Low Serum Maresin-1 Levels Are Associated with Non-alcoholic Fatty Liver Disease: A Cross Sectional Study

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Abstract

Background

Maresin-1 is one of anti-inflammatory pro-resolving mediators, which is considered as a potential regulator of metabolic diseases. However, little information is available on the relationship between Maresin-1 and non-alcoholic fatty liver disease (NAFLD) in humans. Therefore, this study explored the associations between serum Maresin-1 levels and NAFLD.

Methods

A cross-sectional study was conducted in 240 Chinese people, including 116 non-NAFLD subjects and 124 NAFLD patients. NAFLD was diagnosed by abdominal ultrasonography. Serum Maresin-1 levels were determined by ELISA. The association between Maresin-1 and NAFLD was assessed.

Results

Serum Maresin-1 levels in NAFLD patients were markedly lower than those in non-NAFLD subjects (63.63 [59.87–73.93] vs 73.11 [65.12–84.50] pg/mL, $P = 0.000$). The percentages of patients with NAFLD gradually decreased in tandem with increasing quartiles of Maresin-1 ($P < 0.001$). Furthermore, serum Maresin-1 levels were positively associated with AST/ALT, albumin, Albumin-globulin-ratio, and HDL-C (all $P < 0.05$) and negatively associated with BMI, waist circumference, hip circumference, waist-to-hip ratio, ALT, GGT, uric acid, TG, and FBG (all $P < 0.05$) after adjusting for sex and age. We found that serum Maresin-1 levels were significantly associated with NAFLD by binary logistic regression analysis.

Conclusions

Circulating Maresin-1 levels were decreased in patients with NAFLD, and there was a negative correlation between NAFLD and serum Maresin-1 concentrations. Decreased Maresin-1 might be involved in the development of NAFLD.

Background

Non-alcoholic fatty liver disease (NAFLD), which is the most common metabolic liver disease and one of the most common causes of chronic liver disease, is characterized by the ectopic deposition of fat in hepatocytes without secondary causes of liver fat accumulation (e.g., excess alcoholic consumption, medication, viral infection) [1]. NAFLD has a growing impact on world health. The global prevalence of NAFLD is estimated to be as high as 25%, with the highest in the Middle East and South America and lowest in Africa [2]. Given the tremendous changes in lifestyle in the past 20 years, the prevalence of NAFLD in China has reached as high as 22.4%, which is equivalent to that in the United State (24.13%),

Europe (23.71%) and Japan (25%) [2–6]. This rising prevalence of NAFLD will inflict a growing economic burden and will be accompanied by an increasing number of patients with cirrhosis, liver transplantation or/and Hepatocellular Carcinoma (HCC) [7–10]. However, NAFLD is a heterogeneous disease with very different clinical manifestations and different rates of progression among individuals. Some patients are asymptomatic and only found to have simple non-alcoholic fatty liver (NAFL) during physical examination, while others present with severe nonalcoholic steatohepatitis (NASH), liver cirrhosis, or even HCC. Therefore, it is necessary to identify any novel biomarkers in order to better investigate the progression of NAFLD and to screen out the high-risk groups who may be particularly susceptible to NAFLD.

Maresins are the third-largest family of specialized pro-resolving mediators (SPMs) made from docosahexaenoic acid (DHA), which are mainly biosynthesized in M2 macrophages [11–12]. Maresin-1 is the first discovered member of this family. Nowadays, more and more evidence show that Maresin-1 plays vital roles in metabolic diseases. In ob/ob and diet-induced obese mice, Maresin-1 improves insulin sensitivity and reverse adipose tissue dysfunction and inflammation [13]. Maresin-1 ameliorates liver steatosis by inhibiting endoplasmic reticulum stress and lipogenic enzymes and inducing autophagy via AMPK pathway in high fat diet (HFD)-fed mice [14–15]. A recent study by Han et al. showed that Maresin-1 is an endogenous ligand of retinoic acid-related orphan receptor α (RORα). Maresin-1 protected mice from HFD-induced NASH by activating M2 polarization of liver macrophages in a RORα-dependent manner [16]. All these studies indicate that Maresin-1 is closely related to metabolic diseases, especially NAFLD in cells and animal models. However, the relationship between serum Maresin-1 levels and NAFLD in humans is still unclear.

In the present cross-sectional study, we recruited subjects with NAFLD to investigate alterations in circulating Maresin-1 and to explore the potential relationship between Maresin-1 levels and NAFLD subjects.

