Correlation Between the Thyroid Hormone Levels and Nonalcoholic Fatty Liver Disease in Type 2 Diabetic Patients with Normal Thyroid Function

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Abstract

Background: The objective of this study is to retrospectively analyze the correlation between the thyroid hormones and nonalcoholic fatty liver disease (NAFLD) in type 2 diabetes mellitus (T2DM) patients with normal thyroid function.

Methods: Totally 586 T2DM patients with normal thyroid function participated in this research and were divided into T2DM without NAFLD (240 cases) group and T2DM with NAFLD (346 cases) group. The NAFLD fibrosis score (NFS) >0.676 was defined as progressive liver fibrosis and used to categorize the patients into T2DM without progressive liver fibrosis group (493 cases) and T2DM with progressive liver fibrosis group (93 cases).

Results: The results indicated that the levels of free triiodothyronine (FT3) and total triiodomethylamine (TT3) were significantly higher while the free thyroxine (FT4) level was lower in T2DM with NAFLD group than that in T2DM2 without NAFLD group ($p<0.05$). The levels of FT3, FT4 and TT3 in patients with progressive liver fibrosis were significantly lower in patients with progressive liver fibrosis than that in patients without progressive liver fibrosis ($p<0.05$). Logistic regression analysis showed a negative relationship between FT4 level and NAFLD ($p=0.026$), between the levels of FT4,TT3 and total thyroxine (TT4) and the risk of progressive hepatic fibrosis ($p=0.022$, $p=0.007$,$p=0.046$).

Conclusion: There is a certain correlation between thyroid hormone levels and NAFLD in T2DM patients, suggesting that the assessment of thyroid hormone levels in T2DM patients with normal thyroid function is of great significance in the prevention and treatment of NAFLD.

Background

Nonalcoholic fatty liver disease (NAFLD) is a clinical pathological syndrome and one of its key characteristics is hepatic steatosis which can be caused by excessive accumulation of fat in the liver [1]. The disease spectrum includes nonalcoholic steatosis (NAS), liver fibrosis and liver cancer [2]. A meta-analysis of 35,599 patients with type 2 diabetes mellitus (T2DM) from 6 countries indicated that the prevalence of NAFLD was 59.67% [3]. NAFLD can significantly increase the prevalence of chronic complications in patients with T2DM, on the other hand, T2DM can stimulate the development from NAFLD to nonalcoholic steatohepatitis (NASH) and make it easy to progress to liver fibrosis [4].

Nowadays, liver biopsy is the "gold standard" in diagnosing NAFLD and progressive liver disease. However, liver biopsy is an invasive approach, so Angulo et al [5] recommended the use of NAFLD liver fibrosis score (NFS) as preliminary evaluation of liver fibrosis. NFS is a non-traumatic system scoring liver fibrosis and NFS > 0.676 is usually used as marker of progressive liver fibrosis. These patients with NFS > 0.676 are prone to progression of cirrhosis or even liver cancer while NFS<-1.455 is the exception to progressive liver fibrosis. However, NFS is rarely investigated in T2DM patients, and most studies were based on the use of color ultrasound which can only identify NAFLD with liver fat content greater than 30% [6]. Zhang et al [7] pointed out that the liver fat content of T2DM patients decreased while the liver

fibrosis score was higher in these patients, suggesting that the use of liver color ultrasound diagnosis may underestimate the occurrence of NAFLD. Therefore, it is necessary to find other strategy for effective assessment of NAFLD in T2DM patients.

Thyroid hormone is considered to be very important in the metabolism of human glycolipids. Meanwhile, liver is the major organ for the synthesis and metabolism of thyroid hormones. Lower thyroid hormone level can increase blood lipids and increase the prevalence of NAFLD. Byrne et al [8] pointed out that hypothyroidism was a key element for the occurrence of NAFLD. In contrary, Lee et al [9] pointed out that hypothyroidism was not correlated with the occurrence of NAFLD. Van den Berg et al [10] studied people with normal thyroid function and concluded that the free triiodothyronine (FT3) of NAFLD patients was very high, and free thyroxine (FT4) was very low. Kim et al [11] found subclinical hypothyroidism was one of the independent factors for progressive liver fibrosis. A recent study indicated an association between thyroid hormone and liver level of triglyceride in T2DM patients [12]. These studies suggested the necessity to clarify the relationship between thyroid hormones with NAFLD, particularly for these T2DM patients.

Methods

Research subjects

This study is a retrospective clinical analysis of 980 T2DM patients recruited and admitted, from March 2016 to March 2019, to the department of endocrinology of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine. The diagnosis of T2DM followed the criteria proposed by the WHO Diabetes Expert Committee in 1999 and normal thyroid function was defined as the levels of thyroid hormones in the reference range, i.e., thyroid-stimulating hormone (TSH) from 0.35 to 4.94 uIU/L, FT4 from 9.1 to 19.05 pmol/L and FT3 from 2.63 to 5.7 pmol/L. Exclusion criteria included excessive drinking (n = 98), malignant tumor (n = 56), pregnancy (n = 22), acute complications of diabetes (n = 52), acute cardiovascular events (n = 6), severe liver and kidney dysfunction (n = 89), thyroid dysfunction (n = 60), and other acute or chronic liver diseases (n = 11). Finally, 586 T2DM patients (263 men and 323 women) were included in the study.

General clinical information and laboratory test indicators

The general information included gender, age, height, weight, diabetes course, duration of diabetes treatment, history of drinking, past medical history (hypertension, coronary heart disease, tumor, liver disease, etc.), waistline, hipline, systolic pressure (SBP) and diastolic pressure (DBP). All patients were collected samples of venous blood on an empty stomach in the early morning on the second day after admission. The whole blood was centrifuged using a centrifuge. After separation of serum, fasting blood glucose (FBG), blood fat, liver function, kidney function and albumin (ALB) were measured using an automatic biochemical analyzer (Hitachi,7600-020). Fasting C-peptide (FCP) was detected using enzyme-linked immunosorbent assay (Autolumo A2000 Plus). High-performance liquid chromatography was performed for the determination of glycosylated hemoglobin (HbA1c) and flow cytometry (Sysmex

XN9000) for the determination of platelet (PLT). Chemiluminescence microparticle immunoassay (Abbott ARCHITECT i4000) was used to measure FT3, FT4, TT3, TT4 and TSH.

