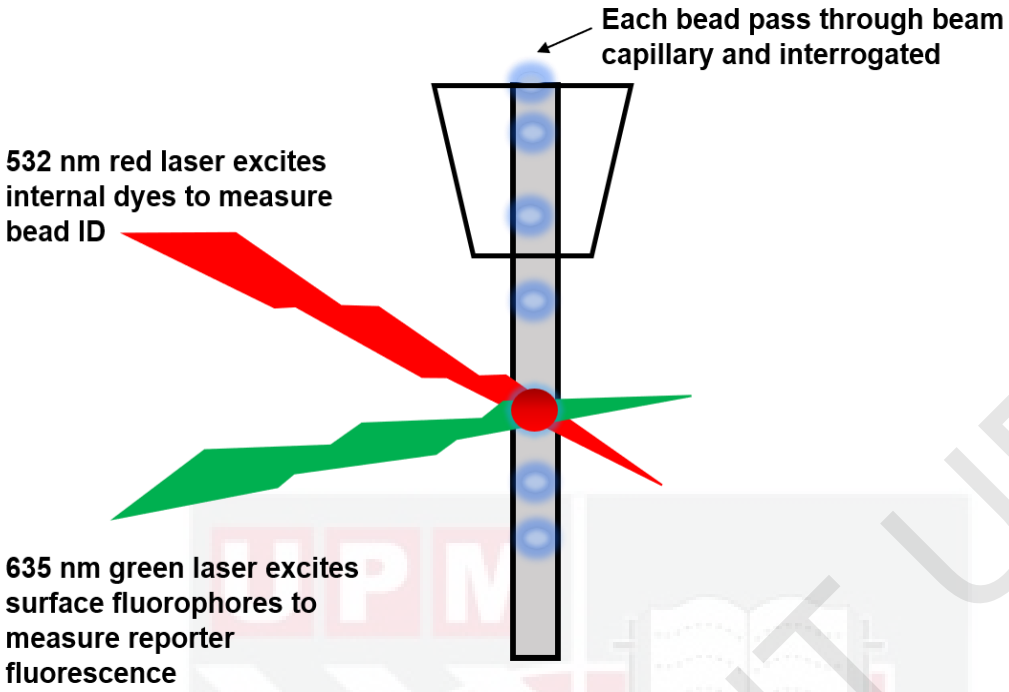


Figure 3.9: Luminex bead passed beam capillary of machine which applied liquid suspension array based on flow cytometry.

3.5.8.1 Sample preparation

The stored plasma was thawed and centrifuged at 16, 000 g for 4 minutes using Sigma 1-14k (Sigma, Germany). Two-fold dilution was done by adding 60 µL of the patient's plasma with 60 µL of Calibrator Diluent RD6-52 and mixing them thoroughly as shown in Figure 3.9. Then , the sample was shaked at 20 g for 5 minutes using a horizontal orbital microplate shaker SK-0180-PRO (DLAB, country).

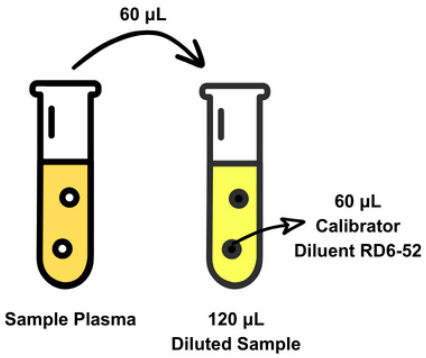


Figure 3.10: Two-fold dilution of the sample. Note: Transferred 60 µL of patient's plasma into 60 µL of Calibrator Diluent RD6-5 and mixed them thoroughly

3.5.8.2 Reagent preparation

500 ml Wash Buffer was prepared by adding 20 ml of Wash Buffer Concentrate to 480 ml of distilled water. Next, reconstitute 225 µL Standard A and 250 µL Standard B with Calibrator Diluent RD6-52. Then, transfer 100 µL each of Standard Cocktail A and Standard Cocktail B into 800 µL Calibrator Diluent RD6-52 in a mixing bottle to create Standard 1 and allow them to sit for 15 minutes as shown in Figure 3.10. To produce a 3-fold dilution series, 200 µL of Calibrator Diluent RD6-52 was pipetted into each of 5 microcentrifuge tubes labeled Standard 2 till 6, then 100 µL Standard 1 was transferred into the Standard 2 tube. Mixed pipetting mixture in Standard 2 tube and transferred 100 µL from Standard 2 tube into the Standard 3 tube. Continued the similar step from the Standard 3 tube till producing the Standard 6 mixture as shown in Figure 3.11.

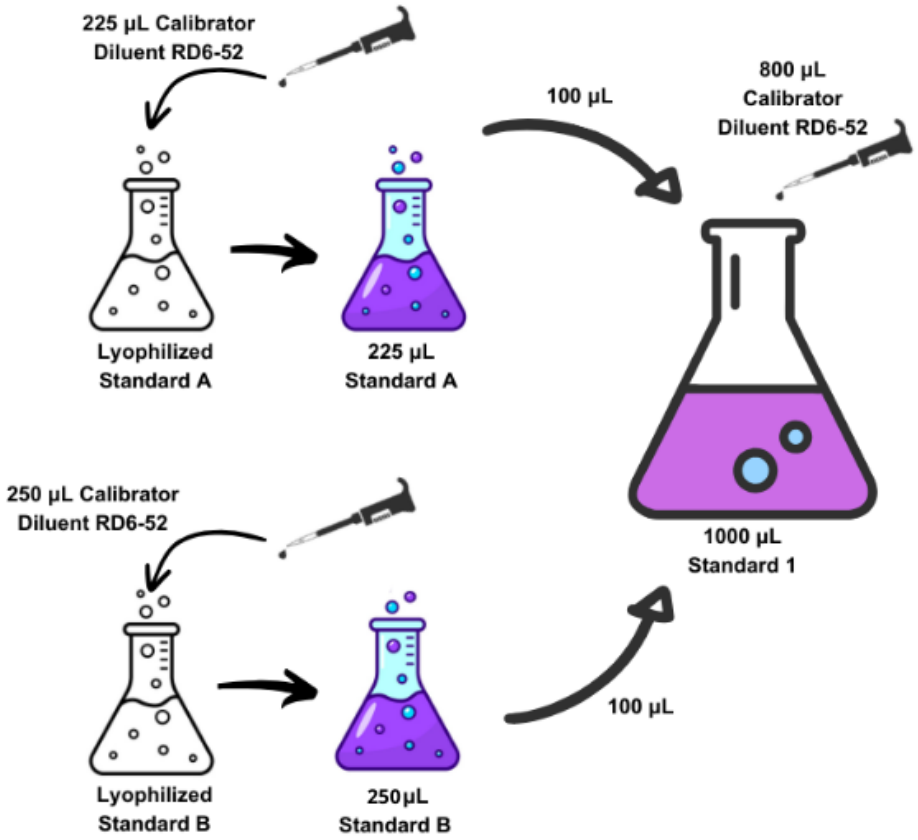


Figure 3.11: Create Standard 1. Note: 100 µL of Standard Cocktail A and Standard Cocktail B was added with 800 µL Calibrator Diluent RD6-52 to create Standard 1.

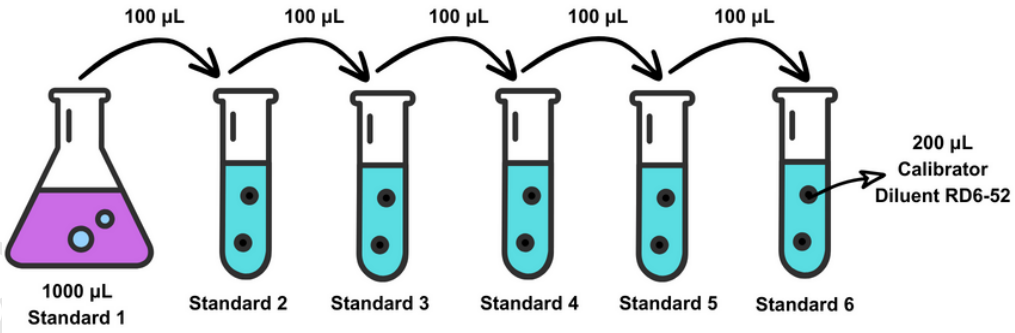


Figure 3.12: Three-fold dilution series. Note: To produce a 3-fold dilution series, 100 µL from the previous tube was transferred into the following tube that contained 200 µL of Calibrator Diluent RD6-52.

3.5.8.3 Diluted Microparticle Cocktail Preparation

Centrifuge microparticle Cocktail vial using Sigma 1-14k (Sigma, Germany) at 1000 g for 30 seconds. Draw 500 µL of it and dilute with 5 ml Diluent RD2-1 into a mixing bottle and vortexed using Vortex Mixers MX-S (DLAB, USA).

3.5.8.4 Diluted Biotin-Antibody Cocktail Preparation

The Biotin-Antibody Cocktail was centrifuged using Sigma 1-14k (Sigma, Germany) at 1000 g for 30 seconds and then 500 µL of it was diluted with 5 ml Diluent RD2-1 into a mixing bottle and vortexed by using Vortex Mixers MX-S (DLAB, USA).

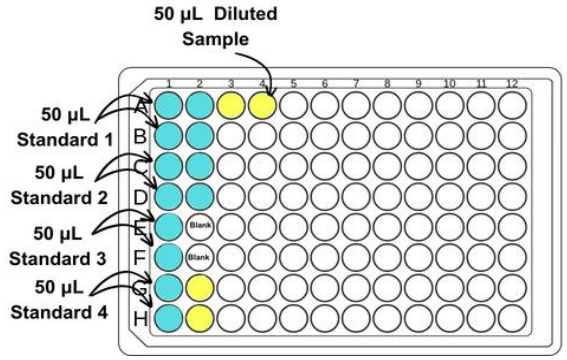
3.5.8.5 Streptavidin-PE Preparation

The Streptavidin-PE vial had a brief centrifuge by using Sigma 1-14k (Sigma, Germany) at 1000 g for 30 seconds, and then 220 µL of it was diluted with 5.35 ml Wash Buffer into a mixing bottle and vortexed by using Vortex Mixers MX-S (DLAB, USA).

