Integrating CCL2 and TNF-α into the Framingham Risk Score for cardiovascular risk prediction: a cross-sectional study in a Malaysian cohort

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ABSTRACT

Introduction: Cardiovascular diseases (CVDs) are the leading cause of death worldwide, significantly contributing to increased healthcare costs and deteriorated health. In Malaysia, CVDs account for 20.79% of deaths in government hospitals. Key risk factors include high blood sugar levels, elevated blood pressure, and increased cholesterol levels. Atherosclerosis frequently serves as the underlying condition for coronary heart disease (CHD), with CCL2 and TNF-α playing a crucial role in recruiting immune cells to inflammation sites. Early diagnosis of CVDs risk is important for preventing severe complications. This cross-sectional study aims to investigate the relationship between biomarker CCL2 and TNF-α expression levels and Framingham Risk Score (FRS) categories in a Malaysian cohort.

Materials and Methods: A total of 333 patients from the Family Medicine Specialist Clinic at Hospital Sultan Abdul Aziz Shah were recruited between March 2022 and February 2023. Blood samples were taken after a 12-hour fasting period, and levels of fasting blood sugar (FBS), triglycerides (TG), total cholesterol (TC), HDL cholesterol, and LDL cholesterol were measured. 150 plasma samples were randomly selected for cytokine analysis of CCL2 and TNF-α using the Human Magnetic Luminex Assay. Patients' cardiovascular risk was assessed using the FRS calculator. The Kruskal-Wallis test was used to analyze the relationship between cytokine levels and FRS categories, followed by a post hoc test with Bonferroni correction. A logistic regression model was implemented to assess the independent effects of these variables.

Results: The results demonstrated a significant association between the level of chemokines CCL2 and pro-inflammatory TNF-α, and FRS categories (low-risk, moderate-risk, and high-risk). CCL2 levels were notably higher in the high-risk group, as were TNF-α levels, with both biomarkers showing increasing trends with higher risk categories, (p<0.001, effect size=0.32) and (p<0.001, effect size-0.29), respectively. Multiple logistic regression analysis showed that dyslipidaemia, FBS, and TNF-α remained significant after adjusting for other variables. Specifically, dyslipidaemia had lower odds of being in the high-risk group (AOR: 0.04), while FBS (AOR: 3.19) and TNF-α (AOR: 1.18).

Conclusion: This study highlights the potential of CCL2 and TNF-α as biomarkers for CVDs risk assessment. Integrating these biomarkers into CVDs risk prediction models may enhance the precision of identifying individuals at elevated risk. However, the study's cross-sectional design and small sample size for cytokine analysis constrain the findings. Future research should explore the long-term predictive value of these cytokines in larger, longitudinal cohorts and explore more advanced techniques for improving CHD risk prediction models.

KEYWORDS:
Cardiovascular diseases, atherosclerosis, Framingham Risk Score, CCL2, TNF-α

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death worldwide and have contributed significantly to increasing healthcare costs and deteriorated health. In Malaysia, CVDs are the number one factor of mortality contributing to 20.79% among the top ten causes of death in government hospitals, according to Malaysian Health Facts 2023.[1] Significant risk factors associated with CVDs include high blood sugar levels, elevated blood pressure, and increased cholesterol levels.[2] These factors are known to drastically increase the risk of developing CVDs, such as heart disease and stroke.

Atherosclerosis frequently serves as the underlying condition for coronary heart disease (CHD). CCL2 plays an important role in migrating various immune cells, such as monocytes, macrophages, and T lymphocytes to the inflammation site.[3] The association between CCL2 and atherosclerosis has been studied since 1991 until the present when researchers found out that CCL2 expression was relatively higher in atherosclerotic vessels than in normal vessels.[4] An increased expression of CCL2 has been strongly associated with macrophage infiltration across multiple layers of the arterial

