Research Article

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Inflammatory biomarkers and risk of atherosclerotic cardiovascular disease

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Abstract: Background: Non-alcoholic fatty liver disease is an increasing health issue that associates with the development of atherosclerotic cardiovascular disease. This study correlates the association between fatty liver and inflammatory biomarkers with cardiovascular risk scores.

Methodology: This cross-sectional study enrolled 10,181 health examination participants from Northern Taiwan and administered a standardized questionnaire with important biochemical tests and abdominal sonography. To assess concentrations of inflammatory markers high sensitivity C-reactive protein (hs-CRP) and fibrinogen were used.

Results: Inflammatory marker levels were significantly increased with increasing fatty liver. In multivariate logistic regression analysis adjusted for major confounding factors, the odds ratios of elevated hs-CRP and fibrinogen were significantly higher in participants with mild or moderate-to-severe fatty liver compared to healthy individuals. The cardiovascular risk scores, above cut-off level 10%, were associated with higher levels of inflammatory biomarkers and fatty liver; odds ratio, 3.52 (2.60-4.77) for non-alcoholic fatty liver disease with hs-CRP, and 2.92 (2.12-4.00) for non-alcoholic fatty liver disease with fibrinogen.

Conclusion: Inflammatory biomarkers (hs-CRP and fibrinogen) are significantly associated with augmentation of fatty liver. Non-alcoholic fatty liver disease may be a predictor of future atherosclerotic cardiovascular disease, and the prediction value increases on adding inflammatory biomarkers levels.

Keywords: Non-alcoholic fatty liver disease; Atherosclerosis; Cardiovascular risk; Inflammatory biomarkers

1 Introduction

In many industrialized countries, non-alcoholic fatty liver disease (NAFLD) is a very common liver disease, with an estimated worldwide prevalence of nearly 25% [1]. A study conducted in Netherlands documented about 22% of the population are suffering from fatty liver [2]. According to the Third National Health and Nutrition Examination Survey performed in the United States, the prevalence was 19.0% [3]. In Taiwan, the prevalence was estimated to be about 21%-43%, according to two cross-sectional studies [4,5].

There is a close relationship between NAFLD, metabolic syndrome and cardiovascular disease; patients with fatty liver often have one or more components of metabolic syndrome [6,7]. Systemic inflammation and subclinical atherosclerosis, which are both predictors of the risk of atherosclerotic cardiovascular disease (ASCVD), are associated with NAFLD [8,9]. Several inflammatory mediators are known to be associated with atherosclerosis, and these include high-sensitivity C-reactive protein (hs-CRP), fibrinogen and TNF-alpha and IL-1b [8-10].

Moreover, clinical associations among NAFLD, systemic inflammation, and ASCVD, the present study aimed to investigate the association between fatty liver and inflammatory biomarkers with cardiovascular risk scores.

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2 Materials and methods

2.1 Participants

This study involved a cross-sectional analysis of a database from a health center in Northern Taiwan. All participants were adults who underwent a health examination in a health center. We excluded those with viral hepatitis, heavy alcohol consumption, and aged more than 65 years. The final study population included 10,181 participants.

2.2 Methods

We conducted this study according to the ethical principles outlined in the Declaration of Helsinki. The study was approved by Institutional Review Board of Mackay Memorial Hospital (ID 12MMHIS092). Informed consent has been obtained from all individuals included in this study.

In the health check-up, well-trained physicians and nurses obtained comprehensive personal history and performed physical assessment and laboratory examination. We extracted personal data, such as age, sex, medical history, cigarette smoking history, and exercise habit. The participants underwent routine physical check-up and systolic and diastolic blood pressure monitoring; their body mass index and waist circumference were recorded. Blood samples were obtained after fasting for 10 h and were evaluated for liver function (alanine aminotransferase), creatinine, electrolytes, complete blood cell count, hemoglobin level, lipid profile, fasting plasma glucose level, hs-CRP level, and fibrinogen level. Participants also underwent abdominal sonography examination to detect the presence of fatty liver. The severity assessment of fatty liver is mainly based on the echogenicity difference between liver and kidney, the extent of blurring of intrahepatic vessel and diaphragm [11,12]. We made the diagnosis of metabolic syndrome based on the presence of ≥3 of the components listed by the revised adult treatment panel III of the National Cholesterol Education Program [13]. We performed ASCVD risk evaluation according to the Framingham risk score, which includes the parameters of age, sex, total cholesterol, high-density lipoprotein cholesterol, smoking, systolic blood pressure, the presence of diabetes and treated or untreated hypertension [14]. A 10-year Framingham risk score of ≦10% is defined as low ASCVD risk [15], and the risk score >10%, is categorized as high risk in our study.