3.5.8.6 Assay Procedure

Standards of 50 µl and diluted samples were added into the microplate accordingly. Two wells were skipped before loading diluted samples for spacing them before loading the sample (Step 1). Vortexed dilute Microparticle Cocktail to resuspend it using MX-S Vortex Mixer (DLAB, USA). Then, 50 µl of the Microparticle Cocktail was added to each well of the microplate. The microplate was covered securely with a foil plate sealer and was incubated for

2 hours at room temperature on a horizontal orbital microplate shaker SK-O180-PRO (DLAB, Germany) which was set at 57 g (Step 2). The plate sealer was taken off after completing the incubation and the microplate was placed onto the magnetic washer MS-ELX50/8M (Biotek, USA) for 2 minutes before filling 100 µl Wash Buffer to wash out the well (Step 3). Take out the microplate from the magnetic washer MS-ELX50/8M and 50 µl of diluted Biotin-Antibody Cocktail was added to each well. The microplate was covered securely with a foil plate sealer and was incubated for 1 hour at room temperature on a horizontal orbital microplate shaker SK-O180-PRO which was set at 57 g (Step 4). Then, the same washing step of using the Wash Buffer earlier was repeated (Step 5). After this second wash, 50 µl of diluted Streptavidin-PE was added to each well. The microplate was covered securely with a foil plate sealer and was incubated for 30 minutes at room temperature on a horizontal orbital microplate shaker SK-O180-PRO which was set at 57 g (Step 6). The last washing step by using the Wash Buffer earlier was done after that (Step 7). 100 µl of Wash Buffer was added into each well to resuspend the microparticles and was incubated for 2 minutes on a horizontal orbital microplate shaker SK-O180-PRO which was set at 57 g (Step 8). Lastly, a microplate was placed into the Luminex® 200™ analysers (Luminex Corporation, USA) to analyse the result. A summary of the assay procedure is shown in Figure 3.12 below.



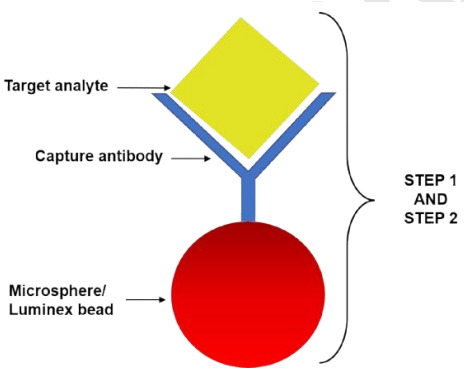
Step 1: Loaded 50 µL of all 6 Standards and diluted samples into the microplate according to its well.

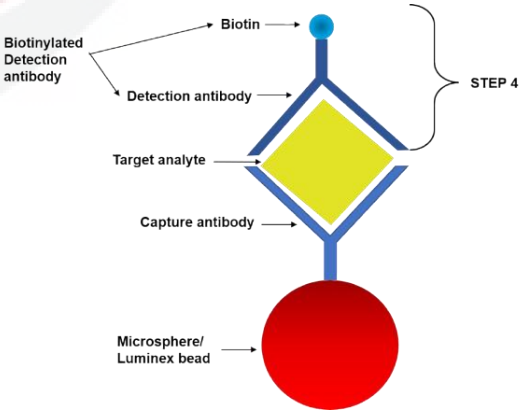
Step 2: Added 50 µL of the Microparticle Cocktail into each well. Covered microplate and incubated for 2 hours at room temperature on a shaker at 57 g.

Step 3: Took off the plate sealer and placed the microplate onto the magnetic washer for 2 minutes. Filled 100 µL Wash Buffer and removed the liquid to wash out the well.

Step 4: Took out the microplate from the magnetic washer and added 50 µL of diluted Biotin-Antibody Cocktail to each well. Covered and incubated for 1 hour at room temperature on a shaker at 57 g.

Step 5: Repeated the wash as in step 3.





Step 6: Added 50 µL of diluted Streptavidin-PE into each well. Covered and incubated for 30 minutes at room temperature on a shaker at 57 g.

↓

Step 7: Repeated the wash as in step 3.

↓

Step 8: Added 100 µL of Wash Buffer to each well and incubated for 2 minutes on shaker at 57 g.

↓

Step 9: Placed the microplate into the Luminex® 200™ analyser to analyse the result.

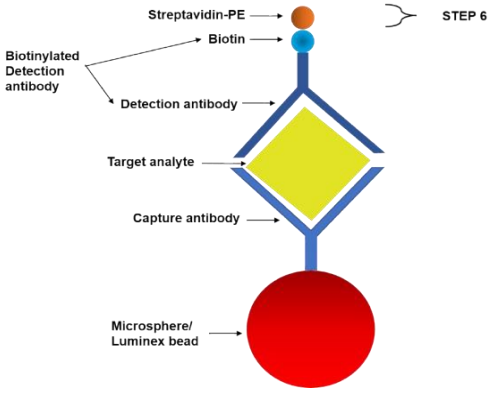


Figure 3.13: Assay summary of the Luminex procedure

3.6 Framingham Risk Score

The FRS (2008) online calculator created by QxMD was applied to calculate patients' risk scores and categorize patients' CVD risk groups. The risk score and categorization of the CVD were calculated using validated algorithms based on parameters of patients' age (30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74 and ≥70 years old), gender (male and female), SBP (<120, 120-129, 130-139, 140-149, 150-159 and ≥160 mmHg), anti-hypertensive medication status (yes and no), TC (<4.14, 4.14-5.15, 5.16-6.19, 6.20-7.24 and ≥7.25 mmol/L), HDL-C (<0.9, 0.9-1.16, 1.17-1.29, 1.30-1.55 and ≥1.56 mmol/L), diabetes status (yes and no), smoking status (yes and no) and history of known vascular disease like CAD, Peripheral Vessel Disease

(PVD) or stroke (yes and no). All parameters required in the FRS calculator we fulfilled. The FRS utilizes these factors to estimate an individual's 10-year risk of experiencing a cardiovascular event. The risk score and groups were divided into three, which were high-risk with a score of more than 20%, moderate-risk with a score of 10-20%, and low-risk with a score lower than 10%. However, patients will be categorized as high-risk if they have diabetes even if the score is not more than 20% as explained by Liew et al. (2018).

3.7 Statistical Analysis

The statistical analysis of the data collected for this study was conducted using IBM SPSS Statistics, version 29.0, from IBM CorporationIBM SPSS Statistics (version 29.0, IBM Corp., Ar-monk, NY, USA) was used to perform statistical analysis. Descriptive data were expressed as frequencies and percentages for categorical variables, mean ± standard deviation (SD) for normally distributed continuous variables, and median with interquartile range (IQR) for non-normally distributed data. Variability in the measurements was captured using both SD and IQR, where larger values indicated greater dispersion. The mean and median were included to represent central tendencies, with the median being particularly suitable for skewed distributions. Statistical significance was established at a p-value threshold of less than 0.05 for all analyses. In addition, potential confounding factors were identified using a multi-step approach to ensure the validity of the statistical analyses. First, an extensive review of the literature was conducted to identify confounding factors that may have influenced the main outcome (FRS) and consultation with clinical experts was sought to ensure that all relevant factors were

considered. These included factors such as age, genders, BMI, smoking status, comorbidities like diabetes, hypertension, hypercholesterolemia and medication used related to these comorbidities. Second, variables with biological plausibility, particularly those known to affect cardiovascular risk or metabolic biomarkers, were prioritized for inclusion such as ABO blood groups and leptin levels.

Analytic analyses, including Kruskal-Wallis, Mann-Whitney, linear regression, and univariate and multiple logistic regression, were performed to explore associations between variables and outcomes. For numerical outcomes, non-parametric tests were utilized due to the non-normal distribution of the leptin level. Specifically, the Kruskal-Wallis test was applied when comparing leptin level across ABO groups (>2 groups), while the Mann-Whitney U test was used for comparing the leptin level between O and non-O blood groups. Linear regression also was conducted to examine the relationship between leptin level with BMI. In the context of categorical outcomes, particularly the FRS categorized into low-risk and moderate-high-risk groups, binary logistic regression was performed. This allowed for the assessment of potential predictors, with results expressed as crude odds ratios (COR) and 95% confidence intervals (CI) in univariate logistic regression (simple binary logistic regression). Each predictor was evaluated individually to identify factors significantly associated with increased cardiovascular risk. The univariate logistic regression was conducted to assess the association between ABO blood groups and FRS for the entire study population. The univariate logistic regression was also conducted to assess the association between leptin and

related factors with FRS for the 150 randomly selected patients. This approach was necessitated by budget constraints limiting the leptin level assay analysis. Thus, leptin and other related factors will be included in a subsequent multiple logistic regression analysis of the binary logistic regression. Finally, the model was constructed using the enter method, incorporating all factors with a p-value less than 0.25 from univariate logistic regression, which assessed the association of leptin and related factors with FRS in 150 randomly selected patients, and further analyzed using multiple logistic regression to adjust for potential confounders. Goodness-of-fit tests, such as Hosmer-Lemeshow, were applied to evaluate the model fit. This approach enabled a comprehensive evaluation of how independent variables influenced the likelihood of moderate-high FRS risk after accounting for other factors. Results were presented as adjusted odds ratios (AOR) with 95% confidence intervals, providing a clearer understanding of the strength and direction of each association.

