ORIGINAL ARTICLE

Association of ABO Blood Group and Leptin Level on Framingham Risk Score in Determining Cardiovascular Disease Risk

Fatin Syazwani Abd Malek[1], Fitri Kahar[1], Nurul Izzati Abdullah[1], Md Parvez Eusof Izzudin[2], Amrina Mohamad Amin[1], Hasnah Bahari[3], Faridah Idris[1], Sazlina Shariff Ghazali[4], Sabariah Md Noor[1]

[1] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2] Family Medicine Clinic, Hospital Sultan Abdul Aziz Shah, Persiaran Mardi, Universiti Putra Malaysia, 43400, Serdang, Selangor Darul Ehsan, Malaysia.
[3] Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor Darul Ehsan, Malaysia.
[4] Department of Family Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor Darul Ehsan, Malaysia.

ABSTRACT

Introduction: Cardiovascular diseases (CVD) remain the leading cause of mortality globally. Blood group antigens have been associated with increased susceptibility to diseases, especially CVD. Leptin offers significant potential to improve the accuracy of CVD risk prediction. Framingham Risk Score (FRS) is the standard risk assessment tool for estimating the 10-year CVD. This study aims to determine the association of ABO blood and leptin level with FRS in assessing CVD risk. **Materials and methods:** A cross-sectional study was conducted among 431 patients without CVD aged from 30-75 years. Blood samples were analyzed for the ABO blood group using antigen-antibody agglutination, and plasma leptin levels were measured using magnetic Luminex with a functional sandwich immunoassay. FRS calculation was categorized into low and moderate-high-risk groups. **Results:** Patients' median (IQR) age was 56 (19) years. Most were females (59.4%), Malays (72.6%), non-diabetic (63.8%), hypertensive (53.1%), dyslipidemia (79.8%), non-O blood group (60.6%), Rh-positive (99.3%) and the moderate-high-risk group of FRS (66.8%). ABO blood group and leptin levels were found not significantly associated with FRS. However, the use of anti-dyslipidemia agents (AOR = 6.44, 95% CI: 2.72–15.26, $p < 0.001$) and fasting blood sugar (FBS) levels (AOR = 1.75, 95% CI: 1.18–2.62, $p = 0.006$) were significantly associated with moderate-to-high FRS risk. **Conclusion:** While non-O blood group and leptin levels were prevalent, neither was associated with FRS. In contrast, anti-dyslipidemia medication use and elevated FBS levels showed strong associations with moderate-to-high FRS risk, emphasizing the importance of managing dyslipidemia and blood sugar in reducing cardiovascular risk.
Malaysian Journal of Medicine and Health Sciences (2024) 20(SUPP11): 16-25. doi:10.47836/mjmhs20.s11.4

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

Corresponding Author:
Sabariah Md Noor, MPath
Email: md_sabariah@upm.edu.my
Tel : +603 97692392

INTRODUCTION

Cardiovascular disease (CVD) is a broad term that encompasses a range of conditions that refer to diseases that affect the blood vessels and heart. It includes heart disease, coronary artery disease (CAD), and other conditions that affect blood vessels like peripheral artery disease (PAD) and elevated blood pressure (1). In 2019, CVD was significantly responsible for a substantial number of deaths worldwide, with 17.9 million fatalities, and accounted for approximately 32% of all global deaths (2). Data in 2019 also showed that 1.7 million Malaysians currently have the three primary CVD risks which are hypertension, diabetes, and hyperlipidemia (3). The findings from the prospective cohort study conducted in 21 countries, including Malaysia, are consistent with the global impact of CVD, indeed it's a major cause of mortality and a significant public health concern in many regions around the world (4).

The ABO system consists of the four major blood groups of A, B, AB, and O. This classification was discovered in 1901 by Karl Landsteiner. The A and B alleles encode N-acetylgalactosamine and D-galactose glycosyltransferase enzymes. In contrast, the O allele is associated with the absence of both A or B antigens on the surface of red cells and glycosyltransferase activity (5). Genome-wide associations (GWAS) study provided compelling evidence connecting the ABO system to

CVD (5). The non-O blood group especially, had been linked to significant cardiovascular risk factors and/or an elevated rate of cardiovascular events (6). This is because the relationship between VWF (von Willebrand factor), a carrier protein for coagulation factor VIII (FVIII), and plasma levels and biological activity is decreased in O blood group individuals as this may also play a role in the associations between the ABO blood group and thrombosis risk (7). Undoubtedly, it is widely recognized that individuals with non-O blood group have plasma levels of both VWF and FVIII that are approximately 25% higher than those with an O blood group (8).

Leptin levels were strongly linked to congestive heart failure (HF) and CVD (9). Several studies have been done highlighting leptin has been associated with cardiovascular events, and hyperleptinemia has been connected to the occurrence of and seriousness of coronary heart disease (CHD) and HF (9-11). Leptin has been discovered as one of the atherosclerotic biomarkers and has proven the sensitivity and specificity of leptin as a good biomarker for subclinical atherosclerosis (12). Further research stated that a high level of leptin could serve as a sign of leptin resistance, influenced by factors such as genetic mutations, autoregulation, limited tissue entry, and molecular or cellular circulatory control (13). Thus, leptin resistance plays a role in implications for heightened cardiac inflammation, increased fibrosis, hypertension, and compromised cardiac metabolism.