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wall and subendothelial space, which causes the accumulation of the immune cells at the inflammatory site. Additionally, highly expressed CCL2 correlates with inflammatory processes in atherosclerotic lesions such as lipid deposition and fibrosis, contributing to the advanced stage of atherosclerosis.[5]

The interaction between CCL2 and its receptor CCR2 plays a critical role in monocyte recruitment, leading to their transformation into foam cells and the formation of fatty streaks in the arterial wall.[6,7] Macrophages activation triggers the release of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), promoting endothelial dysfunction, oxidized LDL accumulation, and atherosclerotic plaque rupture, leading to thrombosis and acute cardiovascular events.[8-10] By measuring TNF-α levels, we can determine the inflammatory dysregulation that contributes to arterial damage and cardiovascular events.

An early diagnosis of CVDs risk may help patients prevent severe complications and provide a personalized treatment. In Malaysia, the application of the FRS scoring system in the CVDs prediction model has been calibrated and validated for the Asian population by Chia et al.[11] However, its predictive accuracy has certain drawbacks, as it underrepresents younger individuals and excludes key factors like family history and diabetes.[12-16] This limitation underscores the need to incorporate additional biomarkers into the FRS prediction model. Therefore, this study aims to investigate the relationship between the expression level of cytokines TNF-α and CCL2, and the classification within the FRS groups in predicting cardiovascular disease risk in 10 years.

MATERIALS AND METHODS

A total of 352 patients attending the Family Medicine Specialist Clinic (FMSC) at Hospital Sultan Abdul Aziz Shah (HSAAS) located in Selangor were recruited in this study from March 2022 to February 2023. However, only 333 patients fulfilled the inclusion and exclusion criteria. Those with a diagnosis of any heart disease like having a New York Heart Association (NYHA) classification, incomplete clinical data in body mass index (BMI), and insufficient samples noticed during laboratory procedures were omitted from the study. Approval from the Institutional Ethics Board was secured from the Ethical Committee for Research Involving Human Subjects (JKEUPM-2021-700) before the start of the study. All eligible patients who participated in the study obtained their verbal and written consent.

Patients with ages ranging from 30 to 75 were recruited. The recruitment process was done by a convenient sampling method, during the early stage, where the researcher screened patients' medical histories to ensure they met the eligibility criteria. This study's inclusion criteria include adult patients 30 to 75 years old, without any cardiovascular events such as coronary heart disease or stroke. Patients with the following criteria were excluded: pregnant women, patients with a history of ischaemic heart disease and stroke, or the presence of conditions including active cancer, liver disorders, active autoimmune disease, and active thyroid. The patient's clinical data including sociodemographic data such as age, gender, race, education level, and marital status, and clinical information such as presence of chronic diseases, smoking status, the use of anti-hypertensive and lipid-lowering agents, based on their first medical entry were retrieved from patient's medical record. The patient's physical examination including systolic blood pressure (SBP) was also recorded. Due to budget limitations, 150 patients were randomly selected using a random number generator from the list of patients who met the inclusion criteria for each category. To ensure the sample represents the overall population, a randomization process was implemented to select 50 participants from each FRS category: low-risk (n=50), moderate-risk (n=50), and high-risk (n=50). This process involved using An a priori power analysis performed using g*power 3.1.9.7[17] to justify the sample size. Using a medium effect size (r=0.0588), power of 80%, and p of 0.05, g*power indicated that at least 50 participants were required.

A total of 10 mL venous samples were collected from the patients after a 12-hour fasting period. The collected blood was subjected to fasting blood sugar (FBS), level of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured. The remaining plasma was stored at -80°C for further plasma biomarker analysis. The study parameters such as gender, age, TC, HDL cholesterol level, SBP, smoking, and diabetes status were used to assess the patients' Framingham general CVDs risk score using the FRS calculator.[10] Their remaining collected blood samples were frozen at -80C for plasma biomarkers concentration measurement of CCL2 and TNF-α cytokines using Human Magnetic Luminex Assay (R&D Systems, Minneapolis, USA). The frozen plasma samples were thawed and diluted before being incubated with fluorescently labeled beads conjugated to specific antibodies for CCL2 and TNF-α. Following incubation, the beads were washed to remove unbound proteins and then incubated with a biotinylated detection antibody cocktail. After another washing step, streptavidin-phycoerythrin was added to bind the biotinylated antibodies, providing a fluorescent signal proportional to the amount of cytokine bound. The beads were then analyzed using the Luminex assay.

