Association between MHR and MAFLD: A Single-center Retrospective Study

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Abstract

Background

Metabolic dysfunction-associated fatty liver disease (MAFLD) has high incidences and is one of the major hepatic diseases. Chronic low-grade inflammation has been considered to be an important pathogenesis of MAFLD. Monocyte /HDL-C ratio (MHR) is a novel marker of inflammation and oxidative stress. This study attempted to explore the correlation between MHR and MAFLD.

Methods

This study enrolled a total of 705 adults with MALFD and 1,505 healthy subjects as the control group. All participants accepted the anthropometric and laboratory tests. MHR was acquired as monocytes count divided by high-density lipoprotein concentration. After adjusting sex, age, BMI, blood pressure, hepatic enzyme, uric acid, fasting glucose, triglyceride, LDL-C, smoking, hypertension, diabetes, hyperlipidemia, we analyzed the correlation between MHR and MAFLD.

Results

An increased MHR was identified in the MAFLD group, and MHR correlated with BMI, diabetes history, and metabolic abnormalities. Univariate and multiple logistic regression analysis showed that MHR was associated with MAFLD. After adjusting for potential confounders, a non-linear relationship was found between MHR and MAFLD, and the inflection point was 0.396 in the non-linear curve. On the left of the inflection point, MHR positively correlated with MAFLD (OR = 1.459, 95% confidence interval (CI): 1.196 to 1.781, p < 0.001). However, there was no obvious relationship on the right (OR = 0.934, 95% CI: 0.797 to 1.096, p = 0.403). Interaction analysis showed that the association between MHR and MAFLD was significant in people less than 60, non-obese, without a history of diabetes, and without severe metabolic abnormalities.

Conclusion

MHR increased in adults with MAFLD. MHR positively correlated with MAFLD when less than 0.396. Therefore, MHR could be used as a predictor of MAFLD.

Background

In 2020, an international panel of experts proposed to replace the definition of non-alcoholic fatty liver disease (NAFLD) with metabolic dysfunction-associated fatty liver disease (MAFLD) [1]. The new diagnostic criteria no longer excluded the state of alcohol consumption and other liver diseases such as viral hepatitis and paid more attention to the roles of metabolic dysfunctions in the prevalence of MAFLD.
According to the new definition, overweight/obesity, type 2 diabetes mellitus (T2DM), and metabolic abnormalities were the etiology of MAFLD. Inflammation, oxidative stress, and insulin resistance are the key mechanisms of MAFLD, and these metabolic indicators play a significant role in the pathogenesis of MAFLD [3, 4]. Importantly, the liver is the most important organ for glucose and lipid metabolism. Lipid droplets accumulate in the liver parenchyma, and MAFLD occurs when the synthesis and uptake of liver fatty acids exceed their oxidation and output capacity [5]. However, it is not a single risk factor, but the interaction of many factors that leads to the progression of MAFLD, and MAFLD can develop in non-obese, non-diabetic people [6]. Uncontrolled inflammation may have a unique role in pathophysiological basis of MAFLD independent of metabolic abnormalities [7, 8]. Patients with MAFLD do not necessarily meet every diagnostic criterion, and the key pathogenesis may differ among different populations according to the updated criteria.

Monocytes accelerate inflammatory response by secreting pro-inflammatory and pro-oxidant cytokines. Macrophages derived from monocytes are the main macrophages in liver, and cytokines produced by macrophages, such as IL-6, TNF-α, IL-1β, directly target hepatocytes and promote steatosis, inflammation, and hepatocyte damage, IL-1β and IL-6 levels were increased in NAFLD peripheral blood monocytes [9, 10]. High-density lipoprotein cholesterol (HDL-C) has anti-inflammatory effects and inhibits the oxidization of endotheliocytes. Monocyte /HDL-C ratio (MHR) is a newly proposed marker representing increased inflammatory response and decreased anti-inflammatory and antioxidant capacity [11]. MHR significantly increases with obesity, polycystic ovary syndrome, metabolic syndrome, and diabetes [12, 13]. In addition, increased MHR positively correlates with metabolic indexes, such as serum uric acid, blood glucose, and serum lipids [14, 15]. Previous studies have demonstrated that MHR is significantly higher in NAFLD and the non-alcoholic hepatic steatosis group [16, 17]. However, whether MHR is involved in the occurrence of MAFLD and how MHR correlates with characteristics of MAFLD warrants further investigation. Therefore, our study aims to observe the level of MHR in patients with MAFLD and identify the relationship between MHR and MAFLD.