As morbidity and mortality from CVD rose, the Framingham study began to explore CVD-related factors and processes, providing a way to categorize the likelihood of a heart-related event over the next decade (14). FRS incorporates well-established risk factors such as gender, age, certain lipid profile parameters, diabetes, and hypertension but it does not encompass genetic factors or biomarkers that are implicated in CVD susceptibility (15). However, FRS does not incorporate a biomarker or genetic determinant as its' parameter. A broader perspective has revealed common prevalent CVDs, such as CAD, atrial fibrillation, heart failure, hypertension, and stroke, are multifaceted conditions influenced by both genetic predisposition and environmental factors (16, 17).

Non-communicable diseases, such as CVD, continue to contribute significantly to mortality rates, despite the availability of numerous CVD risk assessments. Genetic factors and biomarkers, such as ABO blood groups and leptin levels, may serve as valuable parameters for identifying high-risk individuals, enabling early diagnosis and the prevention of disease progression. However, limited studies incorporate these potential parameters alongside the FRS in assessing CVD risk. This study aims to evaluate the role of ABO blood group and plasma leptin levels as additional parameters to FRS in determining CVD risk. Furthermore, additional clinical data, considered confounding factors, were also evaluated for FRS parameters.

MATERIALS AND METHODS

Sampling method and size

A total of 431 patients were selected using convenient sampling and this cross-sectional study was conducted between March 2022 until February 2024 in the Family Medicine Specialist Clinic (FMSC), Hospital Sultan Abdul Aziz Shah (HSAAS), and Primary Care Clinic, Puchong in Selangor. They attended medical check-ups or follow-ups from their previous clinic visits and planned for blood taking. The sample size was calculated based on blood groups and the biggest number of sample size estimations was chosen and applied in this study because the ABO blood group is the indicator of genetic markers which act as an inherited risk factor for CVD. It was calculated by using OpenEpi (www.openepi.com) (18) in which the reference of Malaysian population (P) of blood group O from a previous (19) was taken in applying the formula in Open Epi as blood group O was the highest percentage and 95% confidence level, 5% of precision, and 20% of non-responder rate was take into account. The grouping of ABO blood group was made into 2 categories; O and non-O blood groups as the physiology of A, B, and AB blood groups had A and/or B antigens, whereas the O blood group does not have A and/or B antigens (5). However, due to budget constraints, 150 patients were randomly selected by using an electronic calculator for random selection (20) for leptin immunoassay. OpenEpi was used in sample size estimation by reference to a previous study (21) with consideration of a 95% confidence level, 5% precision, 80% power, and 20% non-responder rate. Thus, 50 patients were randomly selected in the low, moderate, and high-risk groups of FRS.

Study parameters and data collection

The study's inclusion criteria include the participants aged 30 to 75 years old with no history of any cardiovascular diseases such as coronary heart disease, stroke, or peripheral vascular disease, free of any cardiovascular events, have fasted overnight before blood collection, and can provide informed consent. The data collection process for this study used Performa data (demographic details, medical status, clinical examinations, and laboratory measurements). Performa data was gathered from the adapt and adopt form Cardiovascular Risk Assessment Sheet (22) which information includes demographic details (age, gender, ethnicity), medical status (diabetes, hypertension, dyslipidemia and any history of cardiovascular problem), medication status (anti-glycemic, anti-hypertensive, anti-dyslipidemia agent), clinical examinations (height [meter], weight [kilogram], body mass index (BMI) [kg/m2] and blood pressure [mmHg] measurement) were conducted by trained healthcare professionals and measured and calculated by measuring the scale of SECA 286 (Germany) and IntelliVue MX450 Patient

Monitor (Philips, Germany) respectively, and laboratory levels (serum fasting lipid profile [FLP], plasma fasting blood sugar [FBS], ABO blood grouping and plasma leptin immunoassay). Diabetes mellitus was considered when FBS ≥7.0 mmol/L (23). Consistent increase in systolic blood pressure (SBP) of 140 mmHg or higher and/or diastolic blood pressure (DBP) of 90 mmHg or higher classified hypertension and individuals at risk of hypertension when SBP of 130-139mmHg (24). Dyslipidemia cut-off values consist of total cholesterol (TC) > 5.2 mmol/l, low-density lipoprotein cholesterol LDL-C >3.0 mmol/L, triglyceride (TG) > 1.7 mmol/l, high-density lipoprotein cholesterol (HDL-C) < 1.0 mmol/l (25). However, NCD status was determined by the physicians diagnosed.

Sample collection and process

Trained health personnel withdrew patients' venous blood samples. The samples were collected by applying standard venipuncture techniques and stored in a serum separator (SST) tube for FLP, sodium fluoride tube for FBS, and ethylenediaminetetraacetic acid (EDTA) tubes for the ABO blood grouping and leptin levels. The collected blood was also subjected for FBS and FLP of TC, TG, LDL-C, and HDL-C by Alinity ci-series, USA. These biochemistry results were presented in mmol/L with their reference range readings of 3.9-6.1 mmol/L, 0.18-5.19mmol/L, 0.08-1.7mmol/L, 0-0 mmol/L and 1.03-9999 mmol/L respectively. The ABO blood grouping was performed by tube technique (26) where monoclonal antibodies anti-A, anti-B, anti-AB, and anti-D antisera (Novaclone, Canada) were used in forward blood grouping while reverse blood grouping was using known A, B red cells (ID Diacell, Switzerland). The tests were conducted by two independent researchers and the results were confirmed by medical laboratory technologists to ensure the reliability and accuracy of the blood grouping results. Leptin immunoassay was used in Human Magnetic Luminex® Assays (R&D System, USA) to assess the level of biomarkers in ng/ml.

