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Running title:prolactin and fatty liver disease

Correlational study on the levels of prolactin and non-alcoholic fatty liver disease in type 2 diabetic patients

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Abstract:

Objective: This research aims to retrospectively probe the association between the prolactin (PRL) and non-alcoholic fatty liver disease (NAFLD), specifically in type 2 diabetes mellitus(T2DM). **Methods:** There are 406 patients with T2DM have participated. Based on the ultrasound diagnosis of NAFLD, there are two groups, named T2DM without NAFLD (men: 77 cases, women: 66 cases, respectively) and T2DM with NAFLD group (men: 153 cases, women: 110 cases, respectively). Inter-group comparison was operated separately. Multiple logistic regression analysis was used to research the relevance between PRL and NAFLD. **Results:** The results indicated that both men and women, the levels of PRL in the NAFLD group were significantly lower ($p<0.01$). In male subjects, the levels of PRL were negatively correlated with hipline, homeostasis model assessment for insulin resistance (C-peptide) and triglyceeide (TG), and inversely related with high density lipoprotein. In female subjects, PRL levels were negatively related with body mass index, diastolic pressure, waistline, hipline and TG ($p<0.05$ or $p<0.01$). Logistic regression analysis revealed a negative relationship between PRL and NAFLD (men: $p=0.031$, women: $p= 0.004$, respectively). As PRL levels increased, NAFLD prevalence decreased in both genders ($p<0.05$). **Conclusion:** Low levels of PRL in physiological range was a risk factor for NAFLD in T2DM, independent of known metabolic risk factors.

Keywords: Type 2 diabetes mellitus; Non-alcoholic fatty liver disease; Prolactin;

Introduction

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The liver is an important organ of glycolipid metabolism in the body. When the triglyceride deposition in hepatocytes increases and the content exceeds 5%, and other factors causing liver steatosis (such as drinking alcohol and viral hepatitis, etc) are excluded, it can be diagnosed as NAFLD[1]. In China, with the gradual improvement of living standards, NAFLD has surpassed chronic viral hepatitis and become the primary cause of chronic liver diseases[2]. Nowadays, global incidence of NAFLD is 25.2%[3], while the prevalence of NAFLD diagnosed by ultrasound in T2DM patients is 73.7%[4]. T2DM can boost to the development of NAFLD to non-alcoholic steatohepatitis (NASH), even liver fibrosis[1].

Risk factors of NAFLD include central obesity, hypertension, hyperlipidemia, T2DM and metabolic syndrome (Met S), etc[5]. Among Met S related diseases, only NAFLD is considered as a strong predictor of Met S, and the incidence of Met S in fatty liver patients is more than 4 times that of non-fatty liver patients [6]. Therefore, NAFLD is considered as the expression of Met S in liver.

Prolactin (PRL) is a kind of protein hormone mainly secreted by adenohypophysis. Its main physiological function is to stimulate breast development and milk scereion [7]. Its receptors are widely distributed in various tissues and organs of the body, including fat, liver, pancreas and so on [8]. PRL can increase the proliferation of β cells, stimulate insulin secretion and participate in the regulation of glucose metabolism [9]. PRL can also inhibit lipolysis and activate adipocyte differentiation by activating peroxisome proliferator-activated receptor γ [10]. Therefore, studies at home and abroad had found that the decrease of serum PRL at physiological level was closely related to the occurrence of T2DM. Wang et al[11] discovered that the PRL levels of patients with T2DM and impaired glucose regulation were significantly lower than that of people with normal glucose metabolism. The researchers further pointed out that the decrease of PRL physiological level was related to the increased risk of T2DM[9]. Manshaei et al[12] also found that the serum PRL concentration of T2DM patients was lower than that of healthy people. Because of the high incidence of NAFLD in T2DM patients, T2DM is also an important part of Met S. The

relationship among PRL 、 NAFLD and Met Sin physiological level has not been explored. The goal of this research is to explore the relationship among PRL、NAFLD and Met S in patients with T2DM.