All statistical analyses were performed using IBM SPSS Statistics, version 29.0 (SPSS, Chicago, IL). Data are expressed as mean ± standard deviation or median (IQR) for skewed numerical data distribution. Kruskal Wallis test was performed to evaluate the relationship between cytokine level and FRS categories. Two-tailed $p<0.05$ was considered a significant difference. To evaluate the relationship between cytokine levels and FRS categories, the Kruskal-Wallis test, a non-parametric statistical method was used. This test is appropriate for comparing medians across three or more independent groups when the data do not meet the assumptions of normality. If a significant difference was observed (two-tailed $p<0.05$), it indicated that at least one group differed in cytokine levels. Further pairwise comparisons were conducted using a post hoc test with Bonferroni correction to identify specific group differences while controlling for multiple testing. Additionally, the potential confounders such as medications and comorbidities were addressed by modifying these variables in this study.

Multinomial logistic regression analysis was applied to measure parameters that were not included in FRS. In the first stage, univariate logistic regression was used to screen the potential confounders associated with FRS categories, identifying variables with a significance level of p<0.250 for inclusion in the multivariate analysis. These selected cofounders were then included in the multivariate logistic regression model to assess their independent associations with FRS categories. The results were reported as adjusted odds ratios (AOR) with corresponding 95% confidence intervals (CI) and p-values to assess the influence of each confounding factor on FRS categories.

RESULTS

From a total of 352 patients, 333 eligible patients, aged 30 to 75 years, who had no cardiovascular events and documented blood pressure, along with TC, HDL-C levels, smoking status, and presence or absence of diabetes mellitus (DM), were included. Table I shows the clinical characteristics of the total 333 patients with a median age of 55 years old, with gender distribution predominantly female (61.6%) and followed by male (38.4%). The study population is mainly represented by Malay (88%), with a smaller portion of Chinese (6.3%) and Indian (5.7%) ethnicities. Among all subjects, the prevalence of basal diseases shows a high rate of dyslipidaemia (76.6%), followed by hypertension (48.6%) and diabetes (31.8%). The FRS score analysis (Table I) showed that the population was almost equally divided into three risk categories: low-risk (37.8%), moderate-risk (26.7%), and high-risk (35.4%).

Based on the clinical characteristics of patients Table II, the average age increases with risk level, from a median of 46.50 years in the low-risk group to 62 years in the high-risk group. Similarly, the same pattern is observed in gender whereas the proportion of males increased across FRS groups, from 28% in the low-risk group to 52% in the high-risk group. In contrast, smoking prevalence is low overall, with 2% smokers in the low-risk group, and 8% in both moderate-risk and high-risk groups. Hypertension and dyslipidaemia prevalence shows an increasing trend with risk level, from 32% and 56% respectively in the low-risk group to 60% and 90% in the high-risk group. Conversely, DM is more prominent in the high-risk group (90%) compared to the low-risk and moderate-risk groups. TC levels are highest in the moderate-risk with a median of 5.66 mmol/L and lowest in the high-risk group at 4.73 mmol/L. HDL-C levels decreased with risk, from 1.32 mmol/L in the low-risk group to 1.20 mmol/L in the high-risk group but reported the highest median in the moderate-risk group (1.40 mmol/L).

