Association between serum 25-hydroxy vitamin D level and metabolic associated fatty liver disease (MAFLD)—a population-based study

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Abstract. Metabolic associated fatty liver disease (MAFLD) is a new concept proposed in 2020. This study aimed to explore the relationship between serum 25-hydroxy vitamin D (25(OH)D) level and MAFLD based on a population survey dataset (the third National Health and Nutrition Examination Surveys of the United States). Multivariate logistic regression was used to estimate the odds ratio (OR) of serum 25(OH)D level for MAFLD. A total of 12,878 participants were included in this analysis. Among them, 4,027 (31.27%) cases were diagnosed with MAFLD and 8,851 (66.40%) were without MAFLD (non-MAFLD). Patients with vitamin D sufficiency and insufficiency totaled 6,983 (54.22%) and 5,895 (45.78%), respectively. The incidence of MAFLD and the grade of hepatic steatosis were both significantly higher in vitamin D insufficiency group. Multivariate analysis showed that vitamin D insufficiency was an independent risk factor for MAFLD after adjusted for other confounders (OR: 1.130, 95%CI: 1.035 to 1.234). In MAFLD population, the average serum 25(OH)D level decreased with the numbers of metabolic risks in MAFLD cases. Serum 25(OH)D level was not associated with the severity of fibrosis or steatosis in MAFLD group. In Conclusion, lower serum 25(OH)D level is associated with higher prevalence of MAFLD in general population. No relationship was found between serum 25(OH)D level and the severity of hepatic steatosis or fibrosis in MAFLD.

Key words: Vitamin D, Metabolic associated fatty liver disease, NHANES (National Health and Nutrition Examination Surveys)

Methods

Study population
The study dataset comes from a periodic survey of the Centers for Disease Control and Prevention of the United States, the third National Health and Nutrition Examination Surveys 1988–1994 (NHANES III), which is the only survey with both ultrasonography and biochemical examinations and has been frequently used for the following two facts: the diagnosis of MAFLD does not require the exclusion of other chronic liver diseases, while the presence of metabolic disorder is necessary for MAFLD [8]. This new definition helps to identify more cases at high risk of disease progression [9]. However, as the MAFLD population is different from the NAFLD in many aspects, the characteristics of MAFLD, for example the relationship between vitamin D and MAFLD, need to be further clarified. The aim of this study was to investigate the correlation between MAFLD and serum 25(OH)D status based on a population survey database.

VITAMIN D is a key molecule that plays crucial roles in both bone metabolism and immune regulation [1, 2]. 25-hydroxy vitamin D (25(OH)D) is a known biomarker for the evaluation of the vitamin D status in the body [3]. Vitamin D insufficiency is common in the general population and is associated with various health problems [1, 4]. For example, it has been shown to relate to the metabolic syndromes, as well as non-alcoholic fatty liver disease (NAFLD) and its fibrosis [5, 6].

Metabolic (dysfunction) associated fatty liver disease (MAFLD) is a novel definition proposed in 2020, aimed to replace the previous term of NAFLD [7]. The major difference between NAFLD and MAFLD lies on the
According to the definition of vitamin D insufficiency of the Food and Nutrition Board of the Institute of Medicine [15], participants were divided into two groups: the vitamin D sufficiency group (25(OH)D ≥50 nmol/L) and the vitamin D insufficiency group (25(OH)D <50 nmol/L).

Three non-invasive scores, including AST-to-platelet ratio index (APRI), NAFLD fibrosis score (NFS) and Fibrosis-4 index (FIB-4), were used to assess the liver fibrosis in this study. The APRI was calculated as: APRI = 100(FAST/upper limit of normal)/platelet count (10^9/L). The FIB-4 score was calculated as: age × AST (IU/L)/[platelet count (×10^9/L) × ALT (IU/L)^0.5]. The NFS was calculated as: –1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet count (×10^9/L) – 0.66 × albumin (g/dL). The cutoff points of APRI, NFS and FIB-4 to diagnose advanced fibrosis were 1.5, −1.455 and 1.3, respectively [16-18].