3.8 Ethical Approval

Institutional ethics board approval was obtained from the Ethic Committee for Research Involving Human Subject (JKEUPM) prior to the commencement of the study. The JKEUPM ID for this study is JKEUPM-2021-700. All procedures followed the Helsinki Declaration of 1975, revised in 2008.

CHAPTER 4

RESULTS

4.1 Distribution of study population

4.1.1 Demographic and clinical data of the study population on Framingham risk score

This study enrolled 352 patients from March 2022 until February 2023, but only 333 patients fulfilled the inclusion and exclusion criteria and proceeded for further analysis which gave the response rate of 94.6%. Those with a diagnosis of any heart disease like having a NYHA classification, incomplete clinical data in BMI, and insufficient samples noticed during laboratory procedures were omitted from the study. Table 4.1 displayed the distribution of demographic and clinical features of patients in total revealing a median (IQR) age of 55 (19) years old, with females comprising (61.6%) and a majority being of Malay ethnicity (88.0%). However, males were the majority with 71.9% and Indians for 66.7% of individuals in the moderate-high risk FRS, despite females and Malays being the predominant groups in overall. This finding may be attributed to the fact that none of the females in the study reported smoking, whereas smoking as a key risk factor, was significantly higher at 85.7% in the moderate-high risk FRS group compared to the low risk group. Besides that, median BMI (IQR) of patients in the low risk FRS group was 26.90 (6.15) kg/m2, indicating an overweight status, while those in the moderate-high risk FRS group had a median BMI of 28.00 (6.20) kg/m2, reflecting an obese status.

Patients diagnosed with diabetes (100%), hypertension (80.2%), and dyslipidaemia (69.8%) were predominantly in the moderate-high risk FRS category. In total distribution, despite their diagnoses, a notable proportion opted for lifestyle modifications over medication, with only 30.6%, 45.0%, and 54.1% receiving treatment for diabetes, hypertension, and dyslipidaemia, respectively. This showed the highest of patients not keen on anti-dyslipidaemia agents and led to the highest percentage of dyslipidaemia (76.6%) in total compared to diabetes (31.8%) and hypertension (48.6%). Besides that, the higher median levels of TC (5.40mmol/L) and LDL-C (3.40mmol/L) observed in the low risk FRS group compared to the moderate-high risk group which may be attributed to the absence of dyslipidaemia status or anti-dyslipidaemia agent usage as parameters in the FRS calculation, potentially influencing this distribution. In total, the key measurement of diabetes, hypertension, and dyslipidaemia can be seen in median (IQR) FBS level which was within an acceptable range, 5.4 (1.55) mmol/L, mean ± SD SBP measurement at 134.67 ± 16.11 mmHg indicated patients at risk of hypertension, meanwhile, median (IQR) TC, TG and LDL-C was 5.33 (1.58), 1.30 (0.83) and 3.29 (1.50) mmol/L respectively shown beyond of cut off values in FLP.

In total distribution, the non-O blood group (60.1%) was higher than the O blood group (39.9%). However, the O blood group (39.9%) was the highest among each blood group, blood group A (20.4%), B (31.2%), and AB (8.4%) with 99.4% were Rh positive. Notably, blood group AB (71.4%) showed the highest and blood group O (58.6%) showed the lowest in moderate-high risk

FRS. Similarly, the non-blood group (64.5%) presented higher compared to the O blood group (58.6%) in the moderate-high risk FRS. Meanwhile, the O blood group showed the highest in low risk of FRS compared to each of the non-O blood groups (blood groups A, B and AB) and non-O blood group. Moreover, leptin immunoassay was conducted on a randomly selected 150 patients due to budget constraints with the median (IQR) leptin level recorded at 17.84 (21.6) ng/ml overall as in Table 4.2. The median (IQR) leptin level presented higher in low risk FRS with 20.25 (20.72) ng/ml than the moderate-high risk FRS with 17.59 (24.72) ng/ml.

Table 4.1: Patients' socio-demographic and clinical data in Framingham risk score status (N = 333)

Factors	Framingham Risk Score		
	Low risk[a] 126 (37.8)	Moderate-High risk[a] 207 (62.2)	Distribution
Gender[a]			
Male	36 (28.1)	92 (71.9)	128 (38.4)
Female	90 (43.9)	115 (56.1)	205 (61.6)
Ethnicity[a]			
Malay	111 (37.9)	182 (62.1)	293 (88.0)
India	7 (33.3)	14 (66.7)	21 (6.3)
Chinese	8 (42.1)	11 (57.9)	19 (5.7)
Smoking[a]	3 (14.3)	18 (85.7)	21 (6.3)
Age, year[b]	45 (15)	60 (14)	55 (19)
HR, bpm[b]	76 (17)	78 (17)	78 (17)
BMI, kg/m^2[b]	26.90 (6.15)	28.00 (6.20)	27.70 (6.50)
Diabetes	0 (0)	106 (100)	106 (31.8)
FBS (mmol/L)[b]	5.07 (0.65)	5.90 (2.25)	5.40 (1.55)
Hypertension[a]	32 (19.8)	130 (80.2)	162 (48.6)
SBP (mmHg)[c]	126.58 ± 14.51	139.59 ± 15.04	134.67 ± 16.11
DBP (mmHg)[c]	75.10 ± 11.49	76.83 ± 11.31	76.17 ± 11.39
Dyslipidemia[a]	77 (30.2)	178 (69.80)	255 (76.60)
TC (mmol/L)[b]	5.4 (1.19)	5.3 (1.80)	5.33 (1.58)
TG (mmol/L)[b]	1.14 (0.70)	1.37 (0.90)	1.30 (0.83)
LDL-C (mmol/L)[b]	3.40 (1.24)	3.10 (1.70)	3.29 (1.50)
HDL-C (mmol/L)[b]	1.40 (0.48)	1.35 (0.50)	1.37 (0.43)
Use of anti-diabetes agent[a]			
No	126 (54.5)	105 (45.5)	231 (69.4)
Yes	0 (0)	102 (100)	102 (30.6)

Table 4.1: Continued

Use of anti-hypertensive agent[a]						
No		98 (53.6)		85 (46.4)		183 (55.0)
Yes		28 (18.7)		122 (81.3)		150 (45.0)
Use of anti-dyslipidaemia agent[a]						
No		94 (61.4)		59 (38.6)		153 (45.9)
Yes		32 (17.8)		148 (82.2)		180 (54.1)
ABO blood group system[a]						
Non-O Blood group	A	71 (35.5)	23 (33.8)	129 (64.5)	45 (66.2)	200 (60.1) 68 (20.4)
	B		40 (38.5)		64 (61.5)	104 (31.2)
	AB		8 (28.6)		20 (71.4)	28 (8.4)
O Blood group		55 (41.4)		78 (58.6)		133 (39.9)
Rh blood group system[a]						
Positive		126 (38.1)		205 (61.9)		331 (99.4)
Negative		0 (0)		2 (100)		2 (0.6)

Distribution presented in [a]: n (%), [b]: median (IQR), [c]: mean ± SD. HR: Heart rate. BMI: Body mass index. FBS: Fasting blood sugar. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. Bpm: beat per minute. TC: Total cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol.

Table 4.2: Distribution of plasma leptin level in Framingham risk score status (N=150)

Frequency[a]	Leptin level (ng/ml)[b]		Distribution[b]
	Low risk	Moderate-High risk	
150 (100)	20.25 (20.72)	17.59 (24.72)	17.84 (21.6)

Distribution presented in [a]: n (%), [b]: median (IQR)

4.2 ABO blood group on Framingham risk score

Table 4.3 explored the association between each ABO blood group and the FRS, categorizing patients into low-risk and moderate-high risk groups based on their FRS. To gain further insight into potential differences, the ABO blood groups were then re-categorized into two broader categories: non-O and O blood groups. This additional step allowed for a more detailed investigation of how these specific blood groupings might influence cardiovascular risk among the sample population.

The probability of having moderate-high FRS for the A, B, and AB blood groups found no significant with COR=0.82 (95%CI: 0.43, 1.55), COR=1.28 (95%CI: 0.48, 3.34) and OR=0.73 (95%CI: 0.39, 1.33) respectively, indicated that individuals with the A, B and AB blood group had probability 18% less likely, 28% more likely and 27% less likely of being in the moderate-high risk group compared to those with an O blood group. However, those associations were not statistically significant with p-values of 0.537, 0.617 and 0.301 for blood groups A, B and AB respectively.

Similarly, the probability of having moderate-high FRS for the non-O blood group found no significance with COR=0.78 (95%CI: 0.50, 1.23), indicating that individuals with a non-O blood group had 22% less likely to be in the moderate-high risk group compared to those with an O blood group. However, this association was not statistically significant with a p-value of 0.281.

Table 4.3: Association between ABO blood group and Framingham risk score using univariate logistic regression (N=333)

Blood Group System		COR	95% CI		p-value
			Lower	Upper	
ABO Blood group	O	Reference			
	A	0.82	0.43	1.55	0.537
	B	1.28	0.48	3.34	0.617
	AB	0.73	0.39	1.33	0.301
ABO Blood group	O	Reference			
	Non-O	0.78	0.50	1.23	0.281

COR: Crude odd ratio. CI: Confidence Interval.

4.3 ABO blood group and body mass index in plasma leptin level

4.3.1 Association of ABO blood group in plasma leptin level

Table 4.4 on distribution and the association of each non-blood group and non-O blood group with O blood group in plasma leptin level among 150 patients showed B and non-O blood group exhibited the highest median (IQR) of 20.61 (24.78) ng/ml and 17.98 (19.6) ng/ml respectively. Analysis across A, B, AB, and O blood groups with plasma leptin levels yielded a non-significant p-value of 0.467, indicating no significant association between each blood group and leptin level. Similarly, the association of the non-O blood group and O blood group with plasma leptin level exhibited a p-value of 0.327.