Methods

Study Population and Design

non-NAFLD subjects and NAFLD patients were recruited from the Physical Examination Center of the Affiliated Hospital of Southwest Medical University. All ultrasonographic examinations, using a Doppler sonography system (ACUSON Sequoia, SIEMENS, Germany), were performed to diagnose NAFLD by the same group of experienced sonographers following standardized procedures. The diagnosis of NAFLD was made based on the presence of hepatic fat accumulation according to the criteria issued by the Chinese Liver Disease Association [17]. All participants with the following conditions were excluded: 1) excess alcohol consumption (≥ 140 g/week for men, ≥ 70 g/week for women) [18] or alcoholic liver disease, drug- or toxin-induced liver diseases, genetic liver diseases, viral or autoimmune hepatitis, biliary obstructive diseases, renal disease, HIV infection, cancer, acute or chronic inflammatory disease, cardiovascular or cerebral vascular disease, or pregnancy or breastfeeding; 2) systemic corticosteroids

treatment, anti-inflammatory therapy, hypoglycemic or lipid-lowering therapy, and antihypertensive treatment.

After screening, 124 patients with NAFLD and 116 subjects with non-NAFLD aged between 22 and 71 years participated in this clinical study. All participants were categorized into quartiles based on their serum Maresin-1 concentration: quartile 1, Maresin-1 < 62.13 pg/mL; quartile 2, 62.13 pg/mL ≤ Maresin-1 < 68.71 pg/mL; quartile 3, 68.71 pg/mL ≤ Maresin-1 ≤ 77.79 pg/mL; quartile 4, Maresin-1 > 77.79 pg/mL.

All experimental protocols followed the ethical guidelines of the 1964 Declaration of Helsinki and were approved by the Human Research Ethics Committee of the Affiliated Hospital of Southwest Medical University (permission no. KY2021086). Written informed consent was obtained from all participants.

Anthropometric And Biochemical Measurements

In the present study, all anthropometric measurements were performed before breakfast, and all participants wore light clothing and no footwear. After overnight fasting (about 10–12 h), the anthropometric parameters were measured by a designated specialist nurse. After resting for at least 5 minutes, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by using a medical automatic electronic sphygmomanometer (HBP-9020, OMRON Corp., Kyoto, Japan). Height and body weight (BW) were measured by using an ultrasonic analyzer (SK-V7, Shenzhen, China). Waist circumferences (WC, the midpoint between the ilium and the lowest margin of the ribs) and hip circumference (HC, the maximum circumference of the hips) were measured with a cloth measuring tape. Body mass index (BMI) and waist to hip ratio (WHR) were calculated as BW (kg)/height (m^2) and WC (cm)/HC (cm), respectively.

After overnight fasting, blood samples were collected from all participants in the morning of the day of ultrasound examination. Serum samples were collected and stored at -80℃ until analysis. Fasting blood glucose (FBG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), globulin (GLO), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), urea nitrogen (Urea), uric acid (UA), creatinine (Crea), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and homocysteine (HCY) were detected by biochemical autoanalyzer (ADVIA2400, SIEMENS, Germany). Peripheral white blood cell (WBC) and neutrophils (NEU) counts were determined using an automated blood cell counter (Mindray BC-6800, Shenzhen, China).

Serum Maresin-1 Measurement

Serum Maresin-1 levels were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (Human ELISA kit, Senbeijia, NanJing, China) according to the manufacture's protocol. Serum

samples were diluted 5-fold before the assay. The intra- and inter-assay variations were < 9% and < 11%, respectively. The detectable range of the kit was 5 pg/mL-160 pg/mL.

Statistical Analysis

SPSS 22.0 and GraphPad Prism 8.0 were used for all statistical analysis and graphics. Data were expressed as the number (percentage) for categorical variables or as the mean ± standard deviation (SD) or medians [25th, 75th percentiles] for continuous variables, unless otherwise specified. The differences between the two groups were examined using the Student's t-test or Mann-Whitney U-test for continuous variables. The differences among more than two groups were performed with one-way analysis of variance (ANOVA) or Kruskal-Wallis test for continuous variables. Comparisons of categorical variables were made using Chi-square test. The correlation between serum Maresin-1 and other clinical parameters was determined by Pearson correlation or Spearman correlation test according to the distribution of parameters. Partial correlation coefficients were used for age- and sex-adjusted data. Binary logistic regression analyses were used to analyze the association between the serum Maresin-1 levels and NAFLD. *P* value < 0.05 was considered statistically significant.

Results

General Characteristics and Serum Maresin-1 Levels of Study Participants

A total of 116 non-NAFLD subjects and 124 NAFLD patients were enrolled in this study. The average age of the subjects was 43.44 ± 11.36 years, including 134 males (55.8%) and 106 females (44.2%). Table 1 summarizes the general anthropometric, biochemical and clinical parameters and serum Maresin-1 levels of the subjects. Compared to non-NAFLD subjects, NAFLD patients had higher BMI, WC, HC, WHR, SBP, DBP, FBG, WBC count, ALT, AST, GGT, ALP, Urea, UA, Crea, TC, TG, LDL-C, and HCY ($P < 0.05$ or $P < 0.01$ or $P < 0.001$) and lower levels of AST/ALT and HDL-C (both $P < 0.001$). The proportion of male was higher in subjects with NAFLD than in those non-NAFLD ($P = 0.029$). No significant differences were found in age, neutrophil count, TP, ALB, GLO, and A/G.