Materials and Methods

Research objects

From November 2018 to December 2019, 656 T2DM patients were selected for hospitalization in our hospital. The diagnosis of T2DM was based on the diagnostic criteria proposed by the WHO Diabetes Expert Committee in 1999. The PRL levels at physiological level are based on the normal reference range of our hospital, that is 2.78-29.20 ng/mL for premenopausal women, 1.79-20.28 ng/mL for menopausal women and 2.12-17.69 ng/mL for men. This study was a retrospective research, so it was exempted from informed consent with the approval of the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine. Exclusion standard: using drugs that affect PRL (metoclopramide, methyldopa, opiates, andimetidine) (n=15), pituitary diseases (n=4), the levels of thyroid stimulating hormone, cortisol, estradiol and testosterone are higher than the normal range (30), hyperprolactinemia (n=5), too much drink (n=56), cancer (n=11), gestation (n=5) and suffering from type 1 diabetes (n=7), diabetes mellitus complicated with acute complications (n=25), acute cardiovascular accidents (n=15), severe hepatic and renal insufficiency (n=30), suffering from viral (n=8), alcoholic (n=30), drug-damaged (n=5) and autoimmune liver diseases (n=4). Ultimately, 406 patients with T2DM (230 men and 176 women) were taken into the research.

General clinical message and laboratory testtargets

We collected gender, age, menopausal history of women, height, weight, diabetes course, pre-admission hypoglycemic plan (include metformin, insulin and other hypoglycemic drugs such as sulfonylureas, glinides, thiazolidinediones, α-glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl peptidase 4 inhibitors, sodium-glucose cotransporter protein 2 inhibitors), history of drinking, complicated

with cancer, other liver diseases history, waistline, hipline, blood pressure data. All patients' morning venous blood samples were collected on the second day after admission and all blood were extracted with a centrifuge. After separation of serum, fasting blood glucose (FBG), blood fat, liver and kidney function were measured using an automatic biochemical analyzer (7600-020; Hitachi). Fasting C-peptide (FCP) was examined using enzyme-linked immunosorbent assay (A2000 Plus; Autolumo). Adopting automated chemiluminescent immunoassay (Siemens Immulite 2000, UK) was to measure PRL. The coefficients of intra-assay and inter-assay variation were between 2.49-3.47% and 2.91-3.14%, respectively. PRL levels are affected by many conditions including various drugs, stress, and exercise, so we drew fasting blood samples on different days when the patients were at rest, and then took the average of two values. High-performance liquid chromatography was used to check glycosylated hemoglobin (HbA1c) (Variant II; Bio-Rad).

Defining, count and group

NAFLD was diagnosed as ultrasound[13] by a senior technician. The ultrasonic diagnosis of fatty liver is as follows: near-field of liver permeate punctiform hyperechow, the composition of the intrahepatic duct is not clearly demonstrated by ultrasonography, and weak echo in the distal echo. Diagnosis of NAFLD is based on flollowing requirements: no drinking history; in addition to other types of liver diseases; unsolvable serum alanine aminotransferase (ALT) or aspartic acid aminotransferase (AST)、glutamyltransferase (GGT)continued to increase over 6 months[14].

Diagnosis of Met S conforms to the standard which is put forward in the ninth edition of internal medicine in China [15], and the diagnostic standard is the following three or more items: ①central obesity and/or abdominal obesity: waistline is 90cm for men and 85cm for women; ②Hyperglycemia: FBG > 6.1mmol/L or blood glucose 2 hours after sugar load >7.8mmol/L and/or those are confirmed as diabetes and treated with hypoglycemic therapy; ③Hypertension: blood pressure exceeds 130/85 mmHg and/or those are diagnosed as hypertension and treated with an tihypertensive therapy; ④ fasting triglyceeide (TG) surpasses 1.7mmol/l; ⑤ fasting high density lipoprotein (HDL). belows 1.04mmol/l.Body mass index (BMI) was computed by

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dividing the body weight (kg) by the square of the height (m^2). homeostasis model assessment for insulin resistance (C-peptide) (HOMA-IR (CP)) was reckoned by FCP substitute for fasting insulin, HOMA-IR (CP) =1.5+FBG (mmol/L)xFCP (pmol/L)/ 2800. HOMA-β (CP-DM) =0.27x FCP (pmol/L)（FBG (mmol /L) -3.5)[16].