Table 4.4: Association of each blood group and non-O blood group with O blood group in plasma leptin level among patients (N=150)

Blood Group System		Frequency[a]	Leptin Level (ng/ml)[b]	p-value
ABO Blood group	O	57 (38.0)	17.70 (26.21)	
	A	32 (21.3)	18.63 (15.64)	0.467[d]
	B	45 (30.0)	20.61 (24.78)	
	AB	16 (10.7)	16.11 (27.38)	
ABO Blood group	O	57 (38.0)	17.70 (26.2)	
	Non-O	93 (62.0)	17.98 (19.6)	0.327[e]

Distribution presented in [a]: n (%), [b]: median (IQR). The result was derived using [d]: Kruskal-Wallis, [e]: Mann-Whitney

4.3.2 Correlation of body mass index in plasma leptin level (N=150)

The analysis of BMI distribution at the leptin level unveiled notable associations. Linear regression revealed in Table 4.5 showed a significant positive association between BMI and leptin level, with a regression coefficient of 2.28 (95% CI: 1.74, 2.83), indicating that for every 1 unit increase in BMI, leptin level increased by 2.28 ng/ml. The model explained 31.6% of the

variation in leptin level (R = 0.562), reflecting a moderate correlation between the two variables, with a statistically significant result (p < 0.001).

Table 4.5: Correlation of body mass index on plasma leptin level (N =150)

Factor	R	B	95% CI		p-value
			Lower	Upper	
BMI	0.562	2.28	1.74	2.83	<0.001[f]

R: Correlation, B: Regression, R^2 (Coefficient of determination): 31.6%. The result was derived using [f:] linear regression.

4.4 Demographic and clinical data on Framingham risk score

Univariate logistic regression analysis in Table 4.6 presented several factors as potential confounders tested for association with FRS. Among the factors, the probability of having an outcome of moderate-high FRS was found to be significantly higher for those who used the anti-dyslipidaemia agent with 7.76 times increase in odds compared to those not on medication with COR=7.76 (95% CI: 3.39, 17.75) and p-value of less than 0.001. Similarly, for dyslipidaemia patients with 3.84 times increase in odds compared to those who were not with COR=3.84 (95% CI: 1.79, 8.24) and a p-value of less than 0.001. Besides that, FBS level was found to be significantly associated, with 1.97 times increase in odds for each unit increase in FBS with COR=1.97 (95% CI: 1.35, 2.89) and p-value of less than 0.001. The use of an anti-dyslipidaemia agent and FBS factors were included in multiple logistic regression since they showed significance with p less than 0.05 and TG was also included by applying the “enter” method when the p-value less than 0.250. Dyslipidaemia status was excluded even though it showed significance because patients on anti-dyslipidaemia agents were already identified as having dyslipidaemia. To

prevent overlap, the factor of use of anti-dyslipidaemia agent was selected since not all dyslipidaemia patients opted for such treatment

Table 4.6: Association between potential confounders and Framingham risk score using univariate logistic regression (N=150)

Factors		COR	95% CI		p-value
			Lower	Upper	
Ethnicity	Malay	Reference			
	Indian	0.72	0.19	2.70	0.631
	Chinese	0.85	0.23	3.05	0.797
ABO Blood group	O	Reference			
	A	0.82	0.43	1.55	0.537
	B	1.28	0.49	3.34	0.617
	AB	0.73	0.39	1.33	0.301
ABO Blood group	O	Reference			
	Non-O	1.00	0.50	2.01	1.000
Dyslipidaemia	No	Reference			
	Yes	**3.84**	**1.79**	**8.24**	**<0.001***
On anti-dyslipidaemia agent	No	Reference			
	Yes	**7.76**	**3.39**	**17.75**	**<0.001***
HR		1.00	0.97	1.04	0.819
DBP		0.99	0.96	1.02	0.389
FBS level		**1.97**	**1.35**	**2.89**	**<0.001***
TG level		1.65	0.94	2.90	0.080
LDL-C level		0.90	0.66	1.23	0.514
BMI level		1.43	0.94	1.09	0.755
Leptin level		1.00	1.00	1.00	0.752

COR: Crude odd ratio, CI: Confidence Interval. HR: Heart rate, DBP: Diastolic blood pressure, FBS: Fasting blood sugar, TG: Triglyceride, LDL-C: Low-density lipoprotein cholesterol, BMI: Body mass index.

4.5 Potential confounders associated with Framingham risk score

After adjusting demographic and clinical parameters, Table 4.7 showed the factors association of potential confounders on FRS among 150 patients by a multiple logistic regression model. Similarly in the univariate logistic regression model, the odds of having an outcome of moderate-high risk FRS were found to be significantly higher for those who used the anti-dyslipidaemia had 6.44 times increase in odds compared to those not on medication compared with

AOR=6.44 (95% CI: 2.72, 15.26) and a p-value of less than 0.001. And, the FBS level was significantly 1.75 times to ha ve moderate-high risk FRS for each unit increase in FBS level with AOR=1.75 (95% CI: 1.18, 2.62) and a p-value of 0.006. The TG level did not show a statistically significant relationship with the moderate-high risk of FRS with AOR=1.37 (95% CI: 0.32, 1.3) and a p-value of 0.324.

Table 4.7: Association between potential predictors on Framingham risk score using multiple logistic regression (N=150)

Factors		AOR	95% CI		p-value
			Lower	Upper	
On anti-dyslipidaemia agent	No	Reference			
	Yes	6.44	2.72	15.26	**<0.001***
FBS level		1.75	1.75	2.62	**0.006***
TG level		1.37	0.32	1.37	0.324

AOR: Adjusted odd ratio. FBS: Fasting blood sugar, TG: Triglyceride.

CHAPTER 5

DISCUSSION

CVD has been reported as the leading cause of mortality worldwide (Prabhakaran et al., 2023). In this study, the ABO blood group and plasma leptin level were analysed with the FRS group, followed by BMI and other confounders to identify potential CVD risk.

5.1 Samples collection

The sample collection process was time-consuming and took longer than expected due to the meticulous procedures involved. Only 333 samples were gathered from April 2022 to February 2023. The reduction in sample size from 428 to 333 was because the data was sourced only from one study site, namely Hospital Sultan Abdul Aziz Shah (HSAAS). The inclusion of the second study site—Klinik Kesihatan (KK) Puchong—was necessary to cover the Petaling District area as approved by the FRGS (research grant provider). However, securing ethical approval from KK Puchong was indeed a challenging journey that required careful navigation of regulatory processes and considerations. Thus, the recommended sample size was not achieved due to the delayed start of new data collection in KK Puchong.

Another issue concerns the patients' inclusion criteria. While a total of 352 patients enrolled in HSAAS between April 2022 until February 2023, only 333 patients fulfilled the inclusion criteria for further analysis. Incomplete clinical data, such as BMI and insufficient samples noticed during laboratory

procedures, were omitted from the study. A number of patients were unaware of their heart condition; however, the data collected from the clinic system indicated that they were diagnosed with heart-related diseases, such as NYHA classification, abnormal heart rhythm, and cardiomyopathy. Such an issue was also omitted from the data analysis. The reduced number of actual samples than the recommended sample size thus highlights the need for expanded collaboration to ensure sufficient sample collection in future research.

5.2 Study Population Distribution

Table 4.1 shows the study population distribution across low and moderate-high risk of FRS, which is similar to the study done in Singapore by Yeo et al. (2024). Demographically, the Family Medicine Specialist Clinic (FMSC) in Hospital Sultan Abdul Aziz Shah (HSAAS) seems to have more Malay, female, and middle-aged adult patients attending follow-up assessments. This aligns with the data from the Department of Statistics Malaysia (2023) where Malay forms the majority of population in the Petaling District compared to other ethnicities, with many belonging to the middle-age range. However, the overall distribution of this study had more females than males, which could be attributed to the fact that females were keen on extra blood-taking during their blood-drawing sessions. This aligns with Lim et al. (2019) who found that females were more perceiving in healthcare-seeking behaviour. On the other hand, males were more prevalent in the moderate-high risk FRS group. This can be attributed to the fact that no female respondents in this study admitted of smoking as it is a significant risk factor of CVD, which is considerably higher in the moderate-high risk FRS group than the low-risk group. The finding is

consistent with Ponjoan et al. (2024), Adil et al. (2023), Azahar et al. (2022), and Razman et al. (2022) who reported a significant association between smoking and FRS, highlighting that smoking was categorised under high-risk FRS with males demonstrating a higher prevalence of smoking habits compared to females.

This study also found that Malay had the highest risk of FRS, which agrees with the findings by Liew et al. (2018). Nevertheless, India was the highest in moderate-high risk owing to the highest percentage of diabetes, hypertension, and dyslipidaemia. The metabolic syndrome of diabetes, hypertension, and dyslipidaemia was central to many FRS studies as their scoring incorporates key related risk factors (Adil et al., 2023). Older age, high SBP, FBS, TC, TG, LDL-C, and low HDL-C were predominant in moderate-high risk FRS, which is similar to those reported by Dehghan et al. (2024) and Ponjoan et al. (2024). However, the evaluation of HR did not examine both Dehghan et al. (2024) and Ponjoan et al. (2024), which was contrary to this study. In this study, HR was included to evaluate its potential as a predictor of CVD risk factors, particularly among older age. This aligns with a study by Wang et al. (2024), which incorporated HR in the risk factor assessment to evaluate the older age in the population. Kaneko et al. (2022) and Nedkoff et al. (2023) emphasised that population ageing significantly contributed to CVD, while Huo et al. (2018) and Rawshani et al. (2018) identified age-dependent associations between risk factors and CVD. Thus, higher HR was present in moderate-high FRS as it played an important role in CVD risk. This aligns with Tian et al. (2024) concerning non-fatal and fatal CVD events, including valvular heart disease,

myocardial disease, arrhythmia, cerebrovascular disease, and PVD.