Framingham Risk Score Calculation

The Framingham Risk Score (2008) was calculated using validated algorithms based on patients' age, gender, blood pressure, TC, HDL-C, anti-hypertensive agent consumption, diabetes status smoking status, and any history of vascular diseases (27, 28). Patients were categorized as smokers if they reported that they were currently smoking. The FRS utilizes these factors to estimate an individual's 10-year risk of experiencing a cardiovascular event. Based on the FRS, the respondents were categorized into three groups; high-risk (>20%), moderate-risk (10-20%), and low-risk (<10%). The respondents also were grouped as high-risk if they have diabetes even if the score is not more than 20% (29). FRS has been calibrated and validated in the Asian population (30) and Malaysian populations (27).

Statistical Analysis

The statistical analysis of the data collected for this study was conducted using IBM SPSS Statistics, version 29.0, from IBM Corporation. Data were presented in frequency and percentages for categorical data and median (IQR) for skewed numerical data distribution as both ways were to express descriptive data. Logistic regression analysis was applied to analyze parameters that were not measured in FRS. Univariate logistic regression was tested for each potential predictor confounders in association with moderate-high risk of FRS and it presented in an odd ratio. Analysis of potential predictors that did not count in FRS were presented in Tables II, III, and IV for a better understanding of the factors influencing FRS. In Table III for medication and non-communicable diseases (diabetes, hypertension, and dyslipidemia) status, only an anti-dyslipidemia agent and dyslipidemia were analyzed by univariate logistic regression because in FRS the use of an anti-hypertensive agent and diabetes status were counted. This is to prevent redundancy in analysis which may lead to inaccuracy. However, in Table 4, only an anti-dyslipidemia agent was selected instead of dyslipidemia status as those who were on the medication were those diagnosed with dyslipidemia because not all dyslipidemia patients were keen for medication. The enter method was used to initially screen parameters for potential inclusion in the multiple logistic regression model. The significant parameters from the univariate analyses were selected using an enter method with a p-value of < 0.250 of significance levels for an addition of the parameter to predict the outcome and were included in the multiple logistic regression model. The p-value, crude odds ratio (COR) in univariate logistic regression, adjusted odds ratio (AOR) in multiple logistic regression, and 95% confidence level (CI) were reported to determine the strength of contribution from each parameter towards moderate-high risk of FRS.

Ethical consideration

Institutional ethics board approval was obtained from the Ethic Committee for Research Involving Human Subjects (JKEUPM) before 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. Verbal and written informed consent were obtained from the patients after the researchers explained the study and the patients read the participants' information sheet. Patients' participation was voluntary. Patients were assigned non-identifiable identification codes for data entry and data analysis. The patients would not be identified in the report writing or publication.

RESULTS

Demographic and clinical parameters of the study population

The study enrolled 453 patients but only 431 met the inclusion and exclusion criteria and were proceeded for data analysis. Those with a diagnosis of any heart disease like having a New York Heart Association (NYHA) classification, incomplete clinical data in BMI, and insufficient samples noticed during laboratory procedures were excluded from the study. Demographic and clinical data were presented in Table I, revealing a median (IQR) age of 56 (19) years old, with females comprising (59.4%) and the majority of Malay ethnicity (72.6%). The median (IQR) BMI of patients in both low 26.90 (6.20) and moderate-high-risk FRS 27.60 (6.18) kg/m2 were obese. Most of the patients who had been diagnosed with diabetes (36.2%), hypertension (53.1%), and dyslipidemia (79.8%) were not keen to start medication and preferred lifestyle modification as only 35%, 50.3%, and 60.8% were on the medication of diabetes, hypertension, and dyslipidemia respectively. Mostly dyslipidemia patients were not keen on medication and this led to the high median (IQR) of TC with 5.40 (1.31) and LDL-C with 3.40 (1.33) mmol/L in low-risk compared to moderate-high risk of FRS. The measurement of diabetes, hypertension, and dyslipidemia can be seen in median (IQR) FBS level which was within an acceptable range, 5.5(1.7) mmol/L, median (IQR) SBP measurement at 135 (18) mmHg indicated patients at risk of hypertension, meanwhile, median (IQR) TC and LDL-C was 5.30 (1.7) and 3.20 (1.6) mmol/L respectively shown beyond out of cut off values in FLP. The non-O blood group (60.6%) was higher than the O blood group (39.4%). However, the O blood group was the highest among each blood group where A (22.0%), B (30.2%), and AB (8.4%) and 99.3% were Rh-positive. Notably, 66.8% of patients were classified as moderate-high risk based on the FRS. Due to budget constraints, leptin immunoassay was conducted on a randomly selected 150 patients, with the median (IQR) leptin level recorded at 17.84 (21.6) ng/ml. However, the median (IQR) leptin level presented higher in low risk with 20.25 (20.72) ng/ml than the moderate-high risk with 17.59 (24.72) ng/ml of FRS.