In conformity with ultrasonic diagnosis, patients with T2DM who met the inclusion criteria were segmented into without NAFLD group (men: 77 cases, women: 66 cases, respectively) and with NAFLD group(men: 153 cases, women: 110 cases, respectively) .

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Statistical analysis

SPSS21.0 statistical software was used for the data analysis and the Kologorov-Smirnov normality of all data were tested. The measured data of the normal distribution was represented by mean ± SD. Comparisons were conducted between two groups and the comparing process was finished by making full use of independent T test. Measurement data for non-normal distributions were expressed as medians (interquartile intervals) .Under this situation, two groups were compared by using the Mann-Whitney rank sum test. Counting data was shown by the number of cases, the Chi-square test was adopted to demonstrate the differences within two or more groups. Spearman correlation analysis compared the relationship between PRL levels and other variables. The links among PRL、NAFLD and Met S were analyzed by logistic regression. $P<0.05$ or $P<0.01$ represented the obvious differences in statistics.

Results

1.Comparison of general message and laboratory test targetsin each group

The ultrasonic diagnostic rate of NAFLD was 263 cases (153 plus 110 cases) (64.8%) (Table 1). Men cases with NAFLD group had younger age, and higher BMI、waistline、hipline、diastolic pressure (DBP)、GGT、FBG、TG、total cholesterol

(TC)、low density lipoprotein (LDL)、HOMA-IR (CP)、 HbA1C、the incidence of Met S. Women patients with NAFLD group also had higher BMI、ALT、GGT、TG、HOMA-IR (CP) 、 HbA1C 、 the incidence of Met S. While HDL and PRL were markedly reduced in the cases merged-NAFLD than without NAFLD in both genders ($p<0.05$ or $p<0.01$). In terms of medication history, there was no statistical difference between the two groups of male and female patients in the treatment of hypoglycemic programs, which could exclude the influence of hypoglycemic drugs on the study.

Table 1 Comparison of general material and biochemical indexes of each group

	Men			Women		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	77	153		66	110	
Age(years)	63(54-63)	54(48-62)	0.000	65(57-71)	61(55-69)	0.077
Metabolic syndrome(%)	64.9	85.6	0.000	59.100	80.000	0.003
Menopause(%)	NA	NA		99.100	83.600	0.117
Diabetes course(years)	10(3-15)	8(3-12)	0.280	10(5-20)	10(4-15)	0.070
BMI(kg/m^2)	24.90±2.97	27.18±2.94	0.000	24.54±3.35	26.33±3.55	0.000
Systolic pressure(mmHg)	130(125-146)	132(121-145)	0.880	130(124-151)	130(123-144)	0.233
Diastolic pressure(mmHg)	81.48±9.59	85.80±9.94	0.002	80.48±8.59	79.07±8.16	0.277
Waistline(cm)	90.71±8.02	96.29±8.45	0.000	89.02±9.07	91.38±9.41	0.103
Hipline(cm)	96.64±6.77	100.66±6.18	0.000	97.00±6.52	97.73±7.81	0.526
ALT(U/L)	19(13-28)	21(15-32)	0.082	15(12-21)	19(14-33)	0.000
AST(U/L)	18(15-23)	19(15-23)	0.881	17(15-20)	18(15-25)	0.094
GGT (U/L)	24(17-36)	35(23-56)	0.000	19(14-28)	25(19-35)	0.000
FBG (mmol/L)	6.81(5.41-9.49)	7.80(6.21-11.0)	0.002	6.61(5.48-9.34)	7.89(6.05-10.96)	0.050
TG (mmol/L)	1.20(0.79-1.75)	2.01(1.42-3.27)	0.000	1.23(0.93-1.50)	1.81(1.19-2.35)	0.000
TC(mmol/L)	4.32±0.92	4.83±1.10	0.001	4.77±1.24	5.02±1.11	0.158
HDL (mmol/L)	1.04(0.96-1.18)	0.97(0.82-1.11)	0.004	1.23(1.05-1.47)	1.10(0.99-1.28)	0.002
LDL (mmol/L)	2.45±0.78	2.78±0.85	0.004	2.84±1.05	2.98±0.88	0.373
HOMA-IR(CP)	2.90(2.46-3.97)	3.99(3.18-5.20)	0.000	2.97(2.54-3.68)	3.68(2.91-4.41)	0.001
HOMA-β(CP-DM)	46.94(25.29-88.92)	44.33(27.17-83.92)	0.686	38.55(22.52-80.19)	48.27(25.02-73.90)	0.553
HbA1C (%)	7.5(6.7-9.1)	8.3(7.0-9.7)	0.043	7.7(6.7-9.3)	8.5(7.4-9.9)	0.020
PRL(ng/mL)	10.36(9.35-14.72)	9.56(7.81-12.60)	0.001	12.97(10.03-16.58)	10.38(8.43-14.27)	0.001
Hypoglycemic plan						
Metformin	26(33.8%)	62(40.5%)		17(25.8%)	43(39.1%)	
Other hypoglycemic drugs	16(20.8%)	44(28.8%)	0.083	23(34.8%)	27(24.5%)	0.150
Insulin	35(45.4%)	47(30.7%)		26(39.4%)	40(36.4%)	