Furthermore, the evaluation of DBP in this study agrees with Dehghan et al. (2024) but not Ponjoan et al. (2024). This was due to the difference in population whereby the former focused on metabolic syndrome respondents with 10-year CVD risk, which is similar to the non-metabolic and metabolic syndrome patients in this study and produced advocating findings in that DBP was higher in moderate-high risk. However, the research population in Ponjoan et al. (2024) differed from this study as it focused on individuals with Alzheimer's disease and included an FRS assessment. This study also found that the highest recorded BMI fell within the obesity range, which is consistent with MOH's (2023) announcement of BMI as a prevailing health concern. Similarly, Ponjoan et al. (2024) evaluated BMI with FRS and presented higher BMI in moderate-high FRS; in contrast, Dehghan et al. (2024) evaluated WC with FRS rather than BMI.

NHAM (2023) highlighted that atherogenic dyslipidaemia is often linked to obesity, in which both obesity and dyslipidaemia were predominant in this study. In contrast, dyslipidaemia and hypertension were reported as the least and most prevalent NCD NHAM (2023), while this study found diabetes to have the lowest frequency. The difference in sample size might reflect the different NCD distribution in the study population. Moreover, a significant number of patients diagnosed with dyslipidaemia were hesitant to initiate medication, which could explain the elevated TC and LDL-C levels observed in individuals classified as low risk of FRS. This was likely due to the fact that

FRS did not account for dyslipidaemia status or the use of lipid-lowering medications. Similarly, previous studies have reported high lipid profiles in individuals with low risk of FRS (Diputra et al., 2022; Jahangiry et al., 2017). Whereas, studies on the Malaysian population (e.g., Liew et al., 2018; Chia et al., 2015) found that FRS provides a fairly accurate prediction of CVD risk.

To date, approximately 400 blood group systems have been identified, with the ABO and Rh systems being the most prominent as stated by Sabir et al. (2021). It seemed that the variation in sample size of different study locations did not happen in this study for the ABO blood group distribution. Such findings align with those reported by previous studies in Malaysia, Asia, and other countries whereby the O blood group was the highest and the AB blood group was the lowest in each blood group reported by V Vaghela and V Shah (2022), Browne et al. (2021), Sabir et al. (2021), Hajar et al. (2020), Tiruneh et al. (2020), Canizalez-Román et al. (2018), and Jahanpour et al. (2017). Across the non-O blood groups, several studies found that the A blood group was more prevalent than the B blood group in Sun et al. (2022), Browne et al. (2021), Sabir et al. (2021), and Poetsch et al. (2020) studies, while others reported that the B blood group was higher than the A blood group (Browne et al., 2021; Sabir et al., 2021). Cumulatively, the non-O blood group was more prevalent than the O group. This aligns with the review by Groot et al. (2020) whereby most studies focused on comparing the O and non-O blood groups and found that each non-O blood group had similarities in worse cardiovascular health and reduced longevity compared to the O blood group. In agreement with the findings, this study demonstrated that the non-O blood

group and each of the non-O blood groups presented a higher percentage in moderate-high risk of FRS. This is because the plasma vWF level of individuals with the O blood group is approximately 20 to 30% lower than those with non-O blood groups, resulting in reduced thrombotic risk (Ward et al., 2020). Cheung et al. (2018) also highlighted that non-O blood groups are associated with higher levels of vWF and FVIII, with a graded increase in concentrations from blood group O to AB, reflecting their impact on thrombosis risk. While Parente et al. (2020) attributed these differences to ABO glycosyltransferase activity influencing vWF clearance, Cheung et al. (2018) further noted that reduced vWF clearance in non-O blood groups elevates plasma levels, thus increasing the risk of thrombogenic events and CVD. Additionally, all these studies demonstrated a higher prevalence of Rh-positive compared to Rh-negative individuals, aligning with the findings of this study. Variations in observed trends can be attributed to differences in sample size and population characteristics as blood group distributions often vary with ethnic diversity within populations. These contrasting findings likely indicate a complex interaction among geographical location, genetic factors, environmental influences, lifestyle choices, dietary habits, and psychosocial stress, all of which contribute to the development of CVD (Katna et al., 2020).

Despite the strong correlation between leptin and obesity (Vilariño-García et al., 2024; Rangareddy et al., 2023; Krupa-Kotara & Dakowska, 2021) and obesity being the most prevalent condition in the total distribution of this study's population, lower leptin level was observed in Table 4.2 and fell within the low range as defined by Qunq Y A, and Abdel Hamid A Z. (2012) for the

Malaysian population. However, lower leptin level was presented in the moderate-high risk of FRS. It was due to the random selection of 150 patients for leptin immunoassay analysis in each FRS group where only 50 patients were selected for each FRS group. This resulted in a higher proportion of non-diabetic patients being included even in moderate-high risk, particularly as diabetes patients had the lowest frequency compared to hypertension and dyslipidaemia across the 150 patients. Hence, the odds of having lower leptin in moderate-high risk FRS were high as the status of diabetes patients was minimal. This is supported by Al-Shamsi et al. (2019) whereby assessing total cardiovascular risk is straightforward in certain patient subgroups, like those with prior CVD or diabetes where the risk was high. It subsequently suggests that a higher number of non-diabetes patients will result in the higher potential of patients to be at a low risk of FRS. Similarly, the study by Liew et al. (2018) in Malaysia showed that the number of high-risk individuals increased from 46.4% to 70.9% when diabetes was considered high-risk. In this study, leptin level led to a low risk of FRS as leptin was significantly associated with insulin resistance and diabetes (Vilariño-García et al., 2024). This is supported by Wu et al. (2023) who found a positive association in heart disease among diabetes participants. Vilariño-García et al. (2024) further explained that leptin regulates proinflammatory cytokines, which are closely linked to the development of insulin resistance and diabetes as cardiovascular issues. Studies by Zhao et al. (2021), Kang et al. (2020), and Poetsch et al. (2020) also highlighted the association between leptin and cardiovascular events, and hyperleptinemia is connected to the occurrence and seriousness of CHD and heart failure (HF). Additionally, leptin secretion from EAT is related to heart abnormalities, driven

by its links to oxidative injury, inflammation, and mitochondrial dysfunction (Chen et al., 2023).

5.3 ABO blood group with Framingham risk score

Past studies have demonstrated an association between the ABO blood group in CVD, with non-O blood groups being linked to higher risks of venous and arterial thrombosis, ischemic CVD, and cardiovascular complications (Neshat et al., 2024; Parente et al., 2020; Paquette et al., 2018; BA et al., 2017; Chen et al., 2016; Capuzzo et al., 2016). Parente et al. (2020) concluded that the blood group associated with the highest cardiovascular risk varies across different populations and the O blood group is generally regarded as the reference group with the lowest risk of ischemic CVD. Similarly, BA et al. (2017) reported that individuals with non-O blood groups have a higher risk of venous and arterial thrombosis as well as an increased likelihood of CVD and related complications, compared to those with the O blood group. Capuzzo et al. (2016) found that non-O blood groups are associated with nearly a threefold higher risk of subclinical or clinical cardiovascular events compared to individuals with the O blood group. Table 4.1 revealed intriguing patterns regarding the distribution of cardiovascular risk across different blood groups as assessed by the FRS groups. It denotes that the non-O blood groups (i.e., A, B, and AB) had a higher percentage in the moderate-high risk of FRS than the O blood group. However, Table 4.3 showed that the association between blood groups and FRS did not reach statistical significance, hinting a potential link between blood groups and cardiovascular risk. Although no statistically significant association was found, a higher risk of CVD among the non-O blood

group was still in agreement with previous findings whereby the ABO blood group was associated with CVD risk development. These findings imply that other parameters, such as hormonal changes and genetic predisposition, can play a more critical role in determining cardiovascular risk.

Even though, the relationship between the ABO blood group and CVD has been acknowledged for decades and can indirectly influence FRS, such findings have not been consistently replicated across different populations and research designs. For instance, Amrani et al. (2024) and Katna et al. (2020) reported no significant differences in cardiovascular risk factors among the ABO blood group, while Yaghooti-Khorasani et al. (2020) concluded that variations among the ABO blood group are more likely a consequence rather than a cause of CVD risk. To date, no research has directly investigated the association between the ABO blood group and FRS as the majority of past studies only assessed the relationship between the ABO blood group and CVD risk factors or CVD risk. This makes the present study a novel and ground-breaking exploration that delves into the potential impact of the ABO blood group on FRS, shedding light on its role in cardiovascular risk assessment, which has been established several decades ago (Musunuru et al., 2020). This study explored the association between the ABO blood group with FRS, effectively assessing the role of the ABO blood group in CVD risk following the widespread use of FRS as a CVD risk assessment tool. As highlighted by Mickelsson et al. (2024), the ABO blood group is inherited, which are unchanging traits that serve as potential risk markers.