**Methods**

**Participants**

A retrospective study was conducted at the Physical Examination Center of the Second Hospital of Hebei Medical University from July 2021 to March 2022. We selected 705 patients with MAFLD, designated as the MAFLD group, and 1,505 healthy subjects designated as the control group. The ethical approval of this study was obtained from the Ethics Committee of the Second Hospital of Hebei Medical University.

MAFLD was diagnosed using the International Expert Consensus Statement on Fatty liver Disease associated with Metabolic Dysfunction [2]. The diagnosis of MAFLD was based on hepatic ultrasonography of fat accumulation in the liver in addition to one of the following three criteria: overweight or obesity (BMI ≥ 23 kg/m²), type 2 diabetes mellitus (history of T2DM), or two or more of the following metabolic risk abnormalities (blood pressure ≥ 130/85 mm Hg or specific drug treatment;
triglycerides ≥1.70 mmol/L or specific drug treatment; HDL-cholesterol <1.0 mmol/L for men and <1.3 mmol/L for women or specific drug treatment; prediabetes, fasting glucose 5.6 to 6.9 mmol/L or HbA1c 5.7% to 6.4%; high-sensitivity C-reactive protein > 2 mg/L).

Participants diagnosed with the following disorders were excluded from the study: chronic liver diseases caused by drugs, virus and autoimmune diseases, severe cardiac and renal insufficiency, pregnant and lactating women, malignancy, BMI ≥ 40 kg/m².

**Methods**

Demographic and Anthropometric data were collected at baseline, including age, sex, family history, personal history, and smoking history. Height, body mass, systolic blood pressure, and diastolic blood pressure were measured, and body mass index (BMI) was calculated.

Four mL of venous blood was taken from all participants for laboratory tests after fasting overnight for over 8 hours. After separating, the samples were measured for triglyceride, total cholesterol, HDL-C, low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase, aspartate transaminase, fasting glucose, uric acid, and high-sensitivity C-reactive protein. Blood routine indicators were tested using the Abbott 3700 automatic blood cell analyzer (Abbott, Chicago, USA), and biochemical indicators were assessed using the Roche CoBAS8000-C701 automatic biochemical analyzer (Roche Basel, Switzerland). Furthermore, MHR was calculated as monocyte counts \(10^9/L\) divided by HDL-C (mmol/L).

Hepatic ultrasonographic diagnosis: All participants underwent liver ultrasound examinations performed with a 3.5 MHz transducer (Philips, IU22, Erlangen, Germany) by 2 experienced sonographers.

**Study design**

MHR was divided into four groups based on MHR quartile cut-off points: Q1 (0.086-0.234), Q2 (0.234-0.307), Q3 (0.307-0.399), Q4 (0.399-1.271); and three groups based on tri-sector cut-off points: T1 (0.086-0.257), T2 (0.257-0.363), T3 (0.363-1.271). Age was separately divided into three groups: young adults (<40 years), middle-aged adults (40 to 60 years), and aged adults (>60 years). BMI was separately divided into three groups: low group (<24 kg/m²), middle group (24 to 28 kg/m²), and the high group (>28 kg/m²). Alanine aminotransferase or aspartate transaminase greater than 40 U/L was considered abnormal.

A further stratified analysis was performed based on whether meeting the three criteria for MAFLD. All participants were divided into two groups according to condition 1: 0 group (BMI<23 kg/m²), 1 group (BMI≥23 kg/m²); all participants were divided into two groups according to condition 2: 0 group (without a history of diabetes mellitus and fasting glucose <7.0 mmol/L), 1 group (with history of diabetes mellitus or fasting glucose≥7.0 mmol/L); all participants were divided into two groups according to condition 3 (as described before): group 0 (without or with only 1 kind of metabolic risk abnormalities), group 1 (at least with 2 types of metabolic risk abnormalities).
Family histories of hypertension, diabetes, hyperlipidemia, cardiovascular disease, cerebrovascular
disease, and respiratory disease were identified by experienced physicians before the study.

**Statistical Methods**

All data was analyzed using the R statistical software package (http://www.R-project.org, The R
Foundation) and Empower Stats statistical software (http://www.empowerstats.com, X&Y Solutions, Inc.,
Boston, MA). Data in normal distribution were expressed as mean ± standard deviation (SD), data in non-
normal distribution were expressed as median (quartile), and categorical data were expressed as a
percentage (%). T-test was used for comparison between normal data. A nonparametric test was
performed to compare non-normal data. A Chi-square test ($\chi^2$) was used to analyze the categorical data.
Finally, the univariate analysis was used to analyze the correlation between each variate and MAFLD.