2.3 Statistical analysis

All statistical analyses were performed using SPSS 24 package (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as mean (standard deviation) or median with interquartile range. The Student's *t*-test, chi-square test, and Kruskal–Wallis test were used to analyze the difference in demographic and basic characteristics of the study sample according to the presence of fatty liver and to analyze the difference in inflammatory serum marker levels between different fatty liver groups. To investigate the relationship between serum inflammatory markers and fatty liver, we performed multiple logistic regression analysis. The results are presented as odds ratios with 95% confidence interval (95% CI). Results are considered statistically significant if the $p < 0.05$.

3 Results

The demographic and basic characteristics of the participants are shown in Table 1. A total of 10,181 participants were identified and included. Among the participants, 6,491 (63.8%) had NAFLD, including 4,795 (47.1%) with mild NAFLD and 1,696 (16.7%) with moderate-to-severe NAFLD. There were significant differences in age ($p < 0.001$), sex ($p < 0.001$), current smoking ($p < 0.001$), metabolic syndrome ($p < 0.001$), hs-CRP levels ($p < 0.001$), and fibrinogen levels ($p < 0.001$) among the non-NAFLD, mild NAFLD, and moderate-to-severe NAFLD groups.

The results of multiple logistic regression analysis for inflammatory markers and fatty liver severity are shown in Table 2. In model 1, we analyzed the association between hs-CRP (cut-off level: hs-CRP ≥ 75th percentile) and fatty liver severity. The odds ratio between the mild NAFLD and non-NAFLD groups was 2.05 (95% CI: 1.81–2.31, $p < 0.001$), and the odds ratio between the moderate-to-severe NAFLD and non-NAFLD groups was 3.79 (95% CI: 3.18–4.51, $p < 0.001$). In model 2, we analyzed the association between fibrinogen level (cut-off level: fibrinogen ≥ 75th percentile) and fatty liver severity. The odds ratio between the mild NAFLD and non-NAFLD groups was 1.28 (95% CI: 1.16–1.41, $p < 0.001$), and the odds ratio between the moderate-to-severe NAFLD and non-NAFLD groups was 1.55 (95% CI: 1.42–1.81, $p < 0.001$). We adjusted the effects of major confounding variables, including age, sex, and the presence of metabolic syndrome.

The results of multiple logistic regression analysis for the association between higher ASCVD risk (Framingham risk score ≥ 10%) and NAFLD combined with the

Table 1: Demographic and basic characteristics of participants according to NAFLD severity

	Non-NAFLD	Mild NAFLD	Moderate-to-severe NAFLD	p
n (%)	3690 (36.2)	4795 (47.1)	1696 (16.7)	
Age (years)	45.3 ± 8.4	48.6 ± 8.1	48.5 ± 8.4	<0.001
Sex (Female)	2177 (59.0)	1434(29.9)	295 (17.4)	<0.001
hs-CRP* (mg/dL)	0.5 (0.7)	0.9 (1.5)	1.6 (2.3)	<0.001
Fibrinogen** (mg/dL)	297.0 (69.0)	308.0 (72.0)	315.0 (72.5)	<0.001
Current smoker	2052 (55.6)	3040 (63.4)	1157 (68.2)	<0.001
Metabolic syndrome	207 (5.6)	1573 (32.8)	1079 (63.6)	<0.001
Framingham score >10%	181 (11.6)	868 (31.7)	431 (42.3)	<0.001

hs-CRP* and fibrinogen** are presented as median (interquartile range). Continuous variables are shown as mean ± standard deviation. Categorical variables are shown as number (percentage).
hs-CRP, high-sensitivity C-reactive protein; NAFLD, non-alcoholic fatty liver disease.