Table 1
Main clinical parameters and serum Maresin-1 levels in all participants.

Variables	non-NAFLD	NAFLD	*P*-value
Male/Female	57/59	77/47	0.029
Age (year)	41.50 (33.00–51.00)	45.50 (35.00–53.00)	0.286
BMI (kg/m2)	22.54 (21.20–24.20)	27.26 (25.48–29.49)	0.000
WC (cm)	79.68 ± 8.52	90.39 ± 7.92	0.000
HC (cm)	95.00 (91.00–98.00)	100.00 (96.00-104.75)	0.000
WHR	0.84 ± 0.06	0.89 ± 0.05	0.000
SBP (mmHg)	118.00 (110.00-124.00)	130.50 (119.00-138.00)	0.000
DBP (mmHg)	71.45 ± 9.21	77.29 ± 10.68	0.000
FBG (mmol/L)	4.95 (4.68–5.23)	5.32 (4.90–5.75)	0.000
WBC (*10^9/L)	6.11 ± 1.22	6.75 ± 1.35	0.000
NEU (*10^9/L)	3.65 ± 0.92	3.85 ± 0.95	0.099
ALT (U/L)	18.20 (13.30–27.20)	27.90 (21.25–42.05)	0.000
AST (U/L)	20.20 (17.30-26.05)	23.40 (19.43–27.50)	0.002
AST/ALT	1.15 (0.92–1.34)	0.83 (0.66–0.99)	0.000
TP (g/L)	73.00 ± 3.01	72.63 ± 2.93	0.328
ALB (g/L)	46.25 (45.00-47.63)	46.05 (45.03–47.80)	0.970
GLO (g/L)	26.62 ± 2.52	26.25 ± 2.59	0.260
A/G	1.76 (1.62–1.90)	1.77 (1.65–1.91)	0.501
GGT (U/L)	16.05 (13.10-26.85)	31.55 (20.58–48.60)	0.000
ALP (U/L)	67.40 (53.95–79.78)	74.05 (62.55–88.05)	0.003
Urea (mol/L)	4.70 (3.90–5.63)	5.13 (4.59–5.93)	0.004
UA (μmol/L)	320.00 (261.48-380.78)	370.15 (316.65–440.00)	0.000

Continuous variables are mean ± standard deviation (SD) or medians (25th, 75th percentiles). NAFLD: non-alcoholic fatty liver disease, BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, WBC: white blood cells, NEU: neutrophil, ALT: alanine aminotransferase, AST: aspartate aminotransferase, TP: total protein, ALB: albumin, GLO: globulin, A/G: Albumin-globulin-ratio, GGT: gamma-glutamyl transpeptidase, ALP: alkaline phosphatase, UA: uric acid, Crea: creatinine, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, HCY: Homocysteine.

Variables	non-NAFLD	NAFLD	*P*-value
Crea (μmol/L)	63.35 (53.80–71.40)	70.50 (55.75–77.23)	0.006
TC (mmol/L)	4.73 ± 0.77	4.95 ± 0.72	0.022
TG (mmol/L)	1.16 (0.84–1.67)	2.00 (1.42–2.67)	0.000
HDL-C (mmol/L)	1.35 (1.17–1.66)	1.08 (0.96–1.22)	0.000
LDL-C (mmol/L)	3.10 ± 0.83	3.42 ± 0.70	0.002
HCY (μmol/L)	9.55 (8.00-11.50)	10.65 (8.80–12.10)	0.009
Maresin-1 (pg/mL)	73.11 (65.12–84.50)	63.63 (59.87–73.93)	0.000

Continuous variables are mean ± standard deviation (SD) or medians (25th, 75th percentiles). NAFLD: non-alcoholic fatty liver disease, BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, WBC: white blood cells, NEU: neutrophil, ALT: alanine aminotransferase, AST: aspartate aminotransferase, TP: total protein, ALB: albumin, GLO: globulin, A/G: Albumin-globulin-ratio, GGT: gamma-glutamyl transpeptidase, ALP: alkaline phosphatase, UA: uric acid, Crea: creatinine, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, HCY: Homocysteine.

In all study subjects, the distribution of serum Maresin-1 concentration was from 51.65-224.45 pg/mL. There was no significant difference in circulating Maresin-1 levels between men and women (66.99 [61.18–77.40] vs 71.52 [62.57–79.32], $P = 0.078$). In addition, in order to understand the serum levels of Maresin-1 under different metabolic conditions, all participants were divided into normal and abnormal groups according to the levels of FBG, lipids or BMI [19, 20]. As shown in Fig. 1, serum Maresin-1 levels were significantly lower in subjects with elevated FBG, TC and TG levels (all $P < 0.001$, Fig. 1A-C), decreased HDL-C levels ($P < 0.001$; Fig. 1D), and overweight or obese subjects ($P < 0.001$; Fig. 1F) compared to their controls. There was no significant difference in serum Maresin-1 levels among subjects with different LDL-C levels ($P = 0.2587$; Fig. 1E). Importantly, serum Maresin-1 levels in NAFLD patients were significantly decreased compared with those in non-NAFLD subjects ($P = 0.000$, Table 1).