Definition, calculation and group

NAFLD was diagnosed with abdominal color ultrasound by a senior physician in the hospital. The imaging diagnosis of fatty liver requires to meet the following ultrasound findings: high echo in the proximal diffusing point of the liver, higher echo intensity in the liver than in the kidney and unclear intrahepatic tube structure. Under this situation, the distant echo of liver intends to become weaker and weaker. The NAFLD diagnosis complied with the guidelines for NAFLD in China (2010 revision) [13] which requires no history of drinking. In addition to some key acute and chronic liver diseases, unexplainable continuous increase of serum indices of liver function for more than six months was also a requirement for diagnosis of NAFLD.

The body mass index (BMI) was calculated using the body weight (kg) and the square of the height (m^2). Waist to hip ratio was waistline (cm)/hipline (cm). The value of modified Homa-Insulin resistance (C-peptide) (Homa-IR (CP) was calculated by FCP instead of fasting insulin: Homa-IR (CP) = 1.5 + FBG (mmol/L) x FCP (pmol/ L)/2800 [14]. NFS was defined as NFS=(-1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose/presence of diabetes (yes = 1, no = 0) + 0.99 × asparticacid aminotransferase (AST) /alanine aminotransferase (ALT) ratio-0.013 × PLTcount (× 10^9/L)-0.66 × ALB(g/dL).

According to abdominal color ultrasonography, the patients were divided into two different groups: the first group contained 240 cases of T2DM patients without NAFLD and the second group contained 346 cases of T2DM patients with NAFLD. Patient with NFS > 0.676 was defined as progressive liver fibrosis and 493 patients were classified as group of T2DM without progression liver fibrosis and 93 patients were classified as group of T2DM with progressive liver fibrosis.

Statistical analysis

The data of the normal distribution was represented by mean ± SD. SPSS21.0 statistical software was used for the data analysis and the Kologorov-Smirnov normality of all data was tested. Comparisons were conducted within different groups with independent T-test. Measurement data for non-normal distributions were expressed as medians (interquartile intervals). Under this situation, two groups were compared using the Mann-Whitney rank sum test. The Cartridge Test was adopted to demonstrate the differences within two or more groups. The links between thyroid hormone and NAFLD were analyzed by logistic regression. $P < 0.05$ was set as the level for significant difference.

Results

Correlation of NAFLD with clinical data and thyroid hormone levels in T2DM patients

The clinical data and thyroid hormone levels in the participants were listed in Table 1. NAFLD was diagnosed by abdominal ultrasonography in 346 cases (59%). Patients in T2DM with NAFLD group were younger, shorter diabetes course and duration of treatment (all $p < 0.05$) when compared with patients in T2DM without NAFLD. BMI, diastolic pressure, mean arterial pressure, waistline, hipline, waist hip ratio, ALT, AST, glutamyltransferase (GGT), FBG, triglyceride (TG),lipoprotein-B (APO-B), HOMA-IR (CP) and FCP values were all significantly higher in T2DM with NAFLD group (all $p < 0.05$) when compared to T2DM without NAFLD group. FT3 and TT3 levels were significantly elevated ($p < 0.05$) while AST/ALT,high density lipoprotein (HDL), lipoprotein-A1 (APO-A1) and FT4 levels were significantly lower in T2DM with NAFLD group than T2DM without NAFLD group ($p < 0.05$, Table 1). These results suggested that NAFLD is a risk factor in T2DM patients and correlated with the level of thyroid hormone.

Table 1
Comparison of clinical data and thyroid hormone levels in T2DM patients with or without NAFLD

	T2DM without NAFLD (*n* = 240)	T2DM with NAFLD (*n* = 346)	*P value*
Sex (male/female)	101/139	162/184	0.257
Age (years)	62(54–70)	58.5(52–66)	0.002**
Diabetes course(years)	10(5 -14.75)	7(3–12)	0.004**
Duration of treatment (years)	9(2.3–14)	7(1–12)	0.013*
BMI (kg/m^2)	23.81 ± 3.20	26.25 ± 3.37	0.000***
Systolic pressure (mmHg)	132(122–142)	133(124–148)	0.280
Diastolic pressure (mmHg)	80(74–86)	82(76–90)	0.001**
Mean arterial pressure (mmHg)	96.67(91.42-104.67)	99.50(93.33–108.00)	0.009**
Waistline (cm)	88.22 ± 10.51	94.46 ± 9.43	0.000***
Hipline (cm)	97(92–102)	101(96–105)	0.000***
Waist hip ratio	0.91(0.87–0.96)	0.93(0.90–0.97)	0.000***
NFS	-0.2479 ± 1.0663	-0.4283 ± 1.0132	0.038*
ALT (U/L)	16(12–21)	20(15–30)	0.000***
AST (U/L)	16(14–19)	17(15–22)	0.000***
AST/ALT	1.00(0.82–1.25)	0.85(0.68–1.01)	0.000***
GGT (U/L)	18(14–26)	26(19–39)	0.000***
AKP (U/L)	91.00(74.00-113.00)	92.00(75.75–114.00)	0.291
FBG (mmol/L)	7.11(5.75–10.41)	7.70(6.42–9.86)	0.032*
TG (mmol/L)	1.16(0.84–1.64)	1.70(1.26–2.50)	0.000***

Note: NAFLD,Nonalcoholic fatty liver disease;T2DM,Type 2 diabetes mellitus;BMI, Body mass index; NFS, NAFLD fibrosis score; ALT, Alanine aminotransferase; AST, Aspartic acid aminotransferase; GGT, Glutamyltransferase; AKP, Alkaline phosphatase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; APO-A1, Lipoprotein-A1; APO-B,Lipoprotein-B;FT3, Free triiodothyronine; FT4, Free thyroxine; TT3, Total triiodomethylamine; TT4, Total thyroxine; TSH, Thyroid stimulating hormone; Homa-IR (CP),Homa-Insulin resistance(C-peptide);FCP, Fasting C-peptide; HbA1c, Glycosylatedhemoglobin. Abbreviations for other Tables are same.