Univariate and multivariate logistic regression models were conducted to analyze the relationship
between MHR and MAFLD. The non-adjusted, simple-adjusted, and multivariate-adjusted models were
used to analyze the relationship, and the interaction and stratification analyses were performed according
to risk factors. In addition, a generalized additive model (GAM) was used to identify the non-linear
relationship between MHR and MAFLD. We also used stratified logistic regression models to proceed with
subgroup analyses according to the diagnostic criteria of MAFLD. The interaction was performed to test
the statistically significant difference among different stratifications in the subgroups. $P$-values<0.05
were considered statistically significant.

**Results**

**Characteristics of study participants**

This study included 2,210 participants, including 705 (31.9%) with MAFLD at baseline (Table 1). Demographically, male patients in the MAFLD group accounted for more than two-thirds. The prevalence of hypertension, diabetes and dyslipidemia in MAFLD was 51.6%, 16.72%, and 41.42%, compared with 26.76%, 6.83%, and 13.03%, respectively, in the non-MAFLD group. The participants with MAFLD had higher BMI, systolic blood pressure, diastolic blood pressure, uric acid, fasting glucose, total cholesterol, triglyceride, high-sensitivity C-reactive protein, and LDL-C than those without MAFLD ($p<0.01$). MHR, as an inflammatory marker, was higher in the MAFLD group. Furthermore, we found a positive relationship between MHR and NAFLD prevalence, as the latter gradually increased based on MHR quartiles or MHR tri-sector (Table S1).

**Univariate analysis of statistics results and MAFLD**

Univariate analysis was used to analyze the correlation between each indicator and MAFLD (Table S2). We found age, sex, BMI, systolic blood pressure, diabetes family history, uric acid, fasting glucose, high-sensitivity C-reactive protein, triglyceride, total cholesterol, and LDL-C levels were positively correlated with MAFLD. In contrast, HDL-C level was negatively correlated with MAFLD. Furthermore, we found that
females had lower OR levels than males, but the participants with a history of hypertension, diabetes, and hyperlipidemia had higher OR levels than those without.

**Relationship between MHR and diagnostic conditions of MAFLD**

Further analysis of the relationship between MHR and MAFLD was performed based on the three diagnostic criteria of MAFLD. MHR was a risk factor for MAFLD, regardless of whether participants were overweight/obese (BMI $\geq 23 \text{ kg/m}^2$) or had metabolic abnormalities, but MHR of the participants with a history of diabetes was not the risk factor for MAFLD. Even more interesting was that lean MAFLD patients had higher MHR levels (Table 2).

**Association between MHR, MAFLD, and metabolic profiles**

Multivariate logistic regression revealed a correlation between MHR and the prevalent MAFLD (Table 3). With adjustment of sex and age group, each 0.1 SD increase of MHR demonstrated a 47% additional risk of MAFLD. After further adjustment of systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate transaminase, uric acid, fasting glucose, triglyceride, LDL-C, smoking, hypertension, diabetes, hyperlipidemia, the additional risk of prevalent MAFLD was 12% for each 0.1 SD increase of MHR. Moreover, after dividing MHR into three and four equal parts, the top category also demonstrated an increment in the risk of prevalent MAFLD than the bottom category in the full model.

**Smoothing curve fitting of the correlation between MHR and MAFLD**

Next, we use smoothing curve fitting to conduct further analyses. After adjusting sex, age groups, BMI groups, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate transaminase, uric acid, fasting glucose, triglyceride, LDL-C, smoking, hypertension, diabetes, hyperlipidemia, the relationship between MHR and MAFLD was non-linear in a generalized additive model (GAM; Fig 1).

**Threshold effect analysis**

Using the two-piecewise regression model, we found that the inflection point was 0.396. On the left of the inflection point, MHR exhibited a positive correlation with MAFLD (OR=43.408, 95% CI: 5.949 to 316.733, $p=0.0002$), while there was no correlation between MHR and MAFLD on the right of the inflection point (OR=0.504, 95% CI: 0.102 to 2.485, $p=0.4000$) (Table 4). To better explain the relationship between MHR and MAFLD, we enlarged MHR by 10 times, recorded it as MHR*, and observed the change of OR value for every 0.1 increase of MHR. Similar results were identified on the left of the inflection point, and MHR* exhibited a positive correlation with MAFLD (OR=1.459, 95% CI: 1.196 to 1.781, $p=0.0002$). In contrast, on the right of the inflection point, there was no correlation between MHR* and MAFLD (OR=0.934, 95% CI: 0.797 to 1.096, $p=0.4025$).