Clinical and biochemical characteristics by quartiles of serum Maresin-1 in all study participants

Table 2 shows the clinical and biochemical characteristics according to quartiles of serum Maresin-1 levels in all subjects. BMI, WC, HC, WHR, SBP, DBP, FBG, ALT, AST, AST/ALT, GLO, A/G, GGT, ALP, Urea, TG, HDL-C, and Maresin-1 concentrations were significantly different between participants in different serum Maresin-1 quartiles ($P < 0.05$ or $P < 0.01$ or $P < 0.001$). Compared to subjects in the lowest quartile of serum Maresin-1 concentration, participants in the highest quartile had lower levels of BMI, WC, HC, WHR, SBP, DBP, FBG, WBC count, ALT, GLO, GGT, Urea, TC, TG, and LDL-C ($P < 0.05$ or $P < 0.01$ or $P < 0.001$) and higher levels of serum Maresin-1, AST/ALT, A/G, and HDL-C ($P < 0.01$ or $P < 0.001$). As shown in Fig. 2, the prevalence of NAFLD was rapidly decreased in tandem with increasing quartile of serum Maresin-1 levels ($P < 0.001$).

Table 2
Clinical and biochemical characteristics by quartile of serum Maresin-1 level in all subjects.

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-value
	(~ < 62.13)	(62.13 ≤ - < 68.71)	(68.71 ≤ - < 77.79)	(~ ≥ 77.79)	
Sample Size	60	60	60	60	-
Male/Female	37/23	38/22	28/32	31/29	0.198
Age (year)	46.00 (40.25–52.75)	46.00 (34.00–54.00)	38.50 (31.00-51.75)	40.00 (31.25–49.75)	0.075
BMI (kg/m2)	26.13 (24.19–28.93)	26.27 (23.75–28.25)	25.23 (21.99–27.89)	23.06 (21.17–25.60) ***	0.000
WC (cm)	89.20 ± 9.73	87.13 ± 9.39	83.67 ± 9.29 **	80.85 ± 8.83 ***	0.000
HC (cm)	99.00 (94.25–104.00)	98.00 (93.00-102.00)	97.50 (93.25–103.00)	95.00 (92.00–99.00) *	0.017
WHR	0.90 (0.88–0.93)	0.89 (0.84–0.92)	0.85 (0.81–0.90) **	0.85 (0.80–0.88) ***	0.000
SBP (mmHg)	130.50 (118.00-137.00)	123.00 (115.00-134.75)	121.50 (111.00-134.75)	120.00 (112.00-129.50) *	0.038
DBP (mmHg)	76.93 ± 10.84	75.95 ± 10.51	73.78 ± 10.31	71.20 ± 9.15 **	0.012
FBG (mmol/L)	5.47 (4.86–5.81)	5.09 (4.83–5.68)	5.06 (4.73–5.38) *	4.98 (4.68–5.27) **	0.001
WBC (*10^9/L)	6.72 ± 1.45	6.59 ± 1.33	6.30 ± 1.17	6.17 ± 1.29 *	0.079
NEU (*10^9/L)	3.67 (3.08–4.47)	3.69 (3.07–4.58)	3.55 (3.11–4.12)	3.67 (2.99–4.43)	0.830
ALT (U/L)	26.70 (19.45–38.18)	27.40 (19.48–36.70)	20.90 (13.33–30.35) *	19.20 (13.98–27.88) **	0.000
AST (U/L)	23.20 (19.45–29.63)	23.30 (19.98–28.40)	20.15 (16.83–26.38)	20.15 (17.53–24.78)	0.010
AST/ALT	0.85(0.67–1.06)	0.88(0.67–1.14)	1.02(0.83–1.21) *	1.11(0.85–1.28) **	0.000
TP (g/L)	73.10 ± 2.88	73.01 ± 2.54	72.20 ± 3.37	72.95 ± 3.00	0.321
ALB (g/L)	45.95 (45.00–47.00)	45.85 (45.10-47.78)	45.90 (44.90-47.48)	47.25 (45.33–48.83)	0.058

Continuous variables are mean ± standard deviation or medians (25th, 75th percentiles). *P< 0.05, **P< 0.01, ***P< 0.001 versus quartile 1 group.