Note: NAFLD,Nonalcoholic fatty liver disease;T2DM,Type 2 diabetes mellitus;BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartic acid

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aminotransferase; GGT, Glutamyltransferase;FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; HOMA-IR(CP),homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for beta(C-peptide- diabetes mellitus);HbA1c, Glycosylatedhemoglobin;PRL,prolactin.The measured data of the normal distribution was represented by mean±SD. Measurement data for non-normal distributions were expressed as medians (interquartile intervals).[1]

Because women's serum PRL is affected by menopause or not, we analyzed the metabolic status and PRL levels of female patients with or without NAFLD before and after menopause (Table 2). Premenopausal women with NAFLD had higher BMI、FBG 、 TG 、 HbA1C and the incidence of Met S. Postmenopausal women with NAFLD had higher BMI、ALT、GGT、TG、HOMA-IR (CP) and the incidence of Met S, while HDL and PRL were markedly reduced in the cases merged-NAFLD than without NAFLD ($p<0.05$ or $p<0.01$).

Table 2 Comparison of clinical data of women with and without NAFLD before and after menopause

	Premenopause			Postmenpause		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	6	18		60	92	
Age(years)	44.80±3.76	45.20±4.37	0.848	66.15±8.34	64.61±8.16	0.261
Metabolic syndrome(%)	0	77.8	0.001	65	80.4	0.033
Diabetes course(years)	8.180±6.69	4.78±4.12	0.149	12.86±9.02	10.69±6.88	0.116
BMI(kg/m^2)	22.80±3.87	26.70±3.43	0.029	24.71±3.28	26.26±3.59	0.008
Systolic pressure(mmHg)	121.83±7.08	128.50±8.78	0.107	133(127-152)	131(122-145)	0.167
Diastolic pressure(mmHg)	77.50±7.18	84.06±6.78	0.055	80.78±8.71	78.10±8.08	0.054
Waistline(cm)	80.33±12.36	89.06±7.92	0.055	89.88±8.32	91.84±9.65	0.200
Hipline(cm)	94.67±6.83	95.61±7.65	0.791	97.23±6.50	98.14±7.81	0.456
ALT(U/L)	13(11-16)	15(13-45)	0.121	15(12-22)	20(15-33)	0.000
AST(U/L)	16(15-18)	16(13-35)	1.000	17(15-20)	19(16-25）	0.073
GGT (U/L)	20.67±14.28	37.67±31.34	0.217	19(14-29)	26(18-35)	0.002
FBG (mmol/L)	6.61±1.59	10.99±3.10	0.003	7.81±2.97	8.08±2.78	0.566
TG (mmol/L)	1.09(0.63-1.30)	2.02(1.37-2.83)	0.003	1.24(0.93-1.56)	1.74(1.17-2.34)	0.000
TC(mmol/L)	4.77±0.84	5.16±1.46	0.534	4.77±1.28	4.99±1.04	0.229
HDL (mmol/L)	1.23±0.17	1.06±0.21	0.086	1.27±0.29	1.14±0.27	0.005
LDL (mmol/L)	2.92±0.83	2.84±0.86	0.838	2.84±1.08	3.00±0.89	0.301
HOMA-IR(CP)	2.30±0.57	4.87±2.98	0.051	3.28±1.00	3.73±1.43	0.036