The measured data of the normal distribution was represented by mean ± SD. Measurement data for non-normal distributions were expressed as medians (interquartile intervals). *p < 0.05, **p < 0.01,***p < 0.005 T2DM with NAFLD vs T2DM without NAFLD.

	T2DM without NAFLD (*n* = 240)	T2DM with NAFLD (*n* = 346)	*P value*
TC (mmol/L)	4.51 ± 1.00	4.64 ± 0.98	0.107
HDL (mmol/L)	1.19(1.01–1.48)	1.07(0.92–1.31)	0.000***
LDL (mmol/L)	2.65(2.10–3.32)	2.78(2.22–3.32)	0.276
APO-A1 (g/L)	1.29(1.12–1.46)	1.23(1.10–1.38)	0.02*
APO-B (g/L)	0.87 ± 0.23	0.94 ± 0.22	0.000***
FT3 (pmol/L)	3.95 ± 0.51	4.04 ± 0.47	0.021*
FT4 (pmol/L)	13.39 ± 1.53	13.04 ± 1.49	0.007**
TT3 (nmol/L)	1.35(1.21–1.52)	1.39(1.26–1.55)	0.032*
TT4 (nmol/L)	84.47(74.89–97.05)	84.09(73.00-95.80)	0.361
TSH (uIU/L)	2.0209(1.4275–2.9372)	2.0534(1.3637–2.8333)	0.708
HOMA-IR (CP)	2.8335(2.2866–3.4668)	3.4185(2.7337–4.3036)	0.000***
FCP (ng/mL)	1.47(0.99–2.03)	2.15(1.43–2.77)	0.000***
HbA1c (%)	7.7(6.7–9.9)	8.0(6.8–9.6)	0.503

Note: NAFLD,Nonalcoholic fatty liver disease;T2DM,Type 2 diabetes mellitus;BMI, Body mass index; NFS, NAFLD fibrosis score; ALT, Alanine aminotransferase; AST, Aspartic acid aminotransferase; GGT, Glutamyltransferase; AKP, Alkaline phosphatase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; APO-A1, Lipoprotein-A1; APO-B,Lipoprotein-B;FT3, Free triiodothyronine; FT4, Free thyroxine; TT3, Total triiodomethylamine; TT4, Total thyroxine; TSH, Thyroid stimulating hormone; Homa-IR (CP),Homa-Insulin resistance(C-peptide);FCP, Fasting C-peptide; HbA1c, Glycosylatedhemoglobin. Abbreviations for other Tables are same.

The measured data of the normal distribution was represented by mean ± SD. Measurement data for non-normal distributions were expressed as medians (interquartile intervals). *p < 0.05, **p < 0.01,***p < 0.005 T2DM with NAFLD vs T2DM without NAFLD.

Correlation of NFS with clinical information and thyroid level in T2DM patients

According to the criteria of NFS > 0.676, 93 patients (15.9%) were determined to have progressive liver fibrosis (Table 2). Patients with progressive liver fibrosis were older, with longer diabetes course and duration of treatment ($p < 0.05$) when compared to patients without progressive liver fibrosis. Diastolic blood pressure, ALT, GGT, FBG, total cholesterol (TC), low density lipoprotein (LDL) and APO-B were all significantly lower in T2DM with progressive liver fibrosis group; while BMI, waistline, hipline, waist-to-hip ratio and AST/ALT values were significantly higher (all $p < 0.05$) when compared to T2DM without

progressive liver fibrosis. The levels of FT3, FT4 and TT3 were significantly lower while the level of TSH was significantly higher in T2DM with progressive liver fibrosis group when compared to T2DM without progressive liver fibrosis group ($p < 0.05$).

Further analysis indicated that, for these patients with progressive liver fibrosis and NFS > 0.676, there was no significant difference in FT3, TT3, TT4 or TSH levels between these T2DM with or without the NAFLD group (Table 3, all $p > 0.05$). Among these people without progressive liver fibrosis but with NFS < 0.676, significant differences in age and FT4 level were found among T2DM patients with and without NAFLD group (Table 3, $p < 0.05$). However, there is no obvious difference in the FT3, TT3, TT4 and TSH levels between these two different groups (Table 3, $p > 0.05$).

Table 2
Comparison of the clinical data and thyroid hormone levels in T2DM patients with or without progressive liver fibrosis

	T2DM with progressive liver fibrosis	T2DM without progressive liver fibrosis	*p* value
	NFS > 0.676(*n* = 93)	NFS < 0.676(*n* = 493)	
Sex(male/female)	34/59	229/264	0.079
Age(years)	71(66–78)	57(52–65)	0.000***
Diabetes course (years)	10(6-19.5)	7(3–12)	0.000***
Duration of treatment (years)	10(5-18.5)	6(1.25-12)	0.000***
BMI(kg/m2)	26.79 ± 3.87	24.96 ± 3.36	0.000***
Systolic pressure(mmHg)	135(124–149)	132(122-144.5)	0.214
Diastolic pressure(mmHg)	78(70.5–84)	82(76–90)	0.000***
Mean arterial pressure (mmHg)	97.33(90-104.34)	98.67(92.67-106.67)	0.182
Waistline(cm)	96.04 ± 12.13	91.12 ± 9.79	0.000***
Hipline(cm)	102(96-106.5)	99(94–103)	0.001**
Waist hip ratio	0.94(0.90–0.98)	0.92(0.89–0.96)	0.026*
ALT(U/L)	15(11–20)	19(14–28)	0.000***
AST(U/L)	17(15–20)	17(14–21)	0.575
AST/ALT	1.13(0.98–1.35)	0.86(0.70–1.07)	0.000***
GGT(U/L)	18(14-27.5)	24(17–34)	0.001**
AKP(U/L)	90.0(73.0-110.5)	92.0(75.0-113.0)	0.477