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-value
GLO (g/L)	27.11 ± 3.05	26.64 ± 2.34	26.01 ± 2.44 *	25.97 ± 2.20 *	0.039
A/G	1.72 (1.55–1.83)	1.73 (1.60–1.88)	1.79 (1.66–1.91)	1.81 (1.73–1.94) *	0.009
GGT (U/L)	30.90 (19.23–47.33)	29.10 (18.03–42.53)	20.35 (14.68–31.40) *	15.40 (12.68–28.30) ***	0.000
ALP (U/L)	73.85 (56.45–84.73)	73.80 (62.43–84.70)	72.15 (61.23–87.53)	63.20 (52.40-79.13)	0.031
Urea (mol/L)	5.16 (4.83–5.96)	4.98 (4.40–5.90)	4.84 (3.97–5.77)	4.76 (3.96–5.41) *	0.027
UA (μmol/L)	356.55 (296.10-418.60)	362.35 (314.58-434.23)	332.80 (283.48-400.93)	318.55 (269.15-415.45)	0.160
Crea (μmol/L)	68.70 (55.95–75.25)	66.70 (54.38–74.38)	65.40 (52.63–73.78)	65.20 (54.93–74.95)	0.735
TC (mmol/L)	5.00 ± 0.80	4.92 ± 0.75	4.74 ± 0.77	4.71 ± 0.67 *	0.098
TG (mmol/L)	1.81 (1.28–2.65)	1.79 (1.23–2.63)	1.37 (0.91–1.82) **	1.21 (0.90–1.69) ***	0.000
HDL-C (mmol/L)	1.10 (0.99–1.34)	1.11 (0.96–1.24)	1.24 (1.08–1.46)	1.33 (1.17–1.63) **	0.000
LDL-C (mmol/L)	3.42 ± 0.79	3.32 ± 0.82	3.24 ± 0.77	3.07 ± 0.72 *	0.093
HCY (μmol/L)	10.05 (8.40-11.78)	10.10 (8.60–12.20)	10.10 (8.70–11.90)	10.15 (7.63–12.10)	0.866

Continuous variables are mean ± standard deviation or medians (25th, 75th percentiles). *P< 0.05, **P< 0.01, ***P< 0.001 versus quartile 1 group.

Association of Serum Maresin-1 Levels with Clinical Parameters in the Study Population

Next, correlation analysis was performed to investigate the association of serum Maresin-1 levels and other clinical parameters. As shown in Table 3, in all study population, serum Maresin-1 concentrations were positively associated with AST/ALT, ALB, A/G, and HDL-C (P< 0.05 or P< 0.01 or P< 0.001) and were negatively associated with Age, BMI, SBP, WC, HC, WHR, WBC count, ALT, AST, GLO, GGT, Urea, TG, LDL-C, and FBG (P< 0.05 or P< 0.01 or P< 0.001). After adjusting for sex and age, Maresin-1 remained statistically positively associated with AST/ALT, ALB, A/G, and HDL-C (P< 0.05 or P< 0.01 or P< 0.001) and negatively associated with BMI, WC, HC, WHR, ALT, GGT, UA, TG, and FBG (P< 0.05 or P< 0.01 or P< 0.001).

Table 3
The correlations analysis of variables associated with serum Maresin-1 levels in study population.

	Serum Maresin-1		Serum Maresin-1 (age- and sex-adjusted)	
	r	*P*-value	r	*P*-value
Sex	0.114	0.078	-	-
Age	-0.171	0.008	-	-
BMI	-0.331	0.000	-0.318	0.000
WC	-0.329	0.000	-0.294	0.000
HC	-0.204	0.001	-0.224	0.000
WHR	-0.360	0.000	-0.281	0.000
SBP	-0.211	0.001	-0.093	0.152
DBP	-0.113	0.081	-0.079	0.222
FBG	-0.285	0.000	-0.186	0.004
WBC	-0.155	0.016	-0.093	0.151
NEU	-0.060	0.352	-0.015	0.823
ALT	-0.289	0.000	-0.212	0.001
AST	-0.214	0.001	-0.102	0.116
AST/ALT	0.260	0.000	0.240	0.000
TP	0.016	0.810	0.006	0.931
ALB	0.157	0.015	0.172	0.008
GLO	-0.150	0.020	-0.123	0.058
A/G	0.204	0.002	0.156	0.016
GGT	-0.358	0.000	-0.158	0.015
ALP	-0.119	0.065	-0.085	0.193
Urea	-0.211	0.001	-0.125	0.055
UA	-0.126	0.051	-0.129	0.048
Crea	-0.068	0.294	-0.038	0.559
TC	-0.089	0.169	-0.072	0.270
TG	-0.330	0.000	-0.192	0.003

	Serum Maresin-1		Serum Maresin-1 (age- and sex-adjusted)	
HDL-C	0.255	0.000	0.242	0.000
LDL-C	-0.150	0.020	-0.121	0.062
HCY	0.002	0.977	-0.013	0.837

Further, binary logistic regression analysis was performed to investigate the association of Maresin-1 with NAFLD. When no adjustment was made, serum Maresin-1 levels were significantly and inversely associated with the prevalence of NAFLD [OR = 0.945; 95% CI = 0.922–0.968, *P* = 0.000]. After adjusting for sex, age, BMI, SBP, DBP, WHR, and FBG or further adjusting for ALT, AST, AST/ALT and UA, the association between serum Maresin-1 levels and the presence of NAFLD was not affected [OR = 0.958, 95% CI = 0.928–0.989, *P* = 0.008] or [OR = 0.957, 95% CI = 0.926–0.989, *P* = 0.008]. Finally, even after adjusting for lipid profiles (TC, TG, HDL-C, and LDL-C), the Maresin-1 levels were significantly associated with NAFLD [OR = 0.965, 95% CI = 0.933–0.999, *P* = 0.042].