To assess the relationship between FRS categories and levels of the cytokines CCL2 and TNF-α, we performed the Kruskal Wallis test. Figure 1(a) and Figure 1(b) demonstrate statistically significant differences in CCL2 and TNF-α levels, respectively, across low-risk, moderate-risk, and high-risk groups. For CCL2 (Figure 1(a)), the Kruskal-Wallis test indicates a significant difference among groups (p<0.05). Post hoc comparisons using Dunn's test show that CCL2 levels in the high-risk group are significantly higher than those in the low-risk group (p<0.001, effect size=0.32) and the moderate-risk group (p<0.050, effect size=0.15).

Similarly, for TNF-α (Figure 1(b)), the Kruskal-Wallis test confirms significant differences among the risk groups (p=0.001). Dunn's test reveals that TNF-α levels in the high-risk group are significantly higher than those in the low-risk group (p<0.001, effect size=0.46) and the moderate-risk group (p<0.05, effect size=0.29). However, the moderate-risk and low-risk groups also differ significantly (p<0.05) after applying Bonferroni correction.

To identify and quantify the association between potential confounding factors such as age, gender, comorbidities, and cardiovascular risk categories as determined by the FRS, we performed a multinomial logistic regression model. Initially, we screened the variable through univariate analysis with those showing a p-value <0.25 considered for inclusion in the multivariable analysis. While detailed data are not presented in a table, the key findings from the univariate logistic regression analysis are summarized below.

From nine potential cofounders that were screened from the univariate analysis: BMI, ethnicity, dyslipidaemia, heart rate, FBS, TG, LDL, CCL2, and TNF-α, six variables were found to be significant with a threshold of p<0.25. Dyslipidaemia and TNF-α are strongly associated with FRS groups, differentiating high-risk and low-risk groups. Both dyslipidaemia (COR: 0.14, 95% CI: 0.05–0.42, p<0.001), and TNF-α (COR: 1.24, 95% CI: 1.11–1.38, p<0.001) were more likely being in high-risk group. Similarly, FBS (COR: 3.43, 95% CI: 2.07–5.67, p<0.001), CCL2 (COR = 1.01, 95% CI: 1.001–1.10, p=0.004), TG showed a moderate association with odd ratio (COR: 1.90, 95% CI: 1.03–3.51, p=0.039), and LDL had less odd of being in high-risk group (COR: 0.59, 95% CI: 0.39–0.89, p=0.011) compared in moderate-risk group. However, BMI, ethnicity, and HR were not significant (p>0.25) and thus eliminated from further multivariate analysis.

Further analysis using multiple logistic regression analysis is summarised in Table III, dyslipidaemia, FBS, and TNF-α remained significant after adjusting for other variables. Specifically, dyslipidaemia has lower odds of being in high-risk groups, (AOR: 0.04, 95% CI: 0.01–0.28, p<0.001). Conversely, FBS (AOR: 3.19, 95% CI: 1.80–5.63, p<0.001), and TNF-α both had higher odds of being in the high-risk group (AOR: 1.18, 95% CI: 1.03–1.35, p=0.017). Other variables, such as TG, LDL, and CCL2, did not show significant associations after adjustment, indicating that their effects may be influenced by confounding factors.

DISCUSSION

This study demonstrated a significant association between higher FRS categories and elevated levels of chemokines CCL2 and pro-inflammatory cytokines TNF-α. These associations were observed in the study population presented in Table II, which highlighted the risk factors of CVDs such as age, gender distribution, smoking status, DM, hypertension, dyslipidaemia, and blood profile; TG, FBS, and HDL, in the subset of 150 participants. In this study, males at the median age of 55 years old are more susceptible to developing CHD compared to females. The findings are aligned with the previous studies in assessing gender differences in