Table 2: Multiple logistic regression for the association between NAFLD and inflammatory markers (hs-CRP and fibrinogen)

Variable	Odds ratios (95% CI)
Model 1 (hs-CRP ≥ 75th percentile)	
Non-NAFLD	1.00
Mild NAFLD	2.05 (1.81–2.31)
Moderate-to-severe NAFLD	3.79 (3.18–4.51)
Model 2 (fibrinogen ≥ 75th percentile)	
Non-NAFLD	1.00
Mild NAFLD	1.28 (1.16–1.41)
Moderate-to-severe NAFLD	1.55 (1.42–1.81)

Odds ratios were adjusted according to age, sex, and the presence of metabolic syndrome. CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; NAFLD, non-alcoholic fatty liver disease.

Table 3: Multiple logistic regression for the association between high ASCVD risk (Framingham risk score ≥10%) and NAFLD combined with the status of inflammatory markers (hs-CRP and fibrinogen)

Variable	Odds ratios (95% CI)
Model 1 (Framingham risk score ≥10%)	
Non NAFLD with non-higher range of hs-CRP	1.00
NAFLD with non-higher range of hs-CRP	1.90 (1.47-2.45)
NAFLD with higher range of hs-CRP*	3.52 (2.60-4.77)
Model 2 (Framingham risk score ≥10%)	
Non NAFLD with non-higher range of fibrinogen	1.00
NAFLD with non-higher range of fibrinogen	2.08 (1.49-2.90)
NAFLD with higher range of fibrinogen*	2.92 (2.12-4.00)

*Higher range of hs-CRP or fibrinogen: ≥75th percentile of hs-CRP or fibrinogen. Odds ratios were adjusted according to age, sex, and the presence of metabolic syndrome.
ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; NAFLD, non-alcoholic fatty liver disease

status of inflammatory markers are presented in Table 3. In model 1, we analyzed the association between higher hs-CRP levels (cut-off level: hs-CRP ≥ 75th percentile) and fatty liver. The odds ratio between the group of NAFLD plus higher hs-CRP levels and the group of non-NAFLD plus non-higher hs-CRP levels was 3.52 (95% CI: 2.60–4.77, $p < 0.001$), and the odds ratio between the group of NAFLD plus non-higher hs-CRP levels and the group of non-NAFLD plus non-higher hs-CRP levels was 1.90 (95% CI: 1.47–2.45, $p < 0.001$). In model 2, we analyzed the association between higher fibrinogen levels (cut-off level: fibrinogen ≥ 75th percentile) and fatty liver. The odds ratio between the group of NAFLD plus higher fibrinogen levels and the group of non-NAFLD plus non-higher fibrinogen levels was 2.92 (95% CI: 2.12–4.00, $p < 0.001$), and the odds ratio between the group of NAFLD plus non-higher fibrinogen levels and the group of non-NAFLD plus non-higher fibrinogen levels was 2.08 (95% CI: 1.49–2.90, $p < 0.001$). We adjusted the effects of major confounding variables, including age, sex, and the presence of metabolic syndrome.

4 Discussion

Several previous studies have investigated the relationship between hs-CRP and fatty liver [16]. Al Rifai et al. conducted a cross-sectional analysis of 3,976 participants from the Multi-Ethnic Study of Atherosclerosis (MESA), which diagnosed fatty liver through computed tomography imaging, and showed that NAFLD was associated with a prevalence odds ratio for hs-CRP ≥ 2 mg/L [17]. Furthermore, Wang et al. performed a single-center population-based study with 8,618 participants and revealed that the hs-CRP level was independently associated with NAFLD [18].