Table I: Patients' demographic and clinical parameters in Framingham Risk Score (N = 431)

Variables	Framingham Risk Score, n (%)		Total n (%)
	Low 143 (33.2)	Moderate-High 288 (66.8)	
Age, year [a]	45.00 (15)	61.00 (14)	56.00 (19)
Gender			
Male	43 (24.6)	132 (75.4)	175 (40.6)
Female	100 (39.1)	156 (60.9)	256 (59.4)
Ethnicity			
Malay	115 (36.7)	198 (63.3)	313 (72.6)
Chinese	17 (24.6)	52 (75.4)	69 (16.0)
India	11 (22.4)	38 (77.6)	49 (11.4)
Heart rate, bpm [a]	76 (18)	79 (17)	78 (17)
BMI, kg/m² [a]	26.90 (6.20)	27.60 (6.18)	27.50 (6.5)
Diabetes	0 (0)	156 (100)	156 (36.2)
FBS (mmol/L) [a]	5.10 (0.70)	6.00 (2.28)	5.50 (1.7)
Hypertension	37 (16.2)	192 (83.8)	229 (53.1)
SBP (mmHg) [a]	126.00 (21.00)	139 (17.00)	135.00 (18)
Dyslipidemia	89 (25.9)	255 (74.1)	344 (79.8)
TC (mmol/L) [a]	5.40 (1.31)	5.16 (1.88)	5.30 (1.7)
TG (mmol/L) [a]	1.20 (0.70)	1.38 (0.90)	1.30 (0.8)
LDL-C (mmol/L) [a]	3.40 (1.33)	3.10 (1.70)	3.20 (1.6)
HDL-C (mmol/L) [a]	1.40 (0.50)	1.30 (0.47)	1.34 (0.4)
Use of anti-diabetes agent			
No	143 (51.1)	137 (48.9)	280 (65)
Yes	0 (0)	151 (100)	151 (35)
Use of anti-hypertensive agent			
No	110 (51.4)	104 (48.6)	214 (49.7)
Yes	33 (15.2)	184 (84.8)	217 (50.3)
Use of anti-dyslipidemia agent			
No	102 (60.4)	67 (39.6)	169 (39.2)
Yes	41 (15.6)	221 (84.4)	262 (60.8)
Smoking	4 (13.3)	26 (86.7)	30 (7.0)

CONTINUE

Table I: Patients' demographic and clinical parameters in Framingham Risk Score (N = 431). (CONT.)

Variables		Framingham Risk Score, n (%)				Total n (%)	
		Low 143 (33.2)		Moderate-High 288 (66.8)			
ABO blood group system							
Non-O Blood group	A	82 (31.4)	29 (30.5)	179 (68.9)	66 (69.5)	261 (60.6)	95 (22.0)
	B		44 (33.8)		86 (66.2)		130 (30.2)
	AB		9 (25.0)		27 (75.0)		36 (8.4)
O Blood group		61 (35.9)		109 (64.1)		170 (39.4)	
Rh blood group system							
Positive		143 (33.4)		285 (66.6)		428 (99.3)	
Negative		0 (0.0)		3 (100)		3 (0.7)	

[a]: Distribution presented in median (IQR). TC: Total cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol. Bpm: beat per minute.

ABO blood group on Framingham Risk Score

Table II explored the association between the ABO blood group and FRS among 431 patients. The result was not statistically significant, indicating that patients with the non-O blood group had lower odds of having a moderate-to-high FRS compared to those with the O blood group (COR = 0.82, 95% CI: 0.50–1.23, p = 0.336).

Table II: Association between ABO blood group on Framingham Risk Score using univariate logistic regression (N=431)

Variables		COR	95% CI		p-value
			Lower	Upper	
ABO Blood group	O	Reference			
	Non-O	0.82	0.54	1.23	0.336

COR: Crude odds ratio. CI: Confidence interval

Potential confounders on Framingham Risk Score by univariate logistic regression

Table III presented several parameters that were tested for an association with FRS among 150 patients. The association between the ABO blood group and leptin levels on the FRS were found not statistically significant and no difference in the odds of being at moderate-high risk between the non-O blood group and the O blood group and in leptin levels with (COR = 1.00, 95% CI: 0.50–2.01, p = 1.000) and (COR = 1.00, 95% CI: 1.00–1.00, p = 0.752) respectively. However, the odds of having an outcome of moderate-high risk FRS was found to be significantly higher for those who used the anti-dyslipidemia compared to those not on medication (COR = 7.76, 95% CI: 3.390–17.75, p <0.001). Similarly, the FBS level was significantly 1.97 times more likely to have moderate-high risk FRS for each unit increase in FBS level (COR = 1.97, 95% CI: 1.35, 2.89, p < 0.001).

Table III: Association between potential confounders and Framingham Risk Score using univariate logistic regression (N=150)

Potential Confounders		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			
	Non-O	1.00	0.50	2.01	1.000
On anti-dyslipidaemia agent	No	Reference			
	Yes	7.76	3.39	17.75	<0.001*
Dyslipidaemia	No	Reference			
	Yes	3.84	1.79	8.24	<0.001*
HR		1.00	0.97	1.04	0.819
DBP		0.99	0.96	1.02	0.389
BMI level		1.43	0.94	1.09	0.755
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
Leptin level		1.00	1.00	1.00	0.752

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

Potential confounders on Framingham Risk Score by multiple logistic regression

After adjusting demographic and clinical parameters, Table IV showed the 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 was found to be significantly higher for those who used the anti-dyslipidemia compared to those not on medication (AOR=6.44, 95% CI: 2.72-15.26), p <0.001). And, the FBS level was significantly 1.75 times more likely to have moderate-high risk FRS for each unit increase in FBS level (AOR=1.75, 95% CI:

1.18-2.62, p-value = 0.006). The TG level did not show a statistically significant relationship with the moderate-high risk of FRS (AOR=1.37, 95% CI: 0.32-1.3, p-value = 0.324).