5.4 ABO blood group and body mass index in plasma leptin level

5.4.1 ABO blood group in plasma leptin level

This research revealed a scarcity of studies exploring the connection between ABO blood group and leptin level (see Table 4.4). Karagoz et al. (2022) highlighted the novelty in investigating the relationship between anorexigenic hormones like leptin and the ABO blood group, with their investigation among the Turkey population found that the A blood group had high leptin level. Similar research was conducted by Qunq Y A, and Abdel Hamid A Z. (2012) among the Malaysian populace, highlighting a trend whereby individuals with B and AB blood groups exhibit elevated levels of leptin that surpass 20 ng/ml. The seminal works by Qunq Y A, and Abdel Hamid A Z. (2012) and Karagoz et al. (2022) underscore elevated leptin levels among non-O blood groups, hinting a potential correlation between hyperleptinemia and obesity. Furthermore, the relationship between leptin and ABO blood group also suggests a possible link between BMI and ABO blood group. Studies by Amrani et al. (2024), Mukhtar and Abdullahi (2022), Katna et al. (2020), and Ahmed et al. (2019) reported higher BMI levels in non-O blood groups compared to O blood group but were unable to establish a significant association between ABO blood group and obesity prevalence. This shows that an exploration of the association between the ABO blood group and leptin level can offer insights into the genetic underpinnings of obesity. It aligns with this study whereby from the 150 patients in each blood group who were included in the plasma leptin analysis, those with the B blood group demonstrated the highest level of leptin despite the O blood group being the

most. The comparison between non-O and O blood groups also showed that the former had higher leptin level; however, no statistically significant differences in plasma leptin level were observed across these associations. This indicates that blood group status may not directly impact leptin secretion in this cohort. The associations observed in this study may not be directly caused by the ABO antigens but rather resulted from the influence of genes located close to the ABO gene. It is crucial to note that the findings call for further investigation into the underlying mechanisms and clinical implications of these relationships as the connections may not solely hinge on molecular genetic mechanisms. Hence, further exploration with larger sample sizes is imperative to corroborate or disprove these findings definitively. Since the novelty of this study is examining fluctuations in anorexigenic hormone levels based on the ABO blood group, it holds promises for comparison with forthcoming research findings.

5.4.2 Body mass index in plasma leptin level

Since its discovery, leptin's role in the pathophysiology of obesity has been extensively studied, with past research (e.g., Battineni et al., 2021) highlighting its strong association with conditions like obesity, diabetes, insulin resistance, and CVD. The findings by Mohanraj et al. (2022) suggest that overweight and obese Malaysians are in the early stages of leptin resistance, while Rangareddy et al. (2023) reported that leptin resistance, commonly observed in obesity, reduces the hormone's effectiveness due to factors such as inflammation and changes in leptin receptors. According to Battineni et al. (2021), obesity can lead to leptin resistance where the body becomes less

responsive to leptin's appetite-suppressing effects, leading to a weight gain cycle and further increasing cardiovascular risk. In this study, the analysis of BMI distribution to leptin level unveiled a notable connection. The investigation of 150 patients' BMI distribution with normal to obese BMI in Table 4.5 concerning leptin level revealed significant associations reflective of adipose tissue metabolism. A positive correlation was observed between BMI and leptin level, indicating that leptin level increases in parallel with the rise in BMI. This suggests that lower BMI correlates with lower leptin level, which is consistent with the findings by Mohanraj et al. (2020) and Zulfania et al. (2020). Moreover, Vilariño-García et al. (2024) and Obradovic et al. (2021) stated that leptin level is widely recognised to be elevated in obese individuals and serves as a pivotal element in the onset of obesity. Such results highlight the prominent relationship between BMI and leptin level, highlighting the role of adipose tissue in regulating leptin secretion.

In cases of obesity, the level of leptin in the body tends to be high, especially when it is associated with excess body fat. This is because fat cells produce leptin, and as fat mass increases, so does leptin production (Rangareddy et al., 2023). However, despite having high level of leptin, obese individuals may develop a condition called leptin resistance. This occurs when the brain does not respond adequately to the signals of leptin, leading to reduced sensitivity to its effects. As a result, even though leptin level is high, the brain perceives it as insufficient, leading to continued feelings of hunger and decreased energy expenditure. This can contribute to further weight gain and exacerbate obesity-related health issues, including CVD (D'Marco et al., 2020). These findings

contribute to an understanding of the adiposity process and may impose implications for interventions targeting obesity-associated health outcomes. Thus, this research aims to elucidate the genetic factors (e.g., obesity) that contribute to hyperleptinemia. Further investigation may explore the underlying mechanisms driving these conditions.

5.5 Demographic and clinical data on Framingham risk score

5.5.1 Potential confounders and Framingham risk score by univariate logistic regression

This study also analysed several potential confounders that could play a significant role in predicting CVD by FRS, namely ethnicity, ABO blood group, use of anti-dyslipidaemia agents, dyslipidaemia status, HR, DBP, FBS, TG, LDL-C, BMI, and leptin level. These factors were not included in the FRS calculation to avoid potential invalidity in the analysis as the study aims to evaluate their influence on FRS without reanalysing factors that were already incorporated into the calculation. Kasim et al. (2023) stated that Malaysia is home to a diverse ethnic population with roots in Indochina, South Asia, and East Asia. Their study found that FRS showed strong discrimination in predicting CVD risk among Malaysians but demonstrated poor calibration, with predicted risk levels not aligning with observed outcomes. Therefore, the present study included ethnicity, particularly since FRS is widely utilised across many Asian countries (Zhang et al., 2022) and is validated in Malaysia (Chia et al., 2015). This aligns with the study by Liew et al. (2018), which reported a reference for Malay and insignificant results for India and Chinese.

Furthermore, the ABO blood group demonstrated consistently insignificant outcomes across the 150 patients with 50 patients in each FRS. Ward et al. (2020) emphasised that the biological mechanisms, through which ABO affects physiological haemostasis and pathological thrombosis, have been poorly understood until recent years. This explains why the ABO blood group is a risk factor for CVD and remains under investigation (Parente et al., 2020). Although previous studies reported no direct association between ABO blood group on FRS, Al-Taie et al. (2020) and Parente et al. (2020) concluded that genetic studies have provided potential backing for the correlation between ABO blood group phenotypes and cardiovascular risk. For instance, studies by Neshat et al. (2024) and Parente et al. (2020) have linked non-O blood groups to a higher risk of CVD. This underscores the potential influence of blood groups on the risk of CVD and highlights the importance for further investigation into the underlying mechanisms driving these associations. Thus, an in-depth exploration into the relationship between ABO blood group and CVD could aid in developing targeted strategies for CVD prevention.

In this study, both dyslipidaemia and anti-dyslipidaemia agent status were found to have a statistically significant influence over FRS. Dyslipidaemia patients had an increased risk of moderate-high FRS, which is similar to the findings by Jiang et al. (2022). However, Chiveto et al. (2024) and Kim and Jung (2022) reported that most individuals with dyslipidaemia had a significantly higher prevalence of diabetes, a condition directly classified as high-risk in FRS. This is because Wojtasińska et al. (2023) identified diabetes as a catalyst for atherosclerosis, driven by dyslipidaemia, hyperglycaemia,

oxidative stress, and chronic inflammation, thus linking their pathogenesis. Bi et al. (2022) conducted a simulation study involving more than 2 million participants nationwide and found that lipid-lowering therapy is a key strategy for reducing the burden of CVD, thus showing the significance of FRS. This is supported by Baharudin et al. (2022) who reported prominent cases of FRS among Malaysians with elevated lipid profiles than those not on lipid-lowering medication.

Razman et al. (2022) reported that Malaysians who were not on anti-dyslipidaemia agent were at high risk of FRS. In contrast, this study found that those who adhered to anti-dyslipidaemia agent had an increased possibility of moderate-high risk of FRS. This indicates that the use of anti-dyslipidaemia agent is a vital factor to be included in FRS. Furthermore, Mancini et al. (2024) concluded that risk algorithms vary widely in estimating risk, recommending statin use, and incorporating ancillary testing, thus underscoring the limitations of current risk-based approaches for lipid-specific prevention. However, the current FRS did not include the use of anti-dyslipidaemia agent in the calculation parameter, which could lead to underestimation. This is because the hypercholesterolemia status was only reflected on the level, leading to low perceived CVD risk due to the treatment availability options and behavioural lifestyle modifications (Liew et al., 2018).

This study also found that HR was insignificant with FRS. While the finding is similar to that of Huang et al. (2023), their study investigated the association between mean HR with CVD status. However, the study by Marinello et al.

(2024) demonstrated that HR showed an indirect association with FRS as it had a significant age acceleration factor that significantly impacted FRS. Azahar et al. (2022) conducted a study among Malaysians and found that DBP, FBS, TG, and LDL-C had a significant association with FRS. Both FBS and TG also had a significant relationship with FRS (Adil et al., 2023) whereas both variables were found significant with only FBS in this study. It agrees with Azahar et al. (2022) who reported that high FBS is related to diabetes status, which had a high risk in FRS, with a significant increase in DBP, FBS, TG, and LDL-C led to higher risk of FRS. These findings contradict those reported by this study whereby no significant increase in values was observed in the increase of FRS. Such difference in findings might be due to the use of different analysis methods. Similar outcome was presented by Liew et al. (2018) who reported insignificant influence of DBP on FRS among Malaysians, where logistic regression was used as the analysis method.

The significant relationship between obesity status and FRS reported by Jiang et al. (2022) aligns with those of Battineni et al. (2021) whereby overweight and obesity are linked to a higher risk of CVD. However, this study found that BMI had an insignificant link with FRS due to low and moderate-high risks having almost similar BMI values in the obese range. Similarly, Adil et al. (2023) reported an insignificant connection between BMI with FRS as all FRS groups had similar BMI range values of obese value. A study by Kim and Jung (2022) also revealed similar findings whereby both moderate and high risk of FRS had similar BMI values of the overweight range. Therefore, the insignificant differences in the BMI values in FRS risks might lead to

insignificant findings. Innovative adiposity indicators are needed as they can provide valuable insights for assessing CVD risk.