Table I: Demographic description of the study population, N = 333

Demographic	n (%)
Age (year)	55.00 (44.00-63.00)[a]
Gender	
Male	128 (38.4)
Female	205 (61.6)
Ethnicity	
Malay	293 (88.0)
Chinese	21 (6.3)
India	19 (5.7)
Smoking	21 (6.3)
Medical illness	
Diabetes	106 (31.8)
FBS (mmol/L)	5.40 (4.95-6.50)a
Hypertension	162 (48.6)
SBP (mmHg)	134.67 ± 16.11e
Dyslipidaemia	255 (76.6)
Total cholesterol (mmol/L)	5.33 (4.52-6.10)a
High-density lipoprotein (mmol/L)	1.37 (1.17-1.60)a
FRS	
Low	126 (37.8)
Moderate	89 (26.7)
High	118 (35.4)

[a]: median (IQR: 75-25). [e]: mean ± SD.
FRS: Framingham Risk Score
FBS: Fasting Blood Sugar
SBP: Systolic Blood Pressure

Table II: Patients' clinical characteristics according to FRS categories, N= 150

Demographic	Low (%) (n=50)	Moderate (%) (n=50)	High (%) (n=50)
Age (year)	46.50(40.25-54.25)[a]	59.0(50.75-64.0)[a]	62.0(54.0-67.0)[a]
Gender			
Male	14(28)	21(42)	26(52)
Female	36(72)	29(58)	24(48)
Ethnicity			
Malay	42(84)	42(86)	42(88)
Chinese	4(8)	5(10)	2(4)
India	4(8)	2(4)	4(8)
Smoking	1(2)	4(8)	4(8)
Medical illness			
Diabetes	0	0	45(90)
FBS (mmol/L)	5.00 (4.71-5.38)[a]	5.27(4.81-5.84)[a]	7.20(5.98-8.78)[a]
Hypertension	16(32)	26(52)	30(60)
SBP (mmHg)	125.68 ± 13.88[e]	141.14 ± 17.55[e]	137.34 ± 13.19[e]
Dyslipidaemia	28(56)	38(76)	45(90)
TC (mmol/L)	5.38(4.98-6.17)[a]	5.66(4.95-6.62)[a]	4.73(3.75-5.85)[a]
HDL-C (mmol/L)	1.32(1.13-1.61)[a]	1.40(1.20-1.60)[a]	1.20(1.07-1.50)[a]

[a]: median (IQR: 75-25). [e]: mean ± SD
FRS: Framingham Risk Score
FBS: Fasting Blood Sugar
SBP: Systolic Blood Pressure
TC: Total Cholesterol
HDL-C: High-Density Lipoprotein

determining CVDs risk. A study revealed that the incidence of low, moderate, and high-risk CVDs among men is significantly higher than among women ($p<0.05$).[18] Several factors could contribute to the pathogenesis of atherosclerosis such as age, lifestyle, physical activities, and hormones. Males generally have a higher level of testosterone which may contribute to an increasing lipid profile and could increase the likelihood of developing CHD.[19] Additionally, the high-risk group had significantly higher age, SBP, and FBS concentrations compared to those in low-risk and moderate-risk groups. Another study has reported a similar pattern where patients with high SBP and FBS are more likely to be classified in the moderate-risk and high-risk groups of cardiovascular disease.[16]

The prevalence of hypertension and dyslipidaemia increases with higher FRS categories, with nearly 60% of high-risk individuals having hypertension and 90% with dyslipidaemia. These results reflect a common cardiovascular risk profile observed in Asian populations where factors like

Table III: Association between potential confounders on FRS using multivariate logistic regression, N=150

Potential Confounders	Moderate vs. Low		High vs. Low	
	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Dyslipidaemia	0.40(0.16-1.01)	0.054*	0.04(0.01-0.28)	0.001*
FBS	1.18(0.71-1.94)	0.529	3.19(1.80-5.63)	<0.001*
TG	1.04(0.54-2.03)	0.898	1.32(0.63-2.75)	0.458
LDL	1.33(0.85-2.06)	0.210	0.61(0.35-1.08)	0.089
CCL2	1.00(0.99-1.01)	0.325	1.00(0.99-1.02)	0.277
TNF-α	1.12(1.00-1.12)	0.047	1.18(1.03-1.35)	0.017*