Table IV: Association between potential confounders on Framingham Risk Score using multiple logistic regression (N=150)

Variables		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

*: Significant. AOR: Adjusted odds ratio. FBS: Fasting blood sugar, TG: Triglyceride.

DISCUSSION

Demographically, the Malay ethnicity is predominant among the study population, as the majority of Malays reside in the Petaling District. This aligns with the district statistics, which indicate that the Malay population is higher compared to other ethnic groups (31). In our study, the number of males was lower than females. In this cohort, many males declined to give extra blood during their blood draw session. Most patients diagnosed with dyslipidemia were reluctant to start medication, which may explain the elevated total cholesterol (TC) and LDL-C levels observed in individuals classified as low-risk by the FRS. This is because dyslipidemia status or the use of anti-dyslipidemia agents is not accounted for in FRS calculations. Similarly, previous studies (32, 33) have reported high lipid profiles in individuals with low FRS. In addition, studies on the Malaysian population (27, 29) have found that the FRS provides a fairly accurate prediction of CVD risk.

To date, approximately 400 blood group systems have been documented, with the ABO blood group and the RH blood group system being the most significant (34). Our study's blood group distribution showed a similar prevalence as found among the Malaysian population (19), as well as most Asian population studies (34-36) which indicated that the O blood group is more common than the A, B, and AB groups. However, when considered cumulatively, the non-O blood groups were more prevalent than the O group. This pattern is consistent with other countries, where the O blood group tends to be dominant over non-O groups (37, 38). Most previous studies have shown that the A blood group is more prevalent than the B group (19, 34, 36, 39), although a few studies, including our cohort, have found that the B blood group is higher than A (34, 39). These trends are supported by a previous review (37). In addition, all of those studies presented that Rh-positive was higher than negative which also supported our finding. Differences in trends may be attributed to the varying sample sizes and populations of earlier studies, as blood group systems tend to vary with ethnic diversity within a population.

The discovery of leptin has opened a new area of exploration. In this study, leptin levels were higher in the low-risk group than in the moderate- and high-risk FRS groups. This is likely due to the random selection process, where most participants were females, and many with dyslipidemia were classified as low risk. Additionally, fewer patients with diabetes were selected in our cohort of 150 patients, leading to a lower proportion of diabetic patients (only 30%) in the high-risk FRS group. This finding is also demonstrated in previous studies, which have shown that elevated leptin levels are associated with hypercholesterolemia (21), diabetes (9, 15), and females (40).

A study has explored the relationship between ABO blood groups, and health conditions, particularly CVD (6). Previous studies have stated that the prevalence of CVD was considerably higher in the non-O blood group (5, 41). However, a literature search shows there is no published data relating to the ABO blood group system and FRS. This study demonstrated that the prevalence of the moderate-high-risk group in the non-O blood group was higher than in the O blood group. Despite earlier hypotheses and studies indicating a potential link, this study's results highlighted that the ABO blood group did not significantly influence CVD risk as determined by FRS. Although no statistically significant association was found, a higher risk of CVD among the non-O blood group is still in agreement with findings by others where the ABO blood group had an association with CVD risk development. These findings implied that other parameters, such as hormonal changes and genetic predisposition may play a more critical role in determining cardiovascular risk.

Therefore, further analysis of potential confounders were analyzed which likely play a more significant role in predicting CVD by FRS. However, only the anti-dyslipidemia agent and FBS level were presented as statistically significant on FRS. This finding suggested that patients on anti-dyslipidemia agents were more likely to have moderate-to-high FRS. This association highlights a potential limitation of the original FRS, which does not account for the presence of treated dyslipidemia or the use of lipid-lowering medications. Patients receiving anti-dyslipidemia agents were those who have been diagnosed with dyslipidemia, a known risk factor for CVD. Although the medication helps control their dyslipidemia, the underlying risk factor that led to the initiation of treatment may still contribute to a significant CVD risk. Consequently, the FRS may underestimate the true CVD risk in patients receiving anti-dyslipidemia therapy, as similarly reported in a previous study (42). Furthermore, a study conducted in Malaysia (29) also found that the FRS tends to underestimate CVD risk, leading to inaccurate risk estimations. This underestimation has been reported

in other studies as well, particularly among individuals without diagnosed CVD but with metabolic syndrome or other conditions (43-45).