Note:T2DM,Type 2 diabetes mellitus;BMI, Body mass index; NFS, Nonalcoholic fatty liver disease fibrosis score; ALT, Alanine aminotransferase; AST, Aspartic acid aminotransferase; GGT, Glutamyltransferase; AKP, Alkaline phosphatase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; APO-A1, Lipoprotein-A1; APO-B,Lipoprotein-B;FT3, Free triiodothyronine; FT4, Free thyroxine; TT3, Total triiodomethylamine; TT4, Total thyroxine; TSH, Thyroid stimulating hormone; Homa-IR (CP),Homa-Insulin resistance(C-peptide);FCP, Fasting C-peptide; HbA1c, Glycosylatedhemoglobin. Abbreviations for other Tables are same.

The measured data of the normal distribution was represented by mean ± SD. Measurement data for non-normal distributions were expressed as medians (interquartile intervals) *p < 0.05, **p < 0.01, ***p < 0.005 T2DM with NFS > 0.676 vs T2DM without NFS > 0.676.

	T2DM with progressive liver fibrosis	T2DM without progressive liver fibrosis	*p* value
	NFS > 0.676(*n* = 93)	NFS < 0.676(*n* = 493)	
FBG (mmol/L)	6.99(5.59–8.85)	7.70(6.22–10.23)	0.007**
TG(mmol/L)	1.38(0.97–1.89)	1.50(1.02–2.24)	0.087
TC(mmol/L)	4.31 ± 1.08	4.64 ± 0.96	0.003**
HDL(mmol/L)	1.15(0.94–1.37)	1.12(0.94–1.37)	0.971
LDL (mmol/L)	2.41(2.03–3.14)	2.80(2.24–3.36)	0.003**
APOA1(g/L)	1.22(1.10–1.36)	1.26(1.10–1.43)	0.172
APO-B(g/L)	0.85 ± 0.26	0.92 ± 0.22	0.003**
FT3 (pmol/L)	3.88 ± 0.53	4.03 ± 0.48	0.007**
FT4 (pmol/L)	12.79 ± 1.50	13.26 ± 1.50	0.006**
TT3 (nmol/L)	1.33(1.16–1.43)	1.39(1.25–1.55)	0.001**
TT4 (nmol/L)	82.50(73.12–91.30)	84.80(74.28–96.87)	0.12
TSH (uIU/L)	2.2219(1.6204–3.2512)	2.0229(1.3759–2.8217)	0.028*
HOMA-IR (CP)	3.1409(2.4986–3.8983)	3.2306(2.5618–3.9423)	0.441
FCP (ng/mL)	1.82(1.28–2.59)	1.79(1.17–2.58)	0.65
HbA1c (%)	7.60(6.80–8.85)	8.00(6.80–9.90)	0.173

Note:T2DM,Type 2 diabetes mellitus;BMI, Body mass index; NFS, Nonalcoholic fatty liver disease fibrosis score; ALT, Alanine aminotransferase; AST, Aspartic acid aminotransferase; GGT, Glutamyltransferase; AKP, Alkaline phosphatase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; APO-A1, Lipoprotein-A1; APO-B,Lipoprotein-B;FT3, Free triiodothyronine; FT4, Free thyroxine; TT3, Total triiodomethylamine; TT4, Total thyroxine; TSH, Thyroid stimulating hormone; Homa-IR (CP),Homa-Insulin resistance(C-peptide);FCP, Fasting C-peptide; HbA1c, Glycosylatedhemoglobin. Abbreviations for other Tables are same.

The measured data of the normal distribution was represented by mean ± SD. Measurement data for non-normal distributions were expressed as medians (interquartile intervals) *p < 0.05, **p < 0.01, ***p < 0.005 T2DM with NFS > 0.676 vs T2DM without NFS > 0.676.

Table 3
Comparison of thyroid hormone levels in T2DM patients with or without NAFLD based on the NFS level

	T2DM with progressive liver fibrosis		*p* value	T2DM without progressive liver fibrosis		*p* value
	NFS > 0.676 (*n* = 93)			NFS < 0.676 (*n* = 493)		
	without NAFLD (*n* = 45)	with NAFLD (*n* = 48)		without NAFLD (*n* = 195)	with NAFLD (*n* = 298)	
Sex(male/female)	13/32	21/27	0.137	88/107	141/157	0.634
Age (years)	72.27 ± 8.14	69.92 ± 8.92	0.189	60(53–66)	56(51–64)	0.025*
FT3 (pmol/L)	3.81 ± 0.56	3.95 ± 0.50	0.199	3.98 ± 0.50	4.06 ± 0.47	0.081
FT4 (pmol/L)	13.04 ± 1.57	12.55 ± 1.40	0.113	13.47 ± 1.51	13.12 ± 1.49	0.013*
TT3 (nmol/L)	1.29 ± 0.20	1.36 ± 0.20	0.067	1.40 ± 0.22	1.42 ± 0.23	0.215
TT4(nmol/L)	83.67 ± 11.55	83.08 ± 13.58	0.822	85.05(75.20-97.68)	84.61(73.27–95.96)	0.342
TSH (uIU/L)	2.31 ± 0.99	2.51 ± 1.11	0.381	2.02(1.41–2.85)	2.03(1.34–2.75)	0.537

Note:NAFLD,Nonalcoholic fatty liver disease;T2DM,Type 2 diabetes mellitus;BMI, Body mass index; NFS, NAFLD fibrosis score; FT3, Free triiodothyronine; FT4, Free thyroxine; TT3, Total triiodomethylamine; TT4, Total thyroxine; TSH, Thyroid stimulating hormone;

The measured data of the normal distribution was represented by mean ± SD. Measurement data for non-normal distributions were expressed as medians (interquartile intervals) *p < 0.05 T2DM (NFS < 0.676) with NAFLD vs T2DM (NFS < 0.676) without NAFLD.