**Interaction analysis for the relationship between MHR and MAFLD**

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A further subgroup analysis was conducted to evaluate the relationship between MHR and MAFLD. The results were calculated after adjustment for sex, age groups, BMI groups, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate transaminase, uric acid, fasting glucose, triglyceride, LDL-C, smoking, hypertension, diabetes, hyperlipidemia. Moreover, the interactions among different stratifications in the subgroups were also evaluated. As shown in Fig. 2, the interaction test was not statistically significant in different diastolic blood pressure groups, total cholesterol groups, the history of hyperlipidemia (p for interaction: 0.8612, 0.7312, 0.234, respectively). In contrast, the test for interactions was significant among different stratifications in the subgroups of sex, age groups, BMI groups, systolic blood pressure groups, uric acid groups, fasting glucose groups, triglyceride groups, hypertension, diabetes, and smoking. (p<0.05). Therefore, there were interactions between MHR and sex, age, BMI, systolic blood pressure, uric acid, fasting glucose, triglyceride, hypertension, diabetes, and smoking. In the age subgroups, MHR was positively correlated with MAFLD when stratified for younger than 60, but not in the stratification of over 60 years. Meanwhile, a positive relationship between MHR and MAFLD was found in participants that were not obese not smoked, and did not have a history of diabetes. More interesting, there was no relationship between MHR and MAFLD in groups with higher uric acid (≥428mmol/L), fasting glucose (≥7.0mmol/L), and triglyceride (≥2.26mmol/L).

**Discussion**

In this retrospective study, we observed that MHR was significantly and positively associated with MAFLD. MHR in patients with MAFLD was higher than in those without MAFLD. Moreover, in univariate and multivariate logistic regression analysis, MHR was positively related to NAFLD prevalence. Furthermore, after adjusting for potential confounders, the relationship between MHR and MAFLD was non-linear by smoothing curve fitting. There were different correlations between MHR and MAFLD on the left and right sides of the inflection point (MHR = 0.396). MHR was positively associated with MAFLD on the left side of the inflection point, but there was no significant association on the right. Additionally, MHR was positively correlated with MAFLD in the stratification of those younger than 60 but not in those over 60 years of age. Meanwhile, a positive relationship was found in participants without obesity, smoking, and a history of diabetes. Notably, there was no relationship between MHR and MAFLD in participants with serious traditional metabolic disorders.

In 2020, an international panel of experts from 23 countries jointly proposed the diagnostic definition of MAFLD. It was identified that overweight/obesity, type 2 diabetes, and metabolic abnormality as the diagnostic criteria of MAFLD instead of high-risk factors. A long-established diagnostic criterion of NAFLD has been replaced by MAFLD, which emphasizes the importance of metabolic abnormalities in the pathogenesis of fatty liver. Meta-analysis showed that patients with MAFLD were more likely to have metabolic abnormalities than those with NAFLD, and metabolic dysregulation played a vital role in the pathogenesis of MAFLD [18]. In contrast, lean or “metabolic healthy” subjects with MAFLD might have other unique mechanisms contributing to the MAFLD pathology.
MHR was recently defined as a novel marker related to the extent of inflammation, oxidative stress, and metabolic disorder outcomes [19]. Elevated MHR is associated with many disorders such as cardiovascular diseases, metabolic syndrome, and polycystic ovary syndrome [13, 20, 21]. Previous reports had shown that MHR was associated with the prevalence of MAFLD in patients with T2DM and that T2DM patients with higher MHR have a higher probability of being diagnosed with MAFLD [15].

In this study, the prevalence of MALFD was about 31.9%, and male patients accounted for more than 70% of the MAFLD group, the prevalence in males was significantly higher than in females. The prevalence in this study is similar to previous global and Chinese studies, while there was no significant difference between men and women in the North China population, and the prevalence was higher in South Chinese women [18, 22, 23]. Differences in the population may be the main reasons for discrepancies. Regarding the anthropometric parameters, the MAFLD group had remarkably higher levels of BMI, systolic blood pressure, diastolic blood pressure, uric acid, fasting glucose, total cholesterol, triglyceride, high-sensitivity C-reactive protein, and LDL-C than the non-MAFLD group. This indicates that participants in overweight/obese and metabolic disorder groups are more likely to be combined with MAFLD.