The significant association between FBS and FRS is in agreement with others emphasizing impaired glucose metabolism and cardiovascular outcomes. The FBS was presented to be significant in FRS among Malaysians (46, 47) and other countries (33, 48), which was similar to the current study. Besides that, a study among Malaysians (46) and another country (49) presented significant medication adherence and TG levels on FRS. However, further analysis by multiple logistic regression found that TG has remained insignificant in this study. Similarly, a study presented TG was not significant to FRS (33), meanwhile, a previous study from Malaysia (47) and another country (49) demonstrated the significance of TG on FRS. These findings underscore the importance of addressing modifiable metabolic factors, such as blood sugar levels and lipid control which highlighted the importance of managing lipid profiles in cardiovascular prevention strategies, even when genetic and biomarkers, such as ABO blood groups and leptin levels, do not demonstrate a direct impact. On the contrary, a previous study revealed a notable correlation between FRS and biomarkers, particularly emphasizing leptin as a significant biomarker for assessing the risk of CVD in individuals aged 30 years and above (12). Using leptin as a biomarker in conjunction with FRS to predict the risk of CVD offers increased reliability and predictability compared to relying solely on individual biomarkers as summarized suggested the relationship between leptin level and the incidence of CVD remains significant (9, 12, 50). Conclusively, integrating leptin as biomarkers alongside FRS holds substantial promise in enhancing the prediction of CVD risk. We did not find any study that reported exactly similar results of the association of leptin and FRS. However, the lack of statistical significance of leptin and FRS in this study indicated that other factors may contribute to this relationship or that the sample size may not be sufficient to detect a significant difference. This suggested that the influences of leptin on the pathophysiology of CVD risk are complex. Further research is warranted to elucidate the complex interplay between cardiovascular risk, and metabolic biomarkers like leptin, or investigation with additional covariate adjustments may provide clarity on the relationship between them.

CONCLUSION

Cardiovascular diseases are complex and require comprehensive monitoring of various risk factors for effective diagnosis, monitoring, and treatment. This study highlights the significance of an anti-dyslipidemia agent status and FBS on FRS as key parameters in influencing CVD risk. A larger sample size involving a multicenter study is indicated to further verify the association of ABO blood groups and leptin levels with FRS in this study. The management of metabolic health, particularly through lipid-lowering treatments and glycemic control remains crucial in reducing CVD risk.

Identification of novel CVD biomarkers is essential for early diagnosing and preventing disease progression in high-risk individuals. National strategies and policies are needed to address the factors contributing to CVD, along with improved algorithms for healthcare professionals. Future research involving large epidemiological and genetic studies could help in understanding the risks associated with ABO blood groups and leptin to CVD-associated risk, aiming to translate these insights into practical clinical outcomes.

ACKNOWLEDGEMENT

The authors would like to acknowledge heartfelt gratitude to all the participants and collaborators who made this study possible. Particularly thankful to the healthcare professionals and research staff whose dedication and hard work were instrumental in the collection and analysis of data. Sincere thanks to the funding agencies and institutions that provided the necessary financial and logistical support for this research (FRGS/1/2020/SKK0/UPM/02/21) and (GP-IPS 9771800) besides Institutional Ethics board approval was obtained from the Ethic Committee for Research Involving Human Subject (JKEUPM-2021-700) before the commencement of the study. All procedures followed the Helsinki Declaration of 1975, revised in 2008. Invaluable contributions of our colleagues and supervisors, whose guidance and expertise were crucial throughout the study.

REFERENCES

1. IBM Watson Micromedex, Cerner Multum. Is cardiovascular disease the same as heart disease or coronary heart disease? Drugs.com [Internet]. 2022 Oct 11 [cited 2023 Mar]. Available from: https://www.drugs.com/medical-answers/cardiovascular-disease-heart-disease-coronary-3515533/#:~:text=Cardiovascular%20disease%2C%20is%20an%20umbrella,pressure%20and%20peripheral%20artery%20disease.
2. World Health Organization. Cardiovascular diseases (CVD) [Internet]. 2021 Jun [cited 2023 Mar].Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
3. Institute for Public Health. National Health and Morbidity Survey (NHMS) 2019: Non-communicable diseases, healthcare demand, and health literacy—Key Findings. Available from: https://iptk.moh.gov.my/images/technical_report/2020/4_Infographic_Booklet_NHMS_2019_-_English.pdf
4. Dagenais GR, Leong DP, Rangarajan S, Lanas F,