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HOMA-β(CP-DM)	25.07(19.86-28.67)	25.99(13.78-56.47)	0.689	47.00(22.63-85.05)	51.60(28.83-75.27)	0.505
HbA1C (%)	7.62±0.89	9.53±1.66	0.014	8.16±1.82	8.53±1.67	0.196
PRL(ng/mL)	18.92±8.57	14.54±4.64	0.122	13.16±3.79	10.88±3.77	0.000

Note: NAFLD,Nonalcoholic fatty liver disease;T2DM,Type 2 diabetes mellitus;BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartic acid aminotransferase; GGT, Glutamyltransferase;FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; HOMA-IR(CP),homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for beta(C-peptide- diabetes mellitus);HbA1c, Glycosylatedhemoglobin;PRL,prolactin.The measured data of the normal distribution was represented by mean±SD. Measurement data for non-normal distributions were expressed as medians (interquartile intervals).[2]

2. Relationship between PRL levels and related parameters of Met S

We further discussed the relationship between PRL levels and related parameters of Met S (Table 3). We found that in male subjects, the levels of PRL were negatively correlated with hipline, TG and HOMA-IR (CP), and positively associated with HDL, in female subjects, PRL levels were negatively related with BMI, DBP, waistline, hipline and TG ($p<0.05$ or $p<0.01$).

Table3 Relationship between PRL levels and related parameters of Met S

	Men		Women	
	r	P value	r	P value
BMI	-0.092	0.166	-0.192	0.011
Systolic pressure	0.046	0.492	-0.045	0.552
Diastolic pressure	-0.125	0.059	-0.220	0.003
Waistline	-0.056	0.398	-0.152	0.044
Hipline	-0.141	0.032	-0.157	0.037
FBG	-0.109	0.098	-0.034	0.654
TG	-0.252	0.000	-0.258	0.001
TC	-0.096	0.146	-0.061	0.421
HDL	0.147	0.025	0.065	0.390
LDL	-0.042	0.528	-0.110	0.146
HOMA-IR(CP)	-0.141	0.032	-0.049	0.519
HOMA-β(CP-DM)	0.019	0.772	-0.044	0.562
HbA1C	-0.091	0.168	0.057	0.450

Note: Met S,metabolic syndrome;BMI, Body mass index;FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; HOMA-IR(CP),homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for beta(C-peptide- diabetes mellitus);HbA1c, Glycosylatedhemoglobin;PRL,prolactin.;;PRL,prolactin.

3.Multiple factors logistic regression analysis of serum PRL levels and NAFLD risk

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The risk factors for NAFLD were assessed using multiple logistic regression analysis, which included age, BMI, menopause, TG, LDL as well as HOMA-IR (CP), HbA1C and PRL levels as variables. We found that PRL levels were independently negative associated with NAFLD in both men and women (odds ratio (OR): 0.891, 95% confidence interval (CI): 0.803-0.989, p=0.031, for men; OR: 0.874,95% (CI): 0.797-0.957, p=0.004, for women). Other risk factors included age, BMI, LDL and HOMA-IR (CP) for men, and TG for women (Table 4).

Table4 Multivariate logistic regression analysis of serum PRL levels and NAFLD risk

	Men			Women		
	β	OR(95%CI)	p value	β	OR(95%CI)	p value
Age	-0.045	0.956(0.924-0.989)	0.010	-0.044	0.957(0.912-1.004)	0.070
BMI	0.255	1.291(1.122-1.484)	0.000	0.090	1.094(0.97-1.224)	0.120
Menopause				0.213	1.237(0.281-5.441)	0.778
TG	0.176	1.193(0.959-1.483)	0.113	0.981	2.666(1.404-5.064)	0.003
LDL	0.493	1.637(1.046-2.561)	0.031	-0.121	0.886(0.596-1.318)	0.550
HOMA-IR(CP)	0.360	1.134(1.062-1.936)	0.019	0.215	1.240(0.859-1.788)	0.250
HbA1C	0.057	1.059(0.872-1.287)	0.564	0.047	1.048(0.840-1.308)	0.676
PRL	-0.115	0.891(0.803-0.989)	0.031	-0.135	0.874(0.797-0.957)	0.004