Few studies have targeted the relationship between fibrinogen and fatty liver [19]. Sesti et al. performed a cross-sectional study assessing 400 individuals with ultrasonography-diagnosed steatosis and found that individuals with both high and intermediate probabilities of fibrosis showed an unfavorable cardiometabolic risk profile with significantly higher hs-CRP and fibrinogen levels [20]. However, Verrijken et al. conducted a study with 273 patients involving liver biopsy and showed that NAFLD severity independently contributed to an increase in plasminogen activator inhibitor-1 (PAI-1) levels, but not other coagulation factors, including fibrinogen [21].

In our study, hs-CRP and fibrinogen levels were significantly higher in the mild and moderate-to-severe NAFLD groups than in the non-NAFLD group (Table 1). The presence of metabolic syndrome was also significantly higher in the mild and moderate-to-severe NAFLD groups; this was consistent with the findings of a previous study [5]. We further investigated the association between fatty liver and inflammatory markers through multiple logistic regression analysis, and a positive relationship between fatty liver severity and increasing levels of inflammatory markers was established after adjusting for major confounding factors, including age, sex, and the presence of metabolic syndrome (Table 2). Few studies have discussed the relationship between inflammatory marker levels and fatty liver severity. Ajmal et al. performed a cross-sectional study including 104 patients with coronary artery disease and hypertensive heart disease and found that hs-CRP levels were significantly higher in patients with severe NAFLD [9].

Some studies have demonstrated a relationship between ASCVD risk and fatty liver. Sesti et al. showed that individuals with a high probability of fibrosis have a high Framingham risk score [20]. Hamaguchi et al. conducted a 5-year prospective observational study with a total of 1,637 healthy Japanese individuals, and the incidence of cardiovascular disease was higher in 231 participants with NAFLD at baseline (five coronary heart diseases, six ischemic stroke, and one cerebral hemorrhage) than in 990 participants without NAFLD (three coronary heart disease, six ischemic stroke, and one cerebral hemorrhage) [22]. Kim et al. performed a population-based cohort study with 11,154 participants and showed that ultrasonography-diagnosed NAFLD was not associated with increased all-cause and cardiovascular disease mortality; however, NAFLD with advanced fibrosis (defined by the NAFLD fibrosis score) was independently associated with increased all-cause and cardiovascular disease mortality [23]. Furthermore, many previous studies have demonstrated a positive relationship between inflammatory biomarker levels and ASCVD risk [24-27]. In Table 3, we can see that NAFLD itself was a predictor of future ASCVD, and the prediction value increased on the addition of hs-CRP and fibrinogen, which are both non-invasive markers for liver fibrosis.

5 Strengths and limitations

The large number of participants in our study is an important factor that added significant level of statistical confidence in the analyzed data and interpretation, which also is helpful for confident application of the present results in clinical settings. Although fatty liver is a well-known risk factor for ASCVD, the inter-relationship between fatty liver, inflammation markers and ASCVD is not established, and the paucity of studies on fibrinogen and fatty liver further enhances the significance of our present study. However, the present study also has some limitations. First, the diagnosis and severity classification of fatty liver, accomplished using ultrasound, is operator-dependent and the interpretations could be subjective. Second, this is a cross-sectional study, and therefore, the cause-and-effect relationship needs to be further clarified. Lastly, additional inflammation markers, which are associated with atherosclerosis, such as TNF-alpha, should be investigated to make this study more comprehensive.

6 Conclusion

Both NAFLD and ASCVD are growing public health problems; the early reorganization of future ASCVD risk is vital. We found correlations among fatty liver, inflammatory markers, and ASCVD. We further showed that fatty liver severity and inflammatory marker levels play important role in the prediction of ASCVD risk. The increased pre-

diction value of ASCVD risk, by including inflammation markers along with the severity of fatty liver, enhances its utility in clinical practice. Because ultrasound is readily available and is non-invasive, it can be easily-used in combination with simple blood test for inflammation markers. We believe that wide application of the presently described time and cost-efficient tools, and early lifestyle modifications applied to large population can help in reducing the ASCVD risk and the associated socioeconomic burden.

Conflicts of interest: The authors have no conflicts of interest to declare.

Abbreviations: ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio

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