Rama Krishna Reddy et al. (2015) reported a significant correlation between FRS and biomarkers, highlighting leptin as a key predictor for CVD risk. However, their study focused on 30-year rather than 10-year FRS assessments. Poetsch et al. (2020) and Katsiki et al. (2018) suggested that the association between leptin level and CVD incidence remains significant even after adjusting for BMI, indicating that leptin may directly impact cardiovascular health beyond its connection to adiposity. Moreover, hyperleptinemia, commonly seen in individuals with increased adipose tissue and higher BMI, has been associated with CVD outcomes (Krupa-Kotara & Dakowska, 2021). This shows that integrating leptin as a biomarker alongside FRS holds substantial promise in enhancing the prediction of CVD risk. However, despite the positive correlation between BMI and leptin, leptin similar to BMI denoted an insignificant association with FRS in this study. These data suggest that the influences of leptin on the pathophysiology of CVD risk are complex. The lack of statistical significance indicates that other factors may contribute to this relationship or that the sample size may not be sufficient to detect a significant difference. Thus, a prevention strategy could focus on addressing multiple contributing factors to CVD risk beyond leptin alone, emphasising a comprehensive approach that includes lifestyle modifications such as maintaining a healthy diet, regular physical activity, and managing inflammation and metabolic health.

5.5.2 Potential confounders associated with Framingham risk score by multiple logistic regression

After adjusting for demographic and clinical parameters, the analysis identified significant associations between potential confounders and FRS among 150 patients using a multiple logistic regression model. The univariate and multiple logistic regression models consistently showed that the anti-dyslipidaemia agent was associated with FRS and TG remained insignificant. Similarly, a study by Jahangiry et al. (2017) reported that TG was not significant to FRS. However, studies conducted in Malaysia (Azahar et al., 2022) and other countries (Dehghan et al., 2024; Farhood et al., 2023) presented significant medication adherence and TG levels on FRS. This highlights the difference in significance associations as the previous studies assessed untreated individuals and excluded those who were on lipid-lowering medication, whereas this study evaluated patients who were already undergoing treatment. This could have impacted the significant difference.

Concerning the lipid-lowering medication status, the findings of this study suggest that patients on anti-dyslipidaemia agents were more likely to have moderate-high FRS. This association underscores a limitation of the original FRS, which does not account for treated dyslipidaemia or the use of lipid-lowering medications. Patients prescribed with anti-dyslipidaemia agents typically have a prior diagnosis of dyslipidaemia, which is a recognised CVD risk factor. While these medications help manage dyslipidaemia, the underlying risk factor necessitating treatment may still contribute significantly to CVD risk. This is supported by Baharudin et al. (2022) who stated that CVD risk factors and global cardiovascular risk scores were positively correlated

with lipid-lowering medications. Thus, further research is necessary to verify the accuracy of FRS in predicting cardiovascular outcomes for assessing lipid-lowering therapies. This is supported by a review done by Hermansson and Kahan (2017), which concluded that future studies modelling the effects of lipid-modifying therapies (such as medications or treatments that alter cholesterol levels) would benefit from testing and validating the ability of the FRS to accurately predict cardiovascular outcomes.

Furthermore, the significant association between FBS and FRS agreed with others emphasising impaired glucose metabolism and cardiovascular outcomes. FBS was presented as significant in FRS among Malaysians (Azahar et al., 2022; Ahmad et al., 2020) and those in other countries (Dehghan et al., 2024; Adil et al., 2023; Jahangiry et al., 2017). Such results are similar to the current study, which showed that higher FBS led to higher CVD risk. The univariate and multiple logistic regression models consistently demonstrated that FBS was associated with FRS. This is supported by Dehghan et al. (2024) whereby high FBS was seen in individuals with metabolic syndrome, who also had significant of FBS in the 10-year CVD risk groups. Adil et al. (2023) also reported that elevated FBS increases the 10-year CVD risk as assessed by FRS, suggesting that this factor had a significant predictor of cardiovascular risk and should be carefully monitored and managed in individuals with metabolic syndrome to reduce the likelihood of future CVD events. They also emphasised the importance of early identification and intervention in individuals with metabolic syndrome to prevent the onset of CVD. This association underscores a limitation of the

original FRS, which does not account for FBS values.

Despite their significant association, excluding anti-dyslipidaemia agent status and FBS as parameters in FRS suggests a potential underestimation of FRS. As reported in a review study by Hermansson and Kahan (2017), FRS tends to underestimate the true CVD risk in patients. Grand et al. (2022), Barton et al. (2021), and Hermansson and Kahan (2017) further stated that FRS has been found to underestimate cardiovascular risks, including in the Malaysian population. Similar findings were reported by Liew et al. (2018) who highlighted its tendency to misjudge risk levels. Barton et al. (2021) also found an underestimation of FRS while Liew et al. (2018) concluded that most consultations between doctors and patients are based on poor cardiovascular risk assessments.

5.6 Limitation of research

The cross-sectional design of this study limits its ability to establish causality, allowing only an assessment of associations between cardiovascular risk factors and the 10-year risk of future cardiovascular events. The causal relationships and hazard ratios for each risk factor remain unknown, warranting future prospective studies. Longitudinal or prospective studies are required to confirm causal pathways and better understand the temporal relationships between cardiovascular risk factors and outcomes. This study had certain limitations that warrant acknowledgement. The most significant limitation was the budget constraints, which led to the incomplete analysis of all participants who had initially participated in the study. This restricted the

ability to obtain comprehensive data and might have affected the overall findings and conclusions. Additionally, the study was geographically limited to Serdang, Malaysia and did not include participants from other provinces. The lack of broader geographic representation could result in findings that may not be generalisable to the entire Malaysian population. This is because cardiovascular risk factors can vary across regions due to differences in genetics, lifestyle, and environmental factors, and the results may provide an incomplete picture of the entire situation.

The potential role of leptin in cardiovascular risk is another area of limitation. While leptin activates endothelial cells and induces smooth muscle cell proliferation with its receptors expressed in atherosclerotic plaques, the study did not explore leptin resistance or its implications for metabolic dysregulation. Future studies should delve into the complex interplay between leptin, metabolic health, and cardiovascular risk. Similarly, the potential association between ABO blood group and cardiovascular risk, as measured by FRS, was not fully elucidated. While the ABO blood group has been implicated in various cardiovascular outcomes, the underlying mechanisms remain unclear. This study did not investigate the biological pathways or genetic predispositions that might link the ABO blood group to cardiovascular risk factors, such as coagulation profiles or inflammatory markers. Future research should explore these associations in depth to provide a clearer understanding of how the ABO blood group may contribute to cardiovascular health and risk stratification. Finally, this study only focused on key cardiovascular risk factors but did not account for other factors, such as physical inactivity, dietary habits, alcohol

consumption, and socioeconomic status. These elements could significantly impact cardiovascular health and provide a more comprehensive understanding of risk profiles if incorporated into future research.

CHAPTER 6

CONCLUSION AND FUTURE RECOMMENDATIONS

6.1 Conclusion

This study investigated the association between the ABO blood group and leptin level with cardiovascular risk using FRS, addressing a notable gap in existing CVD risk prediction tools. While significant associations were observed between the ABO blood group and leptin in previous CVD research, our findings showed that these factors were not significantly associated with FRS. However, other confounding variables played a more substantial role in predicting cardiovascular risks. These results align with the primary research objectives, emphasising the need for a multidimensional approach to understanding CVD risk.

The study revealed that the ABO blood group and leptin level were not significantly associated with FRS-based cardiovascular risk in this population by multiple logistic regression. Other confounding factors demonstrated stronger predictive associations, reaffirming their critical role in CVD risk stratification. These results highlight the necessity of refining existing risk models by integrating novel biomarkers and tailoring approaches to specific populations. The findings contribute to the growing body of evidence regarding the role of genetic factors and biomarkers in CVD. Despite their non-significant association with FRS, the ABO blood group and leptin may still hold relevance in alternative pathways or specific populations, particularly in the context of geographical, genetic, environmental, and lifestyle influences. This study also

acknowledged potential limitations, such as the influence of anti-dyslipidaemia agents and FBS, which could mask or modify these associations, thus avoiding overreaching interpretations. From a clinical perspective, the findings suggest that the ABO blood group and leptin may have limited standalone utility in FRS-based risk stratification. However, it highlights the broader potential of biomarkers in enhancing CVD risk prediction and management. Biomarkers reflecting diverse aspects of CVD pathobiology can significantly improve early diagnosis, particularly in high-risk individuals or those with subclinical atherosclerosis. Additionally, they may support more precise therapeutic strategies and aid in patient counselling, promoting lifestyle modifications to prevent disease progression.

Despite these findings, significant gaps remain in understanding the interplay between the ABO blood group, leptin, and cardiovascular risk. One major unresolved question is whether these factors play a more pronounced role in alternative biological pathways or specific populations not adequately represented by FRS. Current models of FRS primarily focus on traditional risk factors, potentially overlooking subtler contributions of genetic and biomarker variables. This highlights the need to explore whether the ABO blood group and leptin have more relevant associations in subgroups, such as those with specific comorbidities, ethnic backgrounds, or genetic predispositions. Another key area of uncertainty involves the modulation of these associations by geographical, genetic, environmental, and lifestyle factors. For example, regional dietary habits, psychosocial stress, and varying prevalence of metabolic disorders could influence the relationship between the ABO blood

group, leptin level, and cardiovascular outcomes. Similarly, genetic variations across populations might modify the expression of these factors or their downstream effects, contributing to regional disparities in CVD risk.

6.2 Future Recommendations

The findings of this study underscore the importance of refining and advancing cardiovascular risk prediction methodologies to aid healthcare professionals in clinical practice. Current algorithms, such as FRS, need continuous refinement to integrate emerging insights and novel biomarkers, which may enhance their predictive accuracy and clinical applicability. The role of genetics, particularly ABO blood group phenotypes, and metabolic biomarkers, like leptin in CVD pathophysiology, highlight key areas for future investigation. These factors hold the potential for uncovering new therapeutic strategies and improving prognostic models, even though their direct associations with established risk tools, such as FRS, remain inconclusive.