Correlation of thyroid hormone levels with liver fibrosis and NAFLD in T2DM patients

The level of FT3, FT4 and TT3 was specified as independent variables with different regression models, respectively, and regression models 1, 2 and 3 were constructed, regardless of whether NAFLD was a dependent variable or not. Model 1 did not correct any factors, according to the results (Table 4), suggested that the relationship between FT3 and TT3 level and NAFLD ($p = 0.022$,$p = 0.043$) was considered to be positive, but the correlation between FT4 level and NAFLD was in contrary ($p = 0.007$). Model 2 corrected the gender factor and claimed a positive relationship between FT3 level and NAFLD ($p = 0.034$). Meanwhile, a negative relationship could be found between the FT4 level and NAFLD ($p = 0.006$). TT3 level was not a favorable correlation with NAFLD (p = 0.055). Model 3 corrected for gender,

age, diastolic blood pressure, waistline, diabetes course and BMI. Based on the research results, it was easy to find that only FT4 level was negatively related to NAFLD ($p = 0.026$).

FT3, FT4, TT3, TT4 and TSH were specified as arguments, respectively, and regression models 1, 2 and 4 were constructed regardless of whether they were dependent variables on progressive liver fibrosis (Table 4). In Model 1 which did not correct any factors, FT3, FT4,TT3 and TT4 levels were inversely connected with the risk of progressive hepatic fibrosis ($p = 0.008$, $p = 0.006$, $p = 0.001$,$p = 0.047$ respectively). A positive relationship between TSH and the risk of progressive hepatic fibrosis ($p = 0.035$) was found. Model 2 corrected the factor of gender factors. The results showed that FT3, FT4, TT3 and TT4 levels were inversely related to progressive hepatic fibrosis risk ($p = 0.016$, $p = 0.007$, $p = 0.001$, $p = 0.033$) while TSH was not correlated with progressive hepatic fibrosis risk ($p = 0.062$). Model 4 corrected factors of gender, age, diastolic blood pressure, waistline, diabetes course, BMI, TC and APO-B. As shown in Table 4, the levels of FT4, TT3 and TT4 can be greatly influenced by the risk of progressive hepatic fibrosis ($p = 0.022$, $p = 0.007$, $p = 0.046$, respectively).

When NFS < 0.676, FT4 as independent variable was assigned with NAFLD as dependent variables, the regression models 1, 5 and 6 were constructed. Model 1 did not correct any factors and showed that the FT4 level had negative correlation with the risk of NAFLD ($p = 0.014$). Model 5 corrected gender and age factors and showed that the FT4 level had negative correlation with the risk of NAFLD ($p = 0.013$). Model 6 corrected gender, age, FT3, TT3, TT4 ,TSH and showed that the FT4 levels had negative correlation with the risk of NAFLD ($p = 0.008$).

Finally, all patients were pooled together and the relevance between the levels of FT3, FT4, TT3, TT4 and TSH with the incidence of NAFLD and progressive liver fibrosis was analyzed. The three-point numbers of FT3 were T1(≤3.8 pmol/L)、T2(3.8～4.21 pmol/L) and T3(≥ 4.21 pmol/L). The three-point numbers of FT4 were T1(≤12.41 pmol/L)、T2(12.41～13.7 pmol/L) and T3(≥ 13.7 pmol/L). The three-point numbers of TT3 were T1(≤1.29 nmol/L)、T2(1.29～1.46 nmol/L) and T3(≥ 1.46 nmol/L). The three-point numbers of TT4 were T1(≤77.71 nmol/L)、T2(77.71～91.32 nmol/L) and T3(≥ 91.32 nmol/L). The three-point numbers of TSH were T1(≤1.6312uIU/L)、T2(1.6312～2.5794uIU/L) and T3(≥ 2.5794uIU/L). The results showed that the incidence of NAFLD presented a significant increase trend with level of FT3 increasing ($p < 0.05$; Fig. 1). Following the FT4 level became higher and higher, the NAFLD showed an obvious decrease trend. When the TT3 level progressed from ≤1.29 to 1.29～1.46, the incidence of NAFLD showed an increase trend ($p < 0.05$; Fig. 1). There was no relevance between the level of TT4 and TSH and the incidence of NAFLD ($P > 0.05$; Fig. 1). For the relevance between the levels of thyroid hormones and the incidence of progressive hepatic fibrosis, the results showed a decrease trend with the increase of FT3, FT4 and TT3 levels ($p < 0.05$) but an increase trend with the increase of TSH level (Figure. 2)

Table 4
Multiple factors logistic regression analysis of thyroid hormones and risk of NAFLD and progressive liver fibrosis