Multivariate logistic regression analysis showed that, after adjusting for gender, age, BMI, systolic blood pressure, diastolic blood pressure, alanine aminotransferase, aspartate transaminase, uric acid, fasting glucose, triglyceride, LDL-C, smoking, hypertension, diabetes, and hyperlipidemia, MHR was significantly and positively associated with the prevalence of MAFLD. Previous studies have shown similar results to these findings, supporting the influence and association of these factors [16]. Nevertheless, our study differed from others in that smoothing curve fitting further analyzed the relationship between MHR and MAFLD. The results surprised us as the correlation was non-linear. After using the two-piecewise regression model, we found that the inflection point of MHR was 0.396. On the left of the inflection point, MHR was positively correlated with MAFLD, but there was no correlation between MHR and MAFLD on the right.

As mentioned before, a BMI of 23 as a cut-off point is one of the three criteria for defining MAFLD since being overweight has a strong pathological link to MAFLD [2]. However, MAFLD also exists in normal-weight subjects, Lean MAFLD is defined as MAFLD in lean subjects (BMI < 23 kg/m²) [24]. In our study, whether BMI was more than or less than 23, MHR was positively correlated with MAFLD (OR is 1.7(1.3, 2.2), 1.3(1.2, 1.4), respectively). However, lean MAFLD patients had higher MHR levels than overweight/obese patients (0.53 ± 0.25 vs. 0.39 ± 0.14). This was different from Jia's study, which showed that MHR was higher in the overweight or obese MAFLD group than in the lean MAFLD group [15]. It is likely that in lean MAFLD, inflammation and oxidative stress play a more important role in pathogenesis. However, there were only 13 lean MAFLD patients in this study, so there may be a bias resulting from our participants. Therefore, further studies on lean MAFLD are needed in the future to support this hypothesis.

We further explored the role of other covariables in the association between MHR and MAFLD. Subgroup analysis revealed a consistent pattern in most subgroups, in which MHR is a risk factor for MAFLD. There
was a consistent pattern in diastolic blood pressure groups, total cholesterol groups, history of hyperlipidemia groups, and a consistent trend in sex groups, systolic blood pressure groups, history of hypertension, and diabetes groups, although significant contrast. One of the most important findings of this study was that MHR was not related to MAFLD in groups with the aged group (over 60 years old), significant metabolic disorder group (BMI $\geq 28\text{kg/m}^2$, uric acid $\geq 428\text{mmol/L}$, fasting glucose $\geq 7.0\text{mmol/L}$, and triglyceride $\geq 2.26\text{mmol/L}$), smoking group, and/or diabetes history group. The possible reason is that the roles of these aforementioned characteristics in the pathogenesis of MAFLD are more evident than that of MHR.

Nevertheless, this study has several limitations. First, this study was a single-center retrospective study. It was difficult to reveal the cause and effect, so we only showed the relevance of MHR and MAFLD. Second, we only measured MHR and MAFLD status at the baseline, so we did not obtain a dynamic correlation trend. Third, we did not measure the waist circumference, a criterion of metabolic risk abnormalities. However, BMI was strongly correlated with waist circumference and can effectively reduce the impact of data loss on outcome evaluation. Therefore, this investigation warrants further investigation to determine the reliability of these associations and their influence on MAFLD prevalence.

**Conclusion**

This study demonstrated that patients with MAFLD have higher MHR values. MHR is positively related to MAFLD, which could be used as a convenient and effective biochemical marker in detecting risk factors in MAFLD. Notably, when the MHR is less than 0.396, it has a higher predictive value for the risk of MAFLD. Therefore, MHR can predict the risk of MAFLD in populations without traditional risk factors.

**Abbreviations**

BMI, body mass index; GAM, generalized additive model; HDL-C, high-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; MAFLD, metabolic dysfunction-associated fatty liver disease; MHR, monocyte to high-density lipoprotein cholesterol ratio; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio; CI, confidence interval.

**Declarations**

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**Authors’ contributions**

JH and XL-Z conceived and designed the study. JH, BL-Z and HL classified data and conducted the statistical analysis. JH, YW-L, JB-G and YX-L were the major contributors in writing the manuscript. All
authors read and approved the final manuscript.

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No.

Ethics approval and consent to participate

This study protocol has been approved by the Ethics Committee of the Second Hospital of Hebei Medical University, (IRB Number: 2022-R062).

We confirm that informed consent was obtained from all subjects and/or their legal guardian(s), and all the methods used in this study were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

Data Availability Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

References


**Tables**

Tables 1 to 4 are available in the Supplementary Files section.

**Figures**
Figure 1

See image above for figure legend.
### Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1AssociationofMHRwithprevalencerateofMAFLD.xlsx](#)
- [Table1Thedemographicandclinicalcharacteristicoftheparticipants.xlsx](#)
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• Table 4 Threshold limit value of MHR on the risk of MAFLD using piecewise linear regression.xlsx