Statistical analysis

Categorical variables were expressed as percentages. Continuous variables were expressed as means ± standard deviation (SD). The Student t-test (for variables normally distributed), the Mann-Whitney U-test (for variables non-normally distributed) and the Chi-squared test (for categorical variables) were used to compare the differences between the groups. Multivariate logistic regression was used to estimate the odds ratio (OR) of serum 25(OH)D level for MAFLD [19]. All tests were two-tailed, and a p value less than 0.05 was considered statistically significant. All analyses was conducted by R 3.6.2 (https://www.r-project.org/).

Results

Characteristics of participants

A total of 12,878 participants with complete ultrasound and laboratory data were included in this analysis (Fig. 1), 6,032 (46.84%) of whom were male and the average age was 43.61 ± 15.97 years. Diabetes and hypertension were found in 1,942 (15.08%) and 3,193 (24.79%) participants, respectively. Among them, 4,027 (31.27%) cases were diagnosed with MAFLD and 8,851 (66.40%) without MAFLD (non-MAFLD). The 25(OH)D level in MAFLD (52.29 ± 19.62 nmol/L) was lower in the non-MAFLD group (55.66 ± 21.17 nmol/L), the difference was statistically significant (p < 0.001).

Patients with vitamin D sufficiency and insufficiency totaled 6,983 (54.22%) and 5,895 (45.78%) respectively.

Comparison between vitamin D sufficiency and insufficiency in overall population

As shown in Table 1, in overall population the vitamin D insufficiency group was younger, with a higher proportion of female. The metabolic profiles, including
Fig. 1 The flow chart of cases selection

Table 1 Comparison between vitamin D sufficiency and insufficiency in overall population

| Variables                        | Total     | Sufficiency  | Insufficiency | p   |
|----------------------------------|-----------|--------------|---------------|-----|
| Number of cases                  | 12,878    | 6,983        | 5,895         |     |
| Age (years)                      | 43.61 ± 15.97 | 43.95 ± 16.39 | 43.21 ± 15.45 | 0.008 |
| Male, n (%)                      | 6,032 (46.84) | 3,739 (53.54) | 2,293 (38.90) | <0.001 |
| MAFLD, n (%)                     | 4,027 (31.27) | 2,013 (28.83) | 2,014 (34.16) | <0.001 |
| Hematocrit, n (%)                |           |              |               |     |
| None                             | 8,162 (63.38) | 4,572 (65.47) | 3,590 (60.9)  |     |
| Mild                             | 1,765 (13.71) | 921 (13.19)   | 844 (14.32)   |     |
| Moderate                         | 2,003 (15.55) | 1,004 (14.38) | 999 (16.95)   |     |
| Severe                           | 948 (7.36)   | 486 (6.96)    | 462 (7.84)    |     |
| BMI (Kg/m²)                      | 27.3 ± 5.88 | 26.56 ± 5.22  | 28.17 ± 6.46  | <0.001 |
| Diabetes, n (%)                  | 1,942 (15.08) | 919 (13.16)  | 1,023 (17.35) | <0.001 |
| Hypertension, n (%)              | 3,193 (24.79) | 1,598 (22.88) | 1,595 (27.06) | <0.001 |
| Vitamin D (mmol/L)               | 54.6 ± 20.76 | 70.10 ± 14.30 | 36.25 ± 8.80  | <0.001 |
| ALT (U/L)                        | 18.82 ± 17.86 | 18.75 ± 17.15 | 18.9 ± 18.67  | 0.633 |
| AST (U/L)                        | 22.58 ± 16.50 | 22.23 ± 14.19 | 22.99 ± 18.87 | 0.011 |
| HbA1c (%)                        | 5.53 ± 1.14  | 5.44 ± 1.01   | 5.63 ± 1.27   | <0.001 |
| FPG (mmol/L)                     | 5.63 ± 2.10  | 5.51 ± 1.77   | 5.76 ± 2.43   | <0.001 |
| HOMA-IR score                    | 3.63 ± 8.90  | 3.15 ± 6.56   | 4.19 ± 11.02  | <0.001 |
| TC (mmol/L)                      | 5.28 ± 1.15  | 5.30 ± 1.13   | 5.25 ± 1.17   | 0.029 |
| TG (mmol/L)                      | 1.63 ± 1.34  | 1.65 ± 1.28   | 1.60 ± 1.41   | 0.027 |
| LDL-C (mmol/L)                   | 3.27 ± 0.99  | 3.31 ± 0.99   | 3.23 ± 0.99   | 0.004 |
| HDL-C (mmol/L)                   | 1.32 ± 0.40  | 1.31 ± 0.39   | 1.33 ± 0.41   | <0.001 |
| APRI score                       | 0.23 ± 0.32  | 0.22 ± 0.21   | 0.23 ± 0.41   | 0.060 |
| APRI score >1.5, n (%)           | 67 (0.52)   | 27 (0.39)     | 40 (0.68)     | 0.030 |
| NFS score                        | -2.41 ± 1.48 | -2.45 ± 1.47  | -2.35 ± 1.48  | <0.001 |
| NFS score ≥-1.455, n (%)         | 3,129 (24.30) | 1,640 (23.49) | 1,489 (25.26) | 0.021 |
| FIB-4                            | 0.96 ± 0.97  | 0.91 ± 0.73   | 1.06 ± 1.35   | <0.001 |
| FIB-4 >1.3, n (%)                | 2,533 (19.67) | 1,570 (17.74) | 963 (23.91)   | <0.001 |