	Thyroid hormones and the risk of NAFLD			**Thyroid hormones and the risk of progressive liver fibrosis**			**when NFS < 0.676, FT4 and the risk of NAFLD**		
	OR	95%CI	*p* value	OR	95%CI	*p* value	OR	95%CI	*p* value
FT3									
Model 1	1.489	1.059–2.094	0.022*	0.532	0.334–0.846	0.008**			
Model 2	1.456	1.029–2.058	0.034*	0.560	0.35–0.899	0.016*			
Model 3	1.041	0.7-1.549	0.842						
Model 4				0.654	0.363–1.176	0.156			
FT4									
Model 1	0.86	0.77–0.96	0.007**	0.798	0.679–0.937	0.006**	0.859	0.761–0.969	0.014*
Model 2	0.857	0.767–0.957	0.006**	0.802	0.683–0.942	0.007**			
Model 3	0.865	0.762–0.982	0.026*						
Model 4				0.796	0.655–0.967	0.022*			
Model 5							0.856	0.758–0.968	0.013*
Model 6							0.784	0.656–0.938	0.008*
TT3									
Model 1	2.171	1.025–4.602	0.043	0.142	0.047–0.431	0.001**			
Model 2	2.095	0.985–4.457	0.055	0.152	0.05–0.464	0.001**			
Model 3	1.166	0.495–2.726	0.726						
Model 4				0.151	0.038–0.597	0.007**			

	Thyroid hormones and the risk of NAFLD	Thyroid hormones and the risk of progressive liver fibrosis		when NFS < 0.676, FT4 and the risk of NAFLD
TT4				
Model 1	0.985	0.97-1.0	0.047*	
Model 2	0.984	0.969–0.999	0.033*	
Model 4	0.98	0.962-1.0	0.046*	
TSH				
Model 1	1.252	1.016–1.544	0.035*	
Model 2	1.224	0.99–1.513	0.062	
Model 4	1.13	0.884–1.446	0.330	

Table 4 Multiple factors logistic regression analysis of thyroid hormones and risk of NAFLD and progressive liver fibrosis

Annotation: Model 1 did not correct any factors. Model 2 corrected gender factor. Model 3 corrected gender, age, diastolic pressure, waistline, diabetes course, body mass index, triglyceride, high density lipoprotein, lipoprotein-A1 and lipoprotein-B factors. Model 4 corrected gender, age, diastolic pressure, waistline, diabetes course, and body mass index, total cholesterol and lipoprotein-B factors. Model 5corrected gender and age factors. Model 6 corrected gender, age, FT3, TT3, TT4 factors. NAFLD,nonalcoholic fatty liver disease;NFS,NAFLD fibrosis score;FT3,,free triiodothyronine;FT4,free thyroxine;TT3,total triiodomethylamine;TT4,total thyroxine;TSH,thyroid stimulating hormone.

Discussion

More and more studies showed that the volatility of serum level of thyroid hormones within normal range had a great chance to be associated with the risk of NAFLD [10,15,16].In the present study, the level of FT3 in the NAFLD group was higher than the non-NAFLD group,and the FT4 levels were lower.After correcting the mixed factors such as diabetes course, BMI, systolic pressure, TG, HDL and LDL, the result indicated that the risk of NAFLD showed a significant increase trend with the levels of FT3 increasing,and decline trend with the levels of FT4 increasing.

These studies together suggest an alternative option for diagnosis strategy of NAFLD in clinic, as liver biopsy is actually a traumatic examination that limits its clinical application although it is considered to be the "gold standard" of the diagnosing of NAFLD.It is clear that thyroid hormone is involved in human

lipid metabolism, induces lipolysis in the liver and participates in the storage and degradation of lipid droplets in liver lysosome [17].When the thyroid function is low, the liver's lipase activity and triglyceride elimination is reduced, resulting in intrahepatic triglyceride accumulation and thereby promoting the development of NAFLD. In addition, low thyroid hormone level also affects the adipocytokines in the circulating system, such as tumor necrosis factor-alpha, leptin, adiponectin, etc [18].Adipocytokines promote the formation of hepatitis and liver fibrosis by direct hepatotoxicity or by promoting the formation of free radical[19].The correlation between thyroid hormones and liver fibrosis has been verified in clinic by previous studies[11,15,20–22].Liu etal[21] discussed the results of 1773 health examinations.

Liver fibrosis was assessed by BARD score ≥ 2.Under this situation,the FT3 levels independently have a close relationship with hepatic fibrosis risk which takes place among patients with NAFLD.Bano[15] participated in 9,419 participants, and the liver fibrosis level was detected by using transientselastography. After correcting gender,age,taking lipid-lowering drugs,cardiovascular risk factors and follow-up time described that TSH levels and FT4 levels were positively linked with liver fibrosis risk and risk of liver fibrosis correspondingly.

However, it should be pointed out that these clinical research subjects from above previous studies were all non-diabetic subjects. Currently, it has been clarified that diabetic patients are often accompanied by abnormal level of thyroid hormones. Higher incidence of NAFLD has been confirmed in T2DM patients and lower screening threshold has been proposed for clinicians[23,24].Bril et al [12] demonstrated that low levels of FT4 increased triglyceride levels in T2DM patients followed the increasing risk of NAFLD by taking a study on 232 patients with normal thyroid function in T2DM, which was consistent with the present result that an obvious increase trend of NAFLD incidence was accompanied by the declining of FT4 levels. Zhu etal[25] used the MatchIt function in the R language package to study 184 T2DM patients using 1:1 covariation matching.Here,the level of FT3 and TT3 in the NAFLD group was higher than the non-NAFLD group.After correcting the mixed factors such as diabetes course,BMI, systolic pressure,TG,HDL and LDL,the result found that the serum FT3 levels had a positive relationship with NAFLD’s risk.Meanwhile,the results of NAFLD showed a significant upward trend with the levels of FT3 increasing.which are consistent with our result in the present study.And in our research, when NFS < 0.676, the FT4 level in the T2DM with NAFLD group were actually lower than the T2DM without NAFLD group. The FT4 level was negatively correlated with NAFLD. These results are consistent with the results of previous study by Van den berg [10], suggesting that abnormal thyroid hormone level is one valuable indicator worthy of emphasis for diagnosis of T2DM. The mechanism for the phenomenon may be that metabolic disorder induced by abnormal thyroid hormone functions further increases the cellular resistance to insulin [26].