*: Significant. AOR: Adjusted odds ratio. vs.:Versus

Table IV: Summary of studies related to TNF-α and CCL2 expression in atherosclerosis

TNF-α			
Atherosclerotic progression	**Study Population**	**Findings**	**References**
Early-stage atherosclerosis	apoE-/-/LDL receptor-/- mice	Expression of medial TNF-α and its receptor happens before the atherosclerotic lesions	[26]
Endothelial activation	Human umbilical vein endothelial cells (HUVECs)	TNF-α enhances the movement of LDL across endothelial cells via NF-κB and PPAR-γ activation	[27]
Monocyte recruitment	CHD patients	Overexpression of TNF-α activates the monocyte recruitment forming cholesteryl ester-laden cells	[28]
Plaque destabilization	Patient with symptomatic and asymptomatic carotid stenosis	TNF-α mediates the activation of TREM-1 in VSMCs isolated from symptomatic plaque	[29]
CCL2			
Atherosclerotic progression	**Study Population**	**Findings**	**References**
Early-stage atherosclerosis	CAD patients	CCL2 inhibited the internalization of HDL phospholipids and proteins	[30]
Endothelial activation	Cell culture	CCL2 influences the effects of miR-495 on the proliferation and apoptosis of HUVECs	[31]
Monocyte recruitment	Mice mode	The highest levels of CCL2 are found in neutrophils and circulating monocytes	[32]
Plaque destabilization	Cell culture	Smooth muscle cells (SMCs) secrete CCL2 in the human atherosclerotic plaque and murine SMC line when subjected to inflammatory cytokine in vitro.	[33]

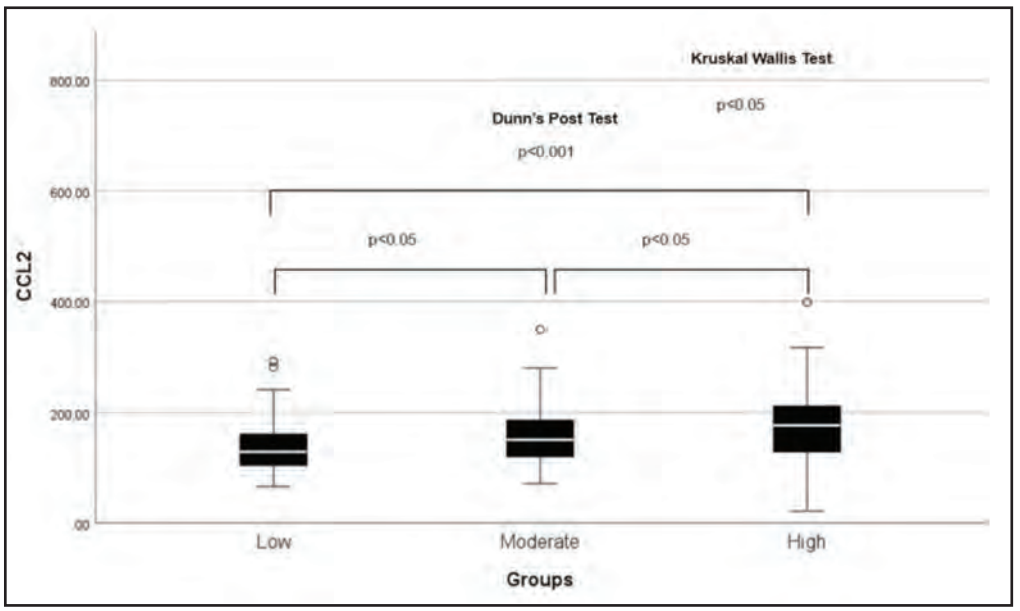

Fig. 1a: Distribution of CCL2 across FRS groups. Higher CCL2 levels in the high-risk group suggest a link to increased cardiovascular risk. Significant differences were found between low-risk vs. high-risk, and moderate-risk vs. high-risk