Abbreviations: MAFLD, metabolic associated fatty liver disease; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; HOMA-IR score, homeostasis model assessment-insulin resistance; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; APRI, AST-to-platelet ratio index; NFS, NAFLD fibrosis score, FIB-4, Fibrosis-4 index.
Table 2  Multivariate analysis for MAFLD in overall population

| Variables* | OR    | 95%CI   | p    |
|------------|-------|---------|------|
| Vitamin D insufficiency | 1.130 | 1.035–1.234 | 0.007 |
| Age        | 1.016 | 1.012–1.019 | <0.001 |
| Male       | 1.375 | 1.258–1.503 | <0.001 |
| Diabetes   | 2.014 | 1.777–2.282 | <0.001 |
| Hypertension | 1.087 | 0.982–1.204 | 0.107 |
| HOMA-IR score | 1.005 | 0.998–1.011 | 0.143 |
| Total cholesterol | 0.945 | 0.906–0.987 | 0.010 |
| Triglyceride | 1.466 | 1.406–1.53 | <0.001 |
| BMI        | 2.251 | 2.147–2.359 | <0.001 |

Abbreviations: BMI, body mass index; HOMA-IR score, homeostasis model assessment-insulin resistance.

*B: All the confounders being adjusted are listed in Table 2.

BMI, fast plasma glucose, glycated hemoglobin (Hba1c), homeostasis model assessment-insulin resistance (HOMA-IR), diabetes and hypertension, were more severe in vitamin D insufficiency group (p < 0.05). Aspartate aminotransferase (AST) was slightly higher in insufficiency group than sufficiency group (22.99 ± 18.87 U/L vs. 22.23 ± 14.19 U/L, p = 0.011), while alanine transaminase (ALT) was similar between the two groups. The difference in blood lipid index between the two groups was statistically significant (p < 0.05).

The prevalence of MAFLD was 2,014 (34.16%) in vitamin D insufficiency group and 2,013 (28.83%) in vitamin D sufficiency group (p < 0.001). Lower serum 25(OH)D level was associated with higher prevalence of MAFLD in the general population and was also inversely associated with the number of metabolic conditions upon the diagnosis of MAFLD. Different from NAFLD [5, 6], no relationship was found between serum 25(OH)D level and the severity of hepatic steatosis or fibrosis in MAFLD cases.