Lopez-Jaramillo P, Gupta R, et al. Variations in common diseases, hospital admissions, and deaths in middle-aged adults in 21 countries from five continents (PURE): a prospective cohort study. Lancet. 2020;395(10226):785–94. doi:10.1016/s0140-6736(19)32007-0.
5. Paquette M, Dufour R, Baass A. ABO blood group is a cardiovascular risk factor in respondents with familial hypercholesterolemia. J Clin Lipidol. 2018;12(2). doi:10.1016/j.jacl.2017.12.001
6. Marie D, Sow MS, Diack A, Dia K, Mboup MC, Fall PD, et al. Cardiovascular disease and ABO blood-groups in Africans. Are blood-group A individuals at higher risk of ischemic disease?: a pilot study. Egypt Heart J. 2017;69(4):229–34. doi:10.1016/j.ehj.2017.03.002.
7. Franchini M, Lippi G. The intriguing relationship between the ABO blood group, cardiovascular disease, and cancer. BMC Med. 2015;13(7). doi:10.1186/s12916-014-0250-y.
8. Capuzzo E, Bonfanti C, Frattini F, Montorsi P, Turdo R, Previdi MG, et al. The relationship between ABO blood group and cardiovascular disease: results from the Cardiorisk program. Ann Transl Med. 2016;4(10):189. doi:10.21037/atm.2016.03.58
9. Poetsch MS, Strano A, Guan K. Role of leptin in cardiovascular diseases. Front Endocrinol. 2020;11:1–13. doi:10.3389/fendo.2020.00354
10. Zhao S, Kusminski CM, Scherer PE. Adiponectin, leptin and cardiovascular disorders. Circ Res. 2021;128(1):136–49. doi:10.1161/CIRCRESAHA.120.314458
11. Kang KW, Ok M, Lee SK. Leptin as a key between obesity and cardiovascular disease. J Obes Metab Syndr. 2020;29(4):248–59. doi:10.7570/JOMES20120.
12. Reddy YVK, Mahendra J, Gurumurthy P, Jayamathi, Babu S. Identification of predictable biomarkers in conjunction to Framingham Risk Score to predict the risk for cardiovascular disease (CVD) in non-cardiac subjects. J Clin Diagn Res. 2015;9(2)-7. doi:10.7860/JCDR/2015/9089.5589.
13. Pérez-Pérez A, Vilarico-García T, Fernández-Riejos P, Martín-González J, Segura-Egea JJ, Sánchez-Margalet V. Role of leptin as a link between metabolism and the immune system. Cytokine Growth Factor Rev. 2017;35:71–84. doi:10.1016/j.cytogfr.2017.03.001.
14. Poznyak AV, Sadykhov NK, Kartuesov AG, Borisov EE, Melnichenko AA, Grechko AV, et al. Hypertension as a risk factor for atherosclerosis: cardiovascular risk assessment. Front Cardiovasc Med. 2022;9:1–8. doi:10.3389/fcvm.2022.959285.
15. Katsiki N, Mikhailidis DP, Banach M. Leptin, cardiovascular diseases and type 2 diabetes mellitus. Acta Pharmacol Sin. 2018;39(7):1176–88. doi:10.1038/aps.2018.40.
16. Musunuru K, Kathiresan S. Genetics of common, complex coronary artery disease. Cell. 2019;177(1):132–45. doi:10.1016/j.cell.2019.02.015.
17. Wojtasińska A, Frąk W, Lisińska W, Sapeda N, Młynarska E, Rysz J, Franczyk B. Novel insights into the molecular mechanisms of atherosclerosis. Int J Mol Sci. 2023;24(17). doi:10.3390/ijms241713434.
18. Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version. www.OpenEpi.com [Internet]. 2023 [cited 2023 Mar]. Available from: https://www.openepi.com/Menu/OE_Menu.htm
19. Musa RH, Ahmed SA, Hashim H, Ayob Y, Asidin NH, Choo PY, et al. Red cell phenotyping of blood from donors at the National Blood Center of Malaysia. Asian J Transfus Sci. 2012;6(1):3. doi:10.4103/0973-6247.95042.
20. CalculatorSoup L. Random number generator [Internet]. 2023 [cited 2023 Mar]. Available from: www.calculatorsoup.com/calculators/statistics/random-number-generator.php.
21. El Khalafawy GK, Elgazzar EM, Eldin ES. Assessment of Framingham Risk Score and serum leptin in patients with skin tag. Arab J Nucl Sci Appl. 2022;0(0):1–7. doi:10.21608/ajnsa.2022.145911.1605.
22. Cardiovascular risk assessment sheet – PCNA [Internet]. 2018 [cited 2023 Mar]. Available from: www.pcna.net/wp-content/uploads/2018/12/1-_patient_assessment.pdf.
23. International Diabetes Federation Diabetes Atlas 2021. IDF Diabetes Atlas. 2022. [Internet]. 2021 [cited 2023 Mar]. Available from: https://diabetesatlas.org/atlas/tenth-edition/
24. Malaysian Society of Hypertension. Clinical practice guidelines management of hypertension. Ministry of Health Malaysia [Internet]; 2018. [cited 2023 Mar]. Available from: https://www2.moh.gov.my/moh/resources/penerbitan/CPG/MSH%20Hypertension%20CPG%202018%20V3.8%20FA.pdf
25. National Heart Association of Malaysia (NHAM). Clinical practice guidelines management of dyslipidaemia 2023 (6th ed.) [Internet]; National Heart Association of Malaysia; 2023. Available from: https://www.malaysianheart.org/index.php?p=highlights&a=1941
26. Ajmani PS. Immunohematology and blood banking: principles and practice. In Immunohematology and Blood Banking: Principles and Practice. 2020. doi:10.1007/978-981-15-8435-0.
27. Chia YC, Gray SYW, Ching SM, Lim HM, Chinna K. Validation of the Framingham general cardiovascular risk score in a multiethnic Asian population: a retrospective cohort study. BMJ Open. 2015;5(5):1–7. doi:10.1136/bmjopen-2014-007324.
28. Framingham Risk Score (2008): Qxmd [Internet]. [cited 2023 Mar]. Available from: www.qxmd.