As for the relationship between TSH and NAFLD, many studies have proved that subclinical hypothyroidism is a risk factor for NAFLD, and And the increase of TSH level is positively correlated with the incidence of NAFLD[27,28].However, the research conclusion for the population with normal thyroid function wss not consistent. Some scholars believed that if TSH was at the high limit of normal value in people with normal thyroid function, it was related to NAFLD[29].But Ittermann etal[30]had found that

TSH level had nothing to do with hepatic steatosis in people with normal thyroid function.In this research,when diagnosing fatty liver by abdominal color doppler ultrasound,the levels of TSH in the NAFLD group was no difference with the non-NAFLD group, however,From the perspective of liver fibrosis,we found that the progressive hepatic fibrosis group had low FT3,FT4,TT3 levels and high TSH levels. Kim etal[22] also concluded that hypothyroidism was an independent predictor of progressive hepatic fibrosis.

Unfortunately, we only found that a positive relationship between TSH and the risk of progressive hepatic fibrosis ($p = 0.035$) which did not correct any factors.While TSH was not correlated with progressive hepatic fibrosis risk which corrected the factor of other factors.There some limitations in the present study. One limitation is that the size of samples is relative small. In addition, the effects of various chronic complications such as cardiovascular and cerebrovascular diseases, hypoglycemic drugs and lipid-regulating drugs on thyroid hormone levels,differences in analytical methods were not considered, and further work is required in the future. The definition of normal thyroid function and differences in the criteria of NAFLD diagnosis might affect the inconsistency of conclusions. Therefore, a correct and thorough understanding of the results in this study requires certain consideration in clinical practice.

Conclusions

The present study indicated that there is closely correlation between the abnormal thyroid hormone levels and liver fibrosis in T2DM patients, i.e., the prevalence of NAFLD increased following the increase of FT3 and decrease of FT4. These results suggested that the change of thyroid hormone level in T2DM patients should be tested routinely for judging the patient's condition and predicting the prognosis.

Abbreviations

NAFLD：Nonalcoholic fatty liver disease

T2DM：Type 2 diabetes mellitus

NFS：Nonalcoholic fatty liver disease fibrosis score

FT3：Free triiodothyronine

TT3：Total triiodomethylamine

FT4：Free thyroxine

TT4：Total thyroxine

TSH：Thyroid-stimulating hormone

NASH：Nonalcoholic steatohepatitis

SBP：Systolic pressure

DBP：Diastolic pressure

FBG：Fasting blood glucose

ALB：Albumin

FCP：Fasting C-peptide

Homa-IR (CP) ：Homa-Insulin resistance (C-peptide)

HbA1c：Glycosylated hemoglobin

PLT：Platelet

AST：Asparticacid aminotransferase

ALT：Alanine aminotransferase

GGT：Glutamyltransferase

AKP：Alkaline phosphatase

BMI：Body mass index

TG：Triglyceride

TC：Total cholesterol

APO-B：Lipoprotein-B

APO-A1：Lipoprotein-A1

HDL：High density lipoprotein

LDL：Low density lipoprotein

SD：Standard Deviation

OR：Odds ratio

CI：Confidence interval

Declarations

Availability of data and materials

The data that support this study are available from the corresponding author only upon reasonable request, once the study has been published.

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Ethics approval and consent to participate

The study was approved by the ethics committee of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine and the informed consent was also signed by all participants in this study (NO.2019MCZQ02).

Consent for publication Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Yuanyuan Zhang and Huaizhen Liu conceived and designed the study. Juyi Li and Ling Li collected clinical data,Yan Wang entered data,Yuanyuan Zhang and Jinjun Zhang analyzed and interpreted the data.Yuanyuan Zhang wrote the manuscript and Zhimian Zhang made a revised version.

Authors information

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Not applicable.