Discussion

Maresin-1 has been identified as a new member of pro-inflammatory regression mediators with an important role in metabolic disorders diseases, including NAFLD [14–16, 21]. However, there is limited evidence to support the potential role of Maresin-1 in the occurrence and development of NAFLD in humans. In the present cross-sectional study, we found that serum Maresin-1 levels were significantly decreased in NAFLD patients compared with those in non-NAFLD subjects. The prevalence of NAFLD was significantly decreased in participants with the highest serum Maresin-1 quartile than in participants with the lowest serum Maresin-1 quartile. In addition, our study demonstrated that serum Maresin-1 concentrations were positively correlated with parameters regarding AST/ALT, ALB, A/G, and HDL-C and negatively correlated with age, obesity, FBG, ALT, GGT, UA, and TG. Importantly, we found that serum Maresin-1 levels were independently associated with NAFLD after adjusting for other potential confounders. Overall, these data indicate that Maresin-1 is an independent protective factor of NAFLD, and the decrease of Maresin-1 levels may play a vital role in the pathophysiological process of NAFLD.

Maresin-1 was the first member of maresins family to be identified. The biosynthesis of maresins mainly occurs in M2 macrophages and is initiated by the key enzyme 12-lipoxygenase (12-LOX) [22–23]. DHA in macrophages is converted to 13S,14S-epoxy-maresin under the action of 12-LOX. Next, the epoxide intermediate forms the final product 7R, 14S-dihydroxydocosa-4Z, 8E, 10E, 12Z, 16Z, 19Z-hexaenoic acid (Maresin-1) through an epoxide-hydrolysis reaction [24]. In NAFLD, activated Kupffer cells release pro-inflammatory cytokines and chemokines [25], which promote the accumulation of pro-inflammatory M1-polarized monocytes in liver tissue [26]. This disrupts the M1/M2 balance, resulting in a decrease in M2 pro-resolving phenotype in liver tissue. Han et al. found that Maresin-1, as an endogenous ligand of RORα, increased the M2 polarity of liver macrophages by enhancing the expression and transcriptional activity of RORα [16]. Collectively, Maresin-1 is synthesized in M2 macrophages and promotes

macrophages to shift to M2 pro-resolving phenotype. When NAFLD occurs, the above loop is destroyed, resulting in the decrease of Maresin-1 synthesis. Our study found that serum Maresin-1 levels in NAFLD patients were significantly decreased than that in non-NAFLD subjects, and with the increase of serum level of Maresin-1, the prevalence of NAFLD decreased. Binary logistic regression analyses showed that Maresin-1 levels were an independent predictor of NAFLD. Thus, these results also suggest that Maresin-1 may be causal factor of NAFLD.

It is well known that NAFLD is a hepatic phenotype of metabolic impairment [27], and our current results are consistent with previous reports [28], which revealed that NAFLD patients had abnormal metabolic features. In the present study, the NAFLD patients had significantly higher BMI, WC, HC, WHR, BP, FBG, ALT, AST, GGT, ALP, Urea, UA, Crea, TC, LDL-C, TG, and HCY levels, but lower AST/ALT ratios and HDL-C than those with non-NAFLD subjects. In addition, our study results showed that serum Maresin-1 levels were negatively associated with BMI, WC, HC, WHR, FBG, ALT, GGT, UA, and TG and positively associated with AST/ALT, ALB, A/G, and HDL-C. Moreover, Maresin-1 has been reported to ameliorate insulin resistance (IR) and dyslipidemia by stimulating the insulin signaling [15, 29–30]. Overall, these results suggest that serum Maresin-1 level is negatively associated with metabolic disorder, indicating that the decrease of serum Maresin-1 may promote the occurrence of NAFLD by aggravating IR and lipid metabolic disorder.

Recently, Félix-Soriano et al. found that Maresin-1 was significantly reduced in adipose tissue in the aged HFD-induced obese mice when compared to the young normal controls [31]. Markworth et al. also showed that the aged mice muscle displayed significantly lower Maresin-1 levels when compared to the young mice muscle [32]. These data suggested that aging is associated with a deficiency of Maresin-1. Our results also showed that serum Maresin-1 levels were significantly negatively correlated with age in all participants.