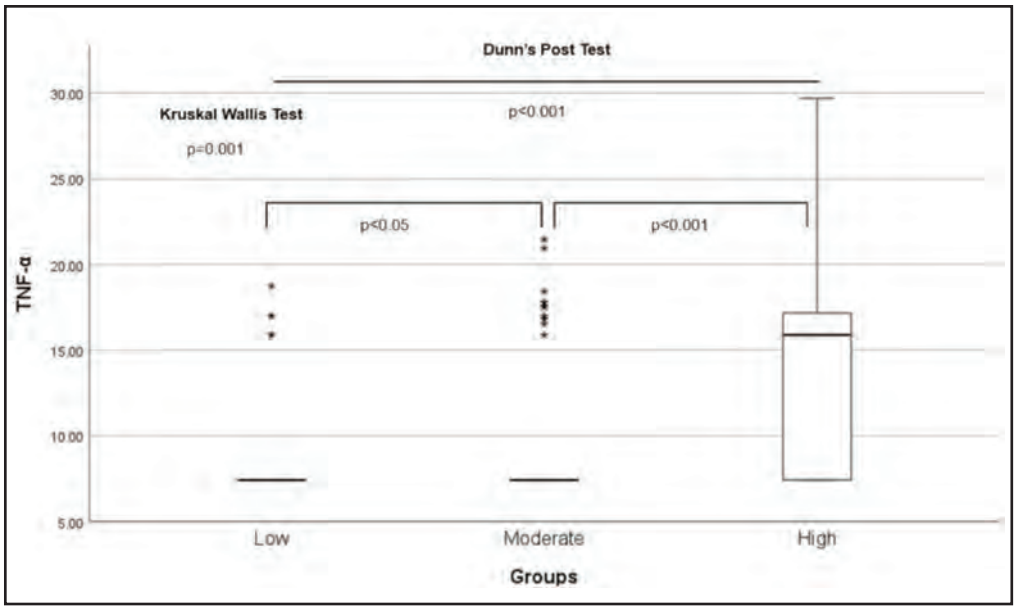

Fig. 1b: Distribution of TNF-α across FRS groups. Elevated TNF-α in a high FRS group may indicate increased inflammation with higher risk. Significant differences were observed between the high-risk and both low-risk and moderate-risk groups

genetics and individual lifestyles significantly influence cardiovascular risk.[20] Hypertension is known to contribute significantly to cardiovascular risk by promoting arterial damage and atherosclerosis. The arterial injury and plaque buildup will trigger the release of chemokines and pro-inflammatory cytokines will work together to regulate the inflammatory responses within the arterial wall.[21,6] The atherosclerotic plaque will secrete more inflammatory cytokines such as TNF-α, interleukin-1, interleukin-6, and interferon-gamma, as well as macrophages at the site of endothelial cells.[22]

As the inflammatory process progresses, CCL2 and TNF-α contribute significantly to the recruitment of immune cells to the site of plaque formation. In our study, we observed an increase in CCL2 and TNF-α levels with higher FRS categories; low-risk, moderate-risk, high-risk. However, TNF-α concentration increased relatively lower than the expression level of CCL2. This is consistent with the general understanding that both cytokines play a part in the inflammatory process, CCL2 tends to have a more prominent role in monocyte recruitment and plaque progression. In contrast, TNF-α contributes more to the inflammatory cascade amplification. The result is aligned with a previous study that showed that TNF-α plays a critical role in initiating and amplifying inflammatory responses by inducing multiple cytokines and chemokines (CCL2, CCL5, ICAM-1, VCAM-1, and IL-6).[23]