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Figures

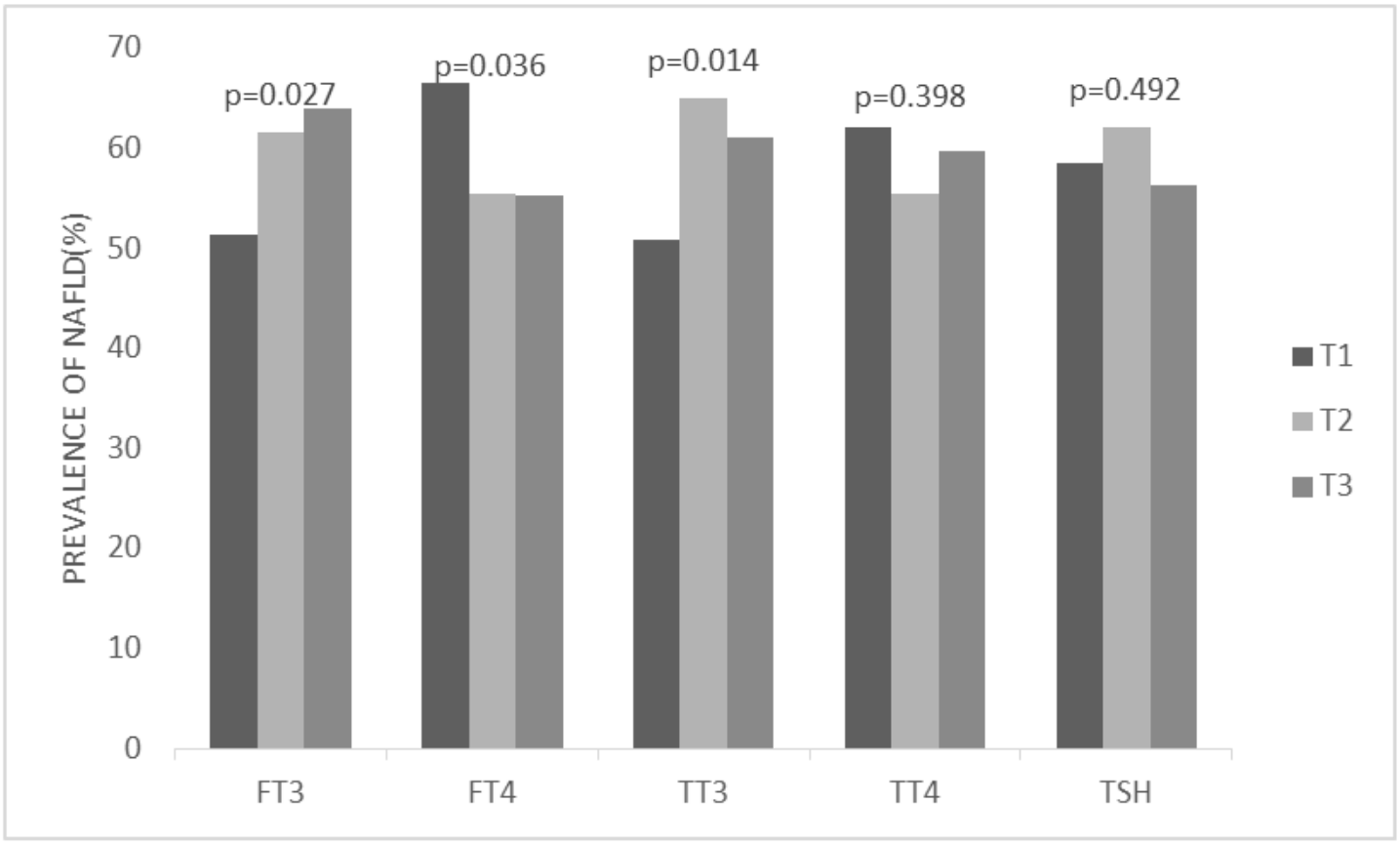

Figure 1

Relevance of the incidence of NAFLD

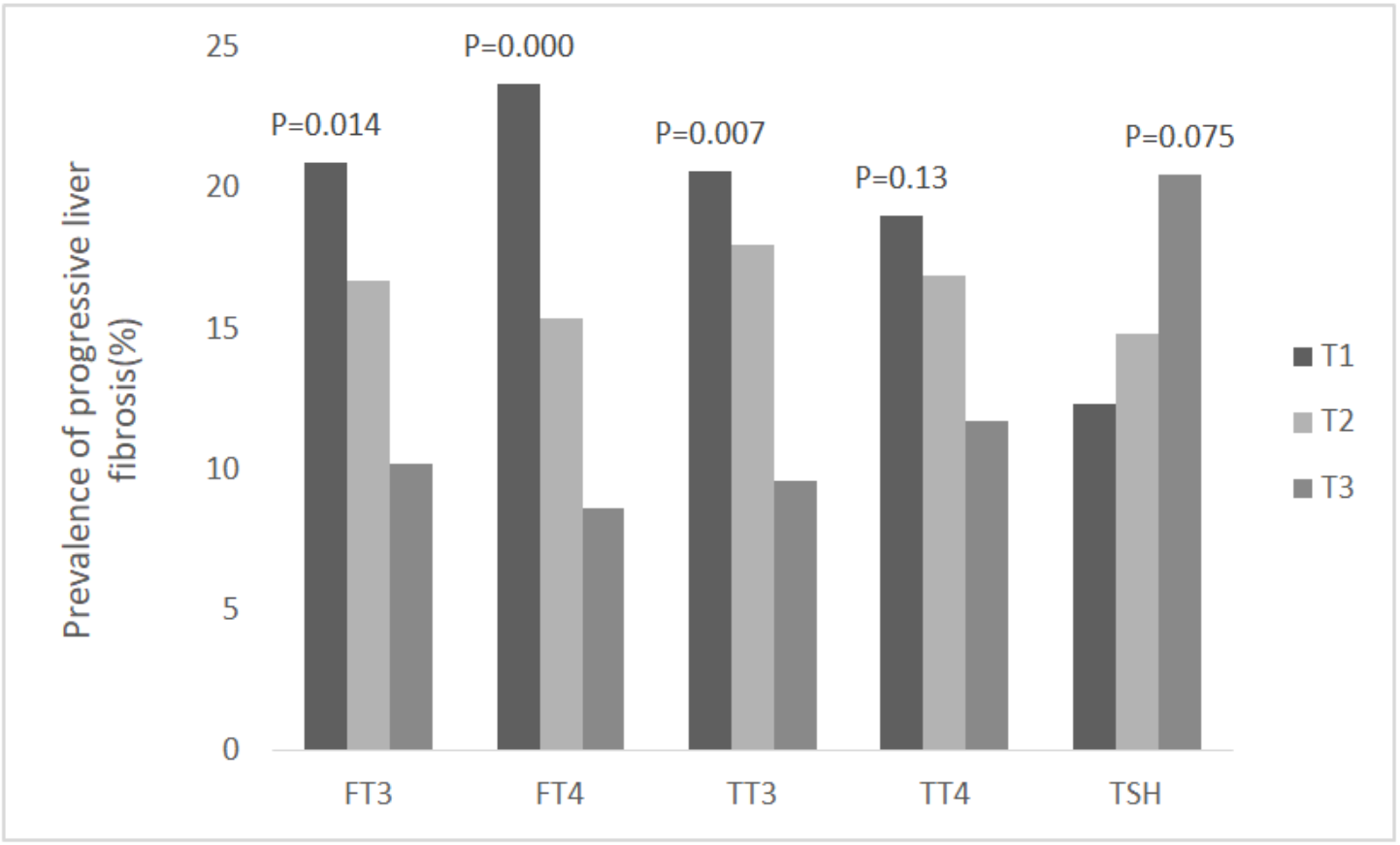

Figure 2

Relevance of the incidence of progressive liver fibrosis