Vitamin D insufficiency was common in MAFLD population in this study. The result of multivariate analysis confirmed the vitamin D insufficiency was with higher odds risk of MAFLD independent of age, sex, metabolic syndromes and other demographic factors. This result was in line with previous studies that the lower serum 25(OH)D level increased the risk of NAFLD [5, 6]. Vitamin D may influence the development of MAFLD in both direct and indirect manners, including the impact on systemic and hepatic inflammation, the maintenance of insulin sensitivity, and the controlling microenvironment by suppressing adaptive immunity and up-regulating innate immunity [20]. The vitamin D supplementation can reduce liver steatosis and improve adipose tissue inflammation on animal model [21]. Additionally, vitamin D supplementation can also alleviate the progression of liver steatosis by alleviating hepatocyte senescence and apoptosis via suppressing p53 pathway [22]. All these evidence may shed light on the treatment of MAFLD as the therapeutic approach is limited in MAFLD.

**Serum 25(OH)D level and the metabolic condition for the diagnosis of MAFLD**

Patients with MAFLD were divided into three groups according to the numbers of metabolic conditions used for the diagnosis of MAFLD. 1,149 (28.53%) cases were diagnosed with MAFLD by single metabolic condition, 2,023 (50.24%) by two conditions and 855 (21.23%) by three conditions. The average serum 25(OH)D levels decreased with the numbers of metabolic conditions in MAFLD cases (54.02 ± 20.87 nmol/L in cases with one condition, 52.35 ± 19.32 nmol/L in cases with two and 49.81 ± 18.31 nmol/L in cases with three). The vitamin D insufficiency was found in 538 (46.82%) cases with one condition, 1000 (49.43%) cases with two conditions and 476 (55.67%) cases with three (p < 0.001).

**Discussion**

The results of this study showed that lower serum 25(OH)D level was associated with higher prevalence of MAFLD in the general population and was also inversely associated with the number of metabolic conditions upon the diagnosis of MAFLD. Different from NAFLD [5, 6], no relationship was found between serum 25(OH)D level and the severity of hepatic steatosis or fibrosis in MAFLD cases.

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In this MAFLD cohort, the serum 25(OH)D was not associated with the severity of fibrosis. The relationship between serum 25(OH)D and liver fibrosis is controversial. Some studies demonstrated an inversely correlation between serum 25(OH)D level and the grade of fibrosis [23, 24]. However, a meta-analysis involving 974 NAFLD patients showed that serum vitamin D level might be not associated with the histologic severity of NAFLD [25]. This discrepancy may result from different sample size, the study population and the measurement of fibrosis. As shown by an in vitro study that vitamin D could inhibit fibrosis by repressing TGF-β signaling pathway in hepatic stellate cells [26], there could be a weak impact of vitamin D on the fibrosis, but its role is not strong enough to overcome other factors contributing to the progression of liver diseases. This view is

Table 3  Comparison between vitamin D sufficiency and insufficiency in MAFLD population

| Variables                      | Sufficiency       | Insufficiency     | p    |
|--------------------------------|-------------------|-------------------|------|
| Number of cases                | 2,013             | 2,014             |      |
| Age (years)                    | 49.27 ± 15.50     | 47.46 ± 14.85     | <0.001|
| Male, n (%)                    | 1,189 (59.07)     | 815 (40.47)       | <0.001|
| BMI (Kg/m²)                    | 29.90 ± 5.63      | 31.50 ± 6.75      | <0.001|
| Diabetes (%)                   | 518 (25.73)       | 633 (31.43)       | <0.001|
| Hypertension (%)               | 690 (34.28)       | 750 (37.24)       | 0.054 |
| Vitamin D (nmol/L)             | 68.24 ± 13.58     | 36.34 ± 8.76      | <0.001|
| Hepatic steatosis, n (%)       |                   |                   | 0.897 |
| Mild                           | 670 (33.28)       | 677 (33.61)       |      |
| Moderate                       | 891 (44.26)       | 897 (44.54)       |      |
| Severe                         | 452 (22.45)       | 440 (21.85)       |      |
| Metabolic conditions, n (%)    |                   |                   | <0.001|
| One                            | 611 (30.35)       | 538 (26.71)       |      |
| Two                            | 1,023 (50.82)     | 1,000 (49.65)     |      |
| Three                          | 379 (18.83)       | 476 (23.63)       |      |
| ALT (U/L)                      | 24.14 ± 22.73     | 23.76 ± 21.76     | 0.583 |
| AST (U/L)                      | 25.07 ± 17.82     | 25.55 ± 20.61     | 0.431 |
| HbA1c (%)                      | 5.79 ± 1.34       | 6.03 ± 1.59       | <0.001|
| FPG (mmol/L)                   | 6.1 ± 2.50        | 6.49 ± 3.28       | <0.001|
| HOMA-IR score                  | 5.02 ± 7.88       | 6.24 ± 12.37      | <0.001|
| TC (mmol/L)                    | 5.55 ± 1.18       | 5.49 ± 1.23       | 0.091 |
| TG (mmol/L)                    | 2.26 ± 1.67       | 2.12 ± 1.89       | 0.011 |
| LDL-C (mmol/L)                 | 3.41 ± 1.03       | 3.34 ± 0.97       | 0.171 |
| HDL-C (mmol/L)                 | 1.17 ± 0.35       | 1.21 ± 0.38       | <0.001|
| APRI score                     | 0.25 ± 0.29       | 0.27 ± 0.55       | 0.394 |
| APRI score >1.5, n (%)         | 12 (0.60)         | 14 (0.70)         | 0.845 |
| NFS score >–1.455, n (%)       | –2.11 ± 1.43      | –2.06 ± 1.50      | 0.278 |
| NFS score >–1.455, n (%)       | 656 (32.59)       | 652 (32.37)       | 0.911 |
| FIB-4                          | 1.06 ± 0.74       | 1.06 ± 1.76       | 0.849 |
| FIB-4 >1.3, n (%)              | 475 (23.60)       | 438 (21.75)       | 0.161 |