Note: The risk factors for NAFLD were assessed using multiple logistic regression analysis in men and women.The ORs with corresponding 95% CIs were adjusted for age, BMI, menopause,TG,LDL as well as HOMA-IR(CP), HbA1C and PRL levels as variables.NAFLD,Nonalcoholic fatty liver disease;BMI, Body mass index;FBG, Fasting blood glucose; TG, Triglyceride; LDL, Low density lipoprotein; HOMA-IR(CP),homeostasis model assessment for insulin resistance(C-peptide); HbA1c, Glycosylatedhemoglobin;PRL,prolactin.;;PRL,prolactin.OR:Odds ratio;CI:Confidence

4.Relationship between PRL levels and the prevalence of NAFLD and Met S

According to the quartile of PRL levels,the subjects were divided into four groups: T1＜8.29 (n= 57 cases)、8.29≤T2＜9.93 (n= 58 cases)、9.93≤T3＜12.68 (n= 57 cases)、T4≥12.68 (n= 58 cases) ng/mL in men (n= 230 cases) and T1＜8.95 (n= 44 cases)、8.95≤T2＜11.32 (n= 44 cases)、11.32≤T3＜14.95 (n= 44 cases)、T4≥ 14.95 (n= 44 cases) ng/mL in women (n= 176 cases). Chi-square test was used to compare the prevalence and composition ratio among different groups. The prevalence of NAFLD had a decreasing trend with the rise of the quartile of PRL in both genders (T1: 84.2%, T2: 63.8%, T3: 59.6%, T4: 58.6%, p=0.012 in men; T1: 79.5%, T2:
65.9%, T3: 54.5%, T4: 50%, p= 0.013 in women). However, the prevalence rates of Met S wereT1: 86%, T2: 79.3%, T3:77.2%, T4: 72.4%, p= 0.354 in men; T1: 84.1%,

T2: 70.5%, T3: 77.3%, T4: 56.8%, p= 0.031 in women. Therefore, in female subjects, the prevalence rates of Met S in the fourth quartile groups of PRL were significantly lower than those in the first, second and third group.

Discussion

At present, due to the rapid increase in the incidence of obesity and obesity-related diseases, NAFLD has become an important public health problem[17] NAFLD is considered as the manifestation of Met S in liver, especially in T2DM patients [18]. In this study, it was found that the incidence of NAFLD diagnosed by abdominal liver color doppler ultrasound was 64.8%. Compared with non-NAFLD patients, NAFLD patients had higher BMI, TG, GGT, HOMA-IR (CP), HbA1C, incidence of Met S and lower HDL in both genders. Equally, Zhang et al[19] also got similar results. Among which BMI, TG and HDL were the components of Met S. Therefore, cases in T2DM complicated NAFLD were easy to promote the abnormality of metabolic indexes.

PRL is considered as a hormone closely related to metabolism [20]. Recent findings have displayed that there had been close association between PRL and T2DM. Across sectional study included 2377 adult community population (excluding hyperprolactinemia), and found that cases with impaired glucose regulation and T2DM had lower PRL levels. Researchers rectified age, sex, BMI and other confounding factors, still discovered that the risk of above-mentioned people with high serum PRL was signifiicantly reduced [11]. Further follow-up of 3.7 years, it was revealed that female cases had a lower risk of T2DM in the highest quartile PRL levels, with a risk ratio of 0.48[9]. Another cross-sectional study also found that the risk of Met S and T2DM in women with lower baseline PRL increased[21]. A large meta-analysis indicated that higher serum PRL levels in normal range were related with low level of T2DM risk[22]. At the same time, Jha et al[23] also found that serum PRL had a significant correlation with liver disease and predicted its mortality. In adipose tissue, PRL intervention can reduce the production of malonyl coenzyme A in human primary adipocytes, thus inhibiting the restart of triglyceride synthesis [24]. PRL receptor can also directly inhibit the expression of fatty acid synthetase and fatty acid synthesis in 3T3L1 cells[25]. PRL reduced the accumulation of triglyceride in

liver through PRL receptor, thus improving liver steatosis [10]. These results indicate that higher PRL levels had positive protective effect on glucose and lipid metabolism.