A couple of limitations of our study should be mentioned. First, the current cross-sectional study is unable to illustrate the causal relationship between the serum Maresin-1 concentration and NAFLD, and it needs to be complemented by prospective studies. Second, we used abdominal ultrasound instead of liver biopsy to diagnosis NAFLD, which can lead to missed or misdiagnosis. Although hepatic steatosis, as an early stage of NAFLD, can be reliably identified by non-invasively imaging tests, including ultrasound, the advanced NAFLD such as NASH and cirrhosis can only be definitely diagnosed by liver biopsy [33]. Thus, the lack of histological confirmation of the degree of hepatic steatosis could be another weakness of the current study. Third, there might exist a selection bias. All the participants, who were recruited from physical examination center, as such, they may have stronger health awareness than non-participants. Finally, our current study may underestimate the true association, because serum Maresin-1 level was only detected once by ELISA kit, which is prone to random measurement error.

Conclusions

In conclusion, our study provides clinical evidence that the serum Maresin-1 concentrations were decreased in NAFLD patients, and that high serum Maresin-1 levels were associated with a reduced prevalence of NAFLD. These findings indicated that Maresin-1 might be a non-invasive molecular biomarker that detects the presence of NAFLD. However, future prospective longitudinal studies are required to confirm the contribution of Maresin-1 to the development of NAFLD.

Abbreviations

NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH, non-alcoholic steatohepatitis; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; WBC, white blood cells; NEU, neutrophil; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; ALB, albumin; GLO, globulin; A/G, Albumin-globulin-ratio; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; UA, uric acid; Crea, creatinine; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HCY, Homocysteine.

Declarations

Ethics approval and consent to participate

The Human Research Ethics Committee of the Affiliated Hospital of Southwest Medical University approved the experimental protocols (permission no. KY2021086). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Guarantors of the article: Jiahao Fan and Yong Xu.

JF and YX conceived, designed and supervised the study; YZ, XT, XF and TY provided research guidance; XF and HW collected the data and biological samples; XF and HW performed the measurements of serum maresin-1 levels; XF and HW analyzed the data and wrote the manuscript; and TY, XF, XT, YZ, JF and YX critically reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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Figures