Conversely, an increased level of CCL2 will facilitate the process of oxidized LDL ingestion by macrophages to form foam cells.[22] CCL2 promotes the monocytes recruitment to the endothelial layer, where they infiltrate and differentiate into macrophages.[24] Georgakis et al. have researched human atherosclerotic plaque exploring the role of inflammatory markers including CCL2 in plaque vulnerability and progression. They found that CCL2 levels correlate with the marker of plaque instability, for example, pro-inflammatory characteristics and matrix turnover which lead to increased cardiovascular events.[25] Another study has reported a similar pattern in CCL2 expression level, where the concentration of CCL2 (>9 fold) is significantly apparent in all artery layers, particularly in adventitial tissue. The results contrast to only 26% in normal arteries and localized to smooth muscle cells.[23]

The significant associations were observed in our Kruskal-Wallis and post hoc Dunn's tests for CCL2 and TNF-α play an important role in inflammation in cardiovascular risk stratification. Increased CCL2 and TNF-α levels in high-risk categories align with other studies that highlight these markers as predictors of adverse cardiovascular events. Given their role in immune cell recruitment and inflammation, both markers offer insight into how immune activation inflammation underpins cardiovascular risk. Table IV summarises studies supporting the role of CCL2 and TNF-α mediating the inflammatory processes involved in atherosclerosis progression at various stages.[26-33]

This study also highlighted the significance of various confounders in predicting CVDs risk as assessed by the FRS. Specifically, TNF-α showed a significant odd ratio of being in high-risk groups even after adjusting for other variables, this predictor may enhance CVDs risk assessment using FRS. Our findings aligned with a previous study conducted by Yuan et al[34], demonstrating that increased TNF levels are associated with a higher risk of common CVDs, with genetically predicted TNF levels showing a positive association with CAD. While FRS provides a well-established framework for predicting CVDs risk based on parameters; age, gender, TC level, HDL, SBP, DM status, smoking, and hypertension medication. This assessment underestimates other cofounders like biomarkers, dyslipidaemia status, TG, HDL, and FBS levels which may lead to inaccurate risk assessment affecting

a patient's CVDs risk profile.[35] A similar case was reported by Qiu et al.,[36] where FRS underestimated CHD events by 22% for the total population, while overestimated for males by 152%.

The current study provides a predictive model to analyze the relationship between inflammatory biomarkers, CCL2 and TNF-α, and cardiovascular risk as stratified by the FRS. Increased levels of CCL2 have been linked to increased plaque vulnerability, while TNF-α contributes to endothelial dysfunction and the amplification of the inflammatory response, both of which are crucial in the atherosclerosis progression in all stages. Therefore, incorporating these biomarkers into risk prediction models FRS could provide a stronger and more accurate result, especially for low-risk and moderate-risk groups. To implement this predictive model into clinical practice, further studies should be conducted to validate the biomarker across diverse populations and evaluate its reliability in different clinical settings.

The strength of the current study is the comprehensive assessment of cytokine levels across a well-characterized cohort with varying cardiovascular risk profiles. The demographic and clinical data provided in Table I and Table II add depth to the analysis performed. However, several limitations of our study should be considered. Firstly, the study was conducted in a cross-sectional design, and we did not follow up with the patients over time. Secondly, the relatively small sample size of 150 for cytokine measurement may reduce the accuracy of our findings. A larger sample size would be needed to increase the sensitivity towards the association within the FRS groups. Thirdly, the study was done within a single centre, the subjects were predominantly Malay population (88%), which does not fully represent the general population. Lastly, potential confounding factors, such as underlying comorbidities and medication use, were not fully controlled, which could influence cytokine levels. Future studies should extend to longitudinal studies to establish the predictive value of CCL2 and TNF-α levels over time and may consider advanced imaging techniques and genetic implications to improve the prediction of CHD risk.

CONCLUSION

This study highlights the potential of CCL2 and TNF-α as biomarkers for CVDs risk assessment. Integrating these biomarkers into CVDs risk prediction models may enhance the precision of identifying individuals at elevated risk, hence supporting clinical intervention and personalized care management. However, the study's cross-sectional design and small sample size for cytokine analysis constrain the findings. Future research should explore the long-term predictive value of these cytokines in larger, longitudinal cohorts and explore more advanced techniques for improving CHD risk prediction models.

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