Abbreviations: MAFLD, metabolic associated fatty liver disease; FLD, fatty liver disease; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; HOMA-IR score, homeostasis model assessment-insulin resistance; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; APRI, AST-to-platelet ratio index; NFS, NAFLD fibrosis score, FIB-4, Fibrosis-4 index.
supported by a meta-analysis which failed to find a positive effect of vitamin D supplements on all-cause mortality, liver-related mortality in adults with chronic liver diseases [27].

A novel finding of this study was that, the serum 25(OH)D level was inversely associated with the numbers of the metabolic conditions upon the diagnosis of MAFLD. It is not a surprising result because lower vitamin D level has been previously shown to increase the HOMA-IR [28, 29], the crux of metabolic syndrome. The new definition of MAFLD emphasizes on the metabolic disorder among patients with liver steatosis and aims to select patients at high risk of disease progression and to treat patients holistically, therefore the control of metabolic syndrome could be more important than the control of liver injury alone in MAFLD cases. Although the serum 25(OH)D level was not associated with fibrosis in MAFLD in this study, high dose of vitamin D and calcium co-supplementation can significantly reduce FBG and HOMA-IR [30], indicating the beneficial effect of vitamin D supplementation in MAFLD needs further investigation.

The strength of this study is that, it is first time to investigate the role of serum 25(OH)D based on the novel definition of MAFLD. The inverse relationship between 25(OH)D level and the numbers of metabolic conditions in MAFLD is the novel finding of this study. There are some limitations of this study. First, we only analyzed the serum 25(OH)D level without taking the vitamin D supplementation, exercise and sunlight into consideration. Second, the data was retrieved from the NHANES III database which was performed 30 years ago. As NHANES is known for its accuracy and objective, the conclusion of this result is robust and unbiased. But further prospective study is still needed to verify this conclusion in contemporary population.

In conclusion, the lower serum 25(OH)D level is associated with higher prevalence of MAFLD in the general population and the number of metabolic conditions in MAFLD population. No relationship was found between serum 25(OH)D level and the severity of hepatic steatosis or fibrosis in MAFLD.

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Authors’ Contributions
Study concept and design: Ruanqin Chen and Bo Wan
Acquisition, cleaning and analysis of the data: Yuan Gao and Yushan Zheng
Drafting of the manuscript: Bo Wan
Critical revision: Ruanqin Chen, Yuan Gao and Yushan Zheng
Study supervision: Ruanqin Chen
All authors contributed to the manuscript for important intellectual content and approved the submission.

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Conflict of Interest
The authors have nothing to declare.

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