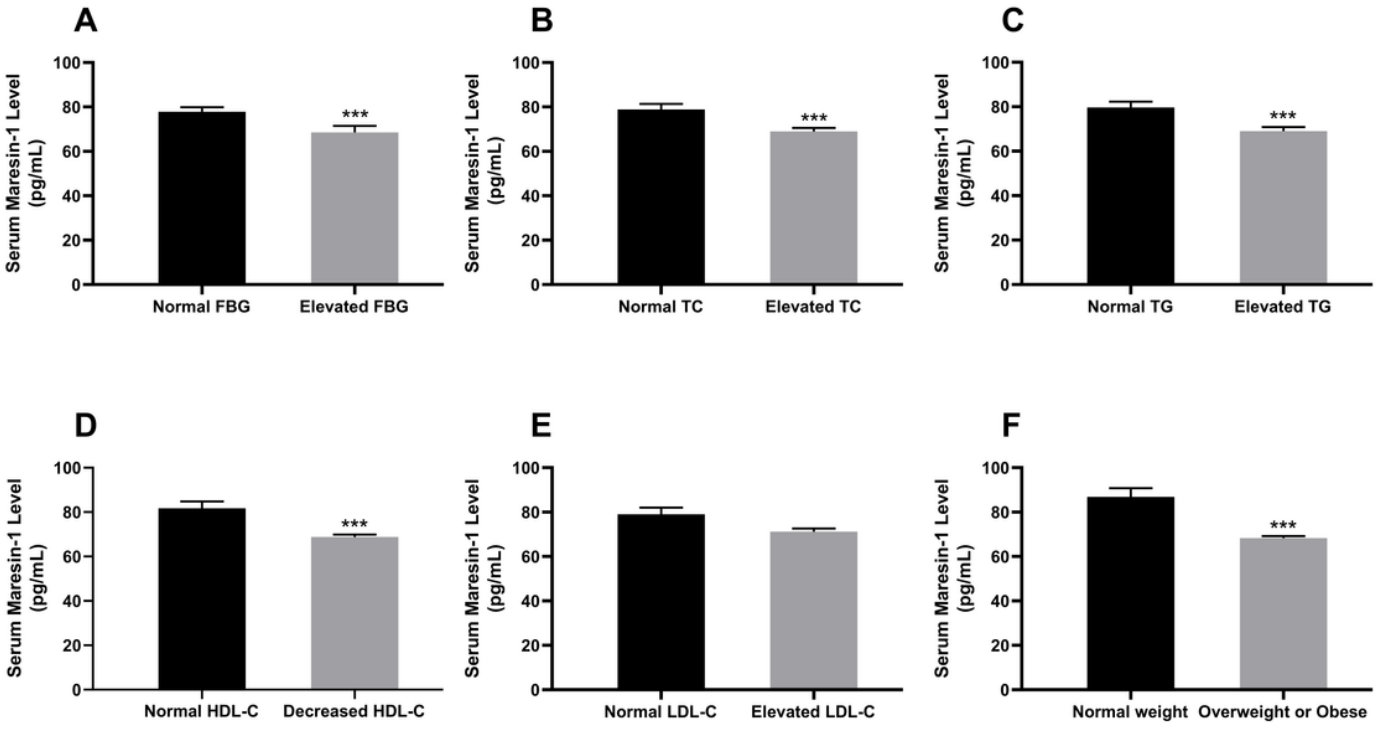

Figure 1

Serum Maresin-1 concentration for study population (A) with (n = 63) or without elevated FBG levels (n = 177), (B) with (n = 84) or without elevated TC levels (n = 156), (C) with (n = 97) or without elevated TG levels (n = 143), (D) with (n = 117) or without decreased HDL-C levels (n = 123), (E) with (n = 112) or without elevated LDL-C levels (n = 128), (F) with normal weight (n = 92) or with overweight or obesity (n = 148). *P < 0.05, **P < 0.01, ***P < 0.001 versus without elevated FBG, TC, TG or LDL-C levels or without decreased HDL-C levels or with normal weight. Data are expressed as means ± SEM.

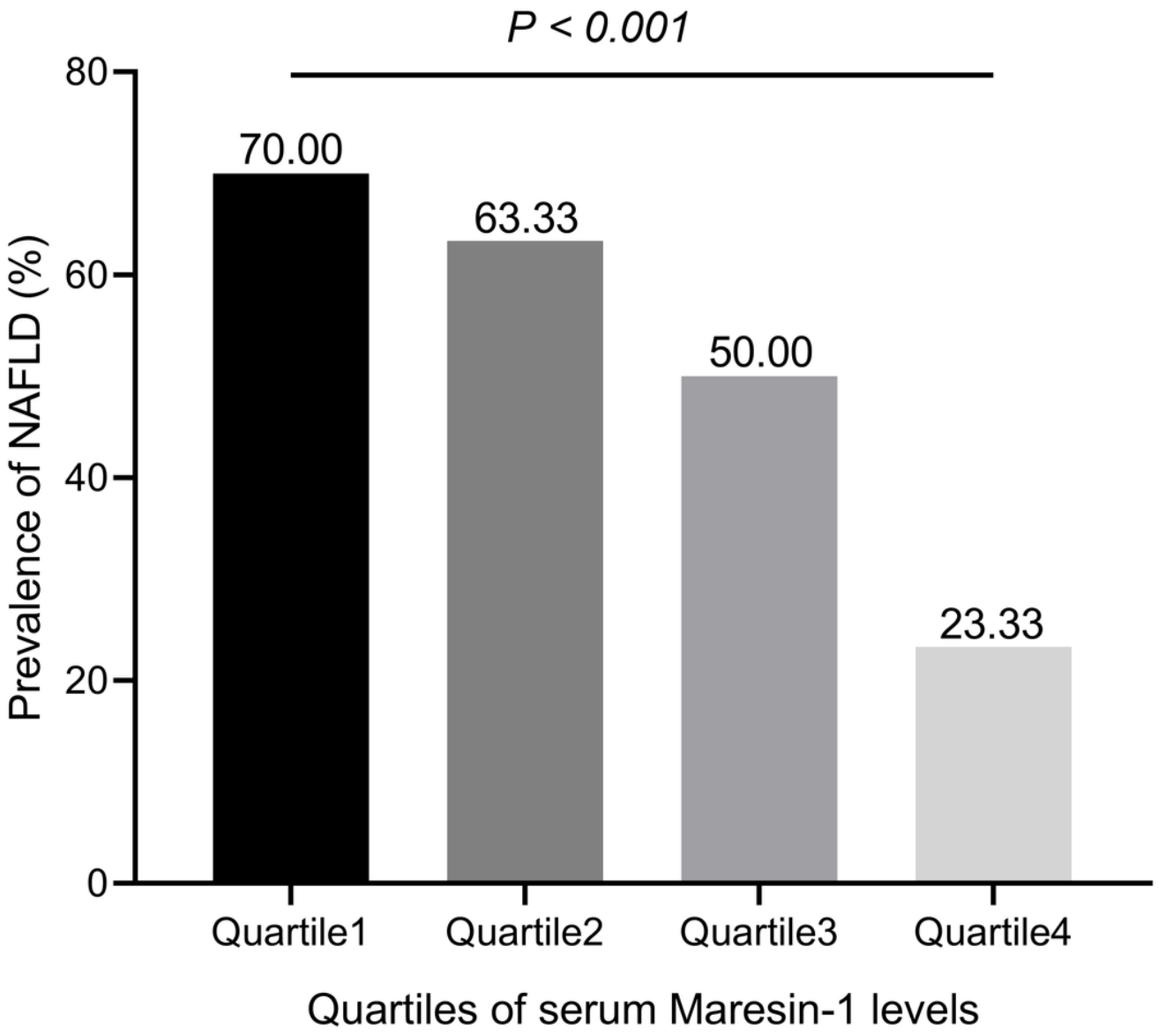

Figure 2

Prevalence of non-alcoholic fatty liver disease (NAFLD) by quartiles of serum Maresin-1 in all participants.