Future research should prioritise elucidating the biological mechanisms underlying the associations between the ABO blood group, leptin, and CVD risk. This includes exploring the interplay between these factors and pathophysiological processes, such as endothelial dysfunction, atherosclerosis progression, and systemic inflammation. Longitudinal studies are critical for assessing how these variables influence cardiovascular outcomes. By tracking dynamic changes in biomarkers and integrating advanced tools for subclinical atherosclerosis assessment, researchers may gain deeper insights into the progression of CVD.

Expanding the study designs to include larger, more diverse cohorts across various cities and provinces is essential for enhancing the generalisability of the findings. In the Malaysian context, replicating similar studies across different populations can provide a more comprehensive understanding on the influence of regional and genetic influences towards cardiovascular risk. Additionally, conducting large-scale epidemiological and genetic studies could help clarify the risks associated with major and minor ABO blood groups and refine the role of leptin in predicting CVD.

Incorporating novel biomarkers into predictive models could significantly improve the assessment of CVD risk. For example, integrating biomarkers linked to inflammation, endothelial function, and subclinical atherosclerosis alongside traditional factors may offer a more holistic view of cardiovascular health. These enhancements can aid in identifying high-risk individuals earlier and tailoring interventions more effectively. Furthermore, non-invasive assessments of coronary atherosclerosis burden and biomarkers of cardiac damage should be explored to refine the stratification of cardiovascular risk.

Finally, translating genetic and biomarker research into actionable clinical tools remains a crucial priority. Developing algorithms that seamlessly integrate traditional and emerging risk factors could transform cardiovascular risk prediction and management. Such advancements will not only support healthcare professionals in clinical decision-making but also encourage proactive lifestyle modifications in patients, ultimately reducing morbidity and mortality associated with CVD. Addressing these priorities through

collaborative research and policy initiatives can contribute to the global effort to mitigate the burden of CVD.

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APPENDICES

Appendix A

Patient's Proforma

SITE ID:	
DATE (DD/MM/YYYY):	
PATIENT REFERENCE NUMBER:	

TO BE FILLED BY PATIENT/ *DIISI OLEH PESAKIT*:

INSTRUCTION FOR PATIENT/ *ARAHAN UNTUK PESAKIT*:

ANSWER ALL THE QUESTION AND PLEASE MARK (✓) OR FILL THE BLANK.

SILA JAWAB SEMUA SOALAN DAN TANDA (✓) ATAU ISI TEMPAT KOSONG.

SECTION/BAHAGIAN A: **SOCIODEMOGRAPHIC AND PERSONAL INFORMATION** ***SOCIODEMOGRAFI DAN BUTIRAN PERIBADI***		
A1	What is your age? *Berapakah umur anda?*	_____ Years (*tahun*)
A2	What is your gender? *Apakah jantina anda?*	☐ Male/ *Lelaki* ☐ Female/ *Perempuan*
A3	What is your race? *Apakah bangsa anda?*	☐ Malay/ *Melayu* ☐ Chinese/ *Cina* ☐ Indian/ *India* ☐ Bumiputera: Please specify/*Sila nyatakan*: _____ ☐ Others/Lain-lain: Please specify/*Sila nyatakan*: _____

TO BE FILLED BY PATIENT/ *DIISI OLEH PESAKIT*:

INSTRUCTION FOR PATIENT/ *ARAHAN UNTUK PESAKIT*:

ANSWER ALL THE QUESTION AND PLEASE MARK (✓) OR FILL THE BLANK.

SILA JAWAB SEMUA SOALAN DAN TANDA (✓) ATAU ISI TEMPAT KOSONG.

SECTION/ BAHAGIAN B: **LIFESYTLES AND CLINICAL INFORMATION** ***GAYA HIDUP DAN MAKLUMAT KLINIKAL***		
B2	Do you smoke? *Adakah anda merokok?*	☐ Yes/ *Ya* ☐ No/ *Tidak* If "Yes"; please specify: *Jika "Ya"; sila nyatakan:* ☐ Less than 20 cigarettes/day/ *Kurang 20 batang rokok/hari*

		☐ More than 20 cigarretes/day/ *Lebih 20 batang rokok/hari* If "No"; please specify: *Jika "Tidak"; sila nyatakan*: ☐ Passive smoker/ *Perokok pasif*
B6	Do you have high blood pressure? *Adakah anda penghidap penyakit darah tinggi?*	☐ Yes/ Ya ☐ No/ Tidak If "Yes"; please state your medication: Jika "Ya"; sila nyatakan nama ubat anda: _____ _____
B7	Do you have diabetes? *Adakah anda pesakit kencing manis?*	☐ Yes/ Ya ☐ No/ Tidak If "Yes"; please state your medication: Jika "Ya"; sila nyatakan nama ubat anda: _____ _____
B8	Do you have high cholesterol problem? *Adakah anda penghidap masalah kolesterol?*	☐ Yes/ Ya ☐ No/ Tidak If "Yes"; please state your medication: Jika "Ya"; sila nyatakan nama ubat anda: _____ _____
B9	Have you been diagnosed with cardiovascular disease, atherosclerosis, previous heart attack and/or previous stroke? *Adakah anda pernah di diagnosis dengan penyakit kardiovaskular, aterosklerosis, sejarah serangan jantung dan/atau sejarah strok/angin ahmar?*	☐ Yes/ Ya ☐ No/ Tidak If "Yes"; please state your medication: Jika "Ya"; sila nyatakan nama ubat anda: _____ _____
B10	Select if you are diagnosed with the following. *Pilih sekiranya anda telah di diagnos dengan penyakit berikut.*	☐ Cancer/ *Kanser* ☐ Liver disorders/*Masalah hati* ☐ Renal disorders/*Masalah buah pinggang/ginjal* ☐ Active autoimmune diseases/ *Penyakit autoimmun*

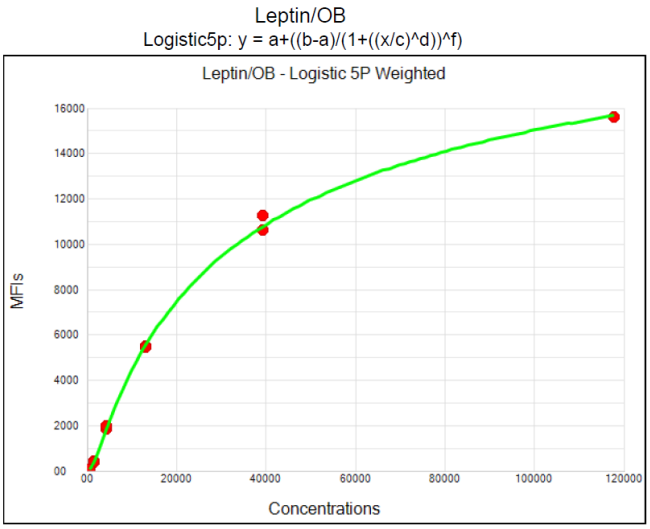
		☐ Thyroid disorder/*Masalah tiroid* ☐ Stroke/*Strok/angin ahmar* ☐ Ischemic heart diseases/Penyakit serangan jantung ☐ None of the above/ Tiada di atas

END / TAMAT

Appendix B

Luminex Leptin standard curve of serial dilution

Test Name: Leptin/OB



Curve Data:

BIODATA OF STUDENT

I, Fatin Syazwani Binti Abd Malek, was born on August 21, 1993, in Klang, Selangor. In 2012, I began my academic journey in healthcare by enrolling in the Bachelor of Nursing (Honours) program at Universiti Teknologi MARA, Puncak Alam, and graduated in 2016.

After graduating, I gained valuable professional experience over five years, working in the Adult Cardiothoracic Intensive Care Unit at the National Heart Institute, Malaysia, and the Paediatric Cardiothoracic Intensive Care Unit at King Fahad Medical City, Saudi Arabia. Upon returning to Malaysia, I pursued a Master of Science in Haematology at Universiti Putra Malaysia, Serdang.

Currently, I conducted research on the potential role of ABO blood groups and leptin levels in predicting CVD risk using the FRS model. I also work as a Research Assistant in collaboration with the University of Edinburgh, United Kingdom, while holding a part-time position as an Assistant Respiratory Technologist at Prince Court Medical Centre, Kuala Lumpur.

Throughout my academic and professional journey, I have developed extensive skills in laboratory techniques and research methodologies. I am proficient in handling biological, chemical, and radiological materials while strictly adhering to safety protocols.

My technical experiences include immunoassays, ABO blood grouping,

Luminex assays, peripheral blood mononuclear cell (PBMC) isolation, DNA extraction, polymerase chain reaction (PCR), PCR purification, microRNA extraction, and high-resolution melting (HRM) technology for gene scanning.

My medical background has strengthened my ability to communicate effectively with patients and encourage their participation in research studies. I have also developed strong problem-solving and conflict-resolution skills through critical and creative thinking.

In addition to my research and professional roles, I have mentored undergraduate interns from Universiti Putra Malaysia, students from international secondary schools, and university students from China. I have also cultivated strong soft skills, data management capabilities, and analytical proficiency, which further support my academic and professional accomplishments.

LIST OF PUBLICATIONS

Malek, F. S. A., Kahar, F., Abdullah, N. I., Izzudin, M. P. E., Amin, A. M., Bahari, H., Idris, F., Ghazali, S. S., & Noor, S. M. (2024). Association of ABO Blood Group and Leptin Level on Framingham Risk Score in Determining Cardiovascular Disease Risk. *Malaysian Journal of Medicine and Health Sciences*, *20*(5), 16–25. https://doi.org/10.47836/mjmhs.20.s11.4