com/calculate/calculator_252/framingham-risk-score-2008.
29. Liew SM, Lee WK, Khoo EM, et al. Can doctors and respondents correctly estimate cardiovascular risk? A cross-sectional study in primary care. BMJ Open. 2018;8:e017711. doi:10.1136/bmjopen-2017-017711.
30. Selvarajah S, Kaur G, Haniff J, Cheong KC, Hiong TG, van der Graaf Y, et al. Comparison of the Framingham Risk Score, SCORE, and WHO/ISH cardiovascular risk prediction models in an Asian population. Int J Cardiol. 2014;176(1):211–8. doi:10.1016/j.ijcard.2014.07.066.
31. Department of Statistics Malaysia. Kawasanku Petaling. [Internet]. OpenDOSM; 2023. Qxmd [cited 2023 Mar]. Available from: https://open.dosm.gov.my/kawasanku/selangor/district/petaling
32. Diputra INY, Lorensia A, Suryadinata RV. Triglycerides and cardiovascular risk: a cross-sectional study. 2022;45(6). Available from: https://www.teikyomedicaljournal.com/volume/TMJ/45/06/triglycerides-and-cardiovascular-risk-a-cross-sectional-study-63059caaa34e1.pdf
33. Jahangiry L, Farhangi MA, Rezaei F. Framingham Risk Score for estimation of 10-years of cardiovascular diseases risk in patients with metabolic syndrome. J Health Popul Nutr. 2017;36(1). doi:10.1186/s41043-017-0114-0.
34. Sabir A, Iftikhar A, Ijaz MU, et al. Retrospective study of frequency of ABO and Rhesus blood group among population of Safdarabad and Faisalabad cities of Pakistan. BMC Res Notes. 2021;14(12). doi:10.1186/s13104-020-05429-z.
35. Vaghela VV, Shah S. Distribution of ABO and RH blood groups in blood donors at the Tertiary Care Centre, Dahod. Biomed Pharmacol J. 2022;15(2):1133–40. doi:10.13005/bpj/2449.
36. Sun Y, Wang L, Niu J, Ma T, Xing L, Song A, et al. Distribution characteristics of ABO blood groups in China. Heliyon. 2022;8(9). doi:10.1016/j.heliyon.2022.e10568.
37. Tiruneh A, Yetneberk T, Gelaw M, Eshetie D. Frequency of ABO and Rh blood group distribution at Debre Tabor Blood Bank, Amhara Region, North-Central Ethiopia: a six-year retrospective survey. J Blood Med. 2020;11:357-361. doi:10.2147/JBM.S266624.
38. Groot HE, Villegas Sierra LE, Said MA, Lipsic E, Karper JC, van der Harst P. Genetically determined ABO blood group and its associations with health and disease. Arterioscler Thromb Vasc Biol. 2020;40(3):830–8. doi:10.1161/atvbaha.119.313658.
39. Browne A, Kinsella A, Keogh M, Morris K, Field S. A snapshot of ABO, RH, and JK blood group systems in modern Ireland. Health Sci Rep. 2021;4. doi:10.1002/hsr2.292.
40. Jenks MZ, Fairfield HE, Johnson EC, Morrison RF, Muday GK. Sex steroid hormones regulate leptin transcript accumulation and protein secretion in 3T3-L1 cells. Sci Rep. 2017;7(1). doi:10.1038/s41598-017-07473-5.
41. Vasan SK, Rostgaard K, Majeed A, Ullum H, Titlestad KE, Pedersen OBV, et al. ABO blood group and risk of thromboembolic and arterial disease: A study of 1.5 million blood donors. Circulation. 2016;133(15):1449–57. doi:10.1161/CIRCULATIONAHA.115.017563.
42. Hermansson J, Kahan T. Systematic review of validity assessments of Framingham risk score results in health economic modelling of lipid-modifying therapies in Europe. Pharmacoeconomics. 2018;36(2):205–13. doi:10.1007/s40273-017-0578-1.
43. Grand M, Dнaz A, Bia D. Cardiovascular risk prediction equations underestimate risk in people living with HIV: Comparison and cut-point redefinition for 19 cardiovascular risk equations. Curr HIV Res. 2022;20(2):137–51. doi:10.2174/1570162x20666220126124149.
44. Barton TJ, Low DA, Bakker EA, Janssen T, de Groot S, van der Woude L, et al. Traditional cardiovascular risk factors strongly underestimate the 5-year occurrence of cardiovascular morbidity and mortality in spinal cord injured individuals. Arch Phys Med Rehabil. 2021;102(1):27–34. doi:10.1016/j.apmr.2020.07.013.
45. Bansal M, Shrivastava S, Mehrotra R, Agarwal V, Kasliwal RR. Low Framingham risk score despite high prevalence of metabolic syndrome in asymptomatic North-Indian population. J Assoc Physicians India. 2009;57(Jan):17–22. Available from: https://pubmed.ncbi.nlm.nih.gov/19753753/
46. Azahar NMZM, Krishnapillai A, Hassan MRC, Dasiman R, Yusoff K. Predictors of cardiovascular disease using Framingham risk score in a rural population. Malays J Med Health Sci. 2022;18(8):340–7. doi:10.47836/mjmhs18.8.43.
47. Ahmad N, Panduragan SL, Chong Hong Soon, Gemini K, Yee San Khor, Bahrin NA, et al. Ten-year cardiovascular disease risk amongst workers in a tertiary healthcare institution in Kuala Lumpur. Borneo Epidemiol J. 2020;1(1):35–45. doi:10.51200/bej.v1i1.2435.
48. Adil SO, Uddin F, Musa KI, Khan A, Shakeel A, Shafique K, et al. Risk assessment for cardiovascular disease using the Framingham risk score and Globorisk score among newly diagnosed metabolic syndrome patients. Int J Gen Med. 2023;16(Aug):4295–305. doi:10.2147/ijgm.s42315.
49. Farhood HH, Abdulridha MK, Hadi H. The association between adverse pregnancy outcomes and laboratory measures as risk for cardiovascular disorders. Al Mustansiriyah J Pharm Sci. 2023;23(2):127–39. doi:10.32947/ajps.v23i2.1014.
50. Mohanraj J, D'Souza UJA, Siat AF, Jegasothy

R, Karkada IR. Predicting plasma leptin with anthropometric & bioelectrical impedance analysis measures of adiposity in a multiethnic young adult population in Malaysia. Eur J Mol Clin Med. 2020;7(6):3075–3088. Available from: https://eprints.ums.edu.my/id/eprint/26709/