Considering that PRL secretion may be different due to gender, we studied male and female subjects separately. We found that compared with non-NAFLD patients, the PRL value of NAFLD patients was lower in both genders. The age, BMI, TG, LDL, HOMA-IR (CP), HbA1C were corrected,at the same time, female subjects corrected menopause factors, the study suggested that PRL levels had negative relatation to the risk of NAFLD. In line with the quartile of PRL, the incidence of NAFLD showed a generally downturn with the increase of PRL levels in both genders. Zhang et al[26] noted that PRL increased by one standard deviation, the risk of male NAFLD patients decreased by 12.3%, and that of female patients decreased by 21.4%. PRL was proved to be a protective factor, which affected the existence and progress of NAFLD.In another study, Zhang et al[19] also found that the PRL levels of NAFLD patients diagnosed by ultrasound were significantly lower than that of non-NAFLD patients, whether male or female. In addition, with the increase of PRL quartile, the incidence of NAFLD decreased. All subjects were corrected for age, sex, BMI, insulin resistance, HbA1C, diabetes and other factors. The results showed that PRL had contrary associated with NAFLD. We took into consideration that PRL levels are affected by many conditions including various drugs, stress, and exercise. We ruled out the following cases: such as using drugs that affect PRL (metoclopramide, methyl-
dopa, opiates, andcimetidine), the levels of thyroid stimulating hormone, cortisol, estradiol and testosterone are higher than the normal range. In terms of medication history, there was no statistical difference between the two groups of male and female patients in the treatment of hypoglycemic programs, which could exclude the influence of hypoglycemic drugs on the study.

In the meantime, the secretion of PRL may be affected by whether women were menopausal or not, this paper analyze the menopausal and non-menopausal subtypes of women, we found that postmenopausal women with NAFLD had lower PRL levels. In addition, Zhang Zhuzi et al[27] divided the included women into

premenopausal group and postmenopausal group, and also found that in both groups, PRL of patients with NAFLD was lower than that of patients without NAFLD, the decrease of PRL levels in postmenopausal women with NAFLD was more significant. It was suggested that the decrease of PRL in NAFLD patients was affected by menopausal factors.

Researchs have shown a correlation between PRL levels and the components of Met S, which could explain the role of PRL in NAFLD. According to the basic researchs, in the obese mouse model induced by high-fat diet, severe metabolic changes would occur in mice with PRL receptor failure. Injection of PRL could improve insulin sensitivity and prevent visceral adipocyte hypertrophy [28]. Clinical studies had found that low serum PRL levels in physiological range was related to poor metabolic outcome of Met S and T2DM[11]. In overweight and obese men, serum PRL levels were lower [28]. Friedrich et al[29] found that PRL levels were negatively correlated with waistline in 1857 healthy women aged 20-79. The endocrine characteristics of Met S and polycystic ovary syndrome (PCOS) have a relatively high coincidence rate [30]. A systematic retrospective analysis of 2052 PCOS patients found that the lower the serum PRL, the higher the BMI.PRL had opposite related with TG, TC and LDL-C [31]. Arterial hypertension is a component of Met S. A prospective study of 874 postmenopausal women found that PRL levels increased by 1 standard deviation during 8 years of follow-up, and the relative risk of hypertension was 1.31[32]. Our study found that in male subjects, the levels of PRL were negatively correlated with hipline, TG and HOMA-IR(CP), and positively associated with HDL, in female subjects, PRL levels were negatively related with BMI, DBP, waistline, hipline and TG. In female subjects, the prevalence rates of Met S in the fourth quartile groups of PRL were significantly lower than those in the first, second and third group. Furthermore, premenopausal and postmenopausal women with NAFLD had higher BMI、TG and the incidence of Met S. As we all know, NAFLD is very common in obese and dyslipidemia patients. Obese individuals produce relatively excessive proinflammatory factors, some of which inhibit the treatment of liver fat and promote the accumulation of lipid in hepatocytes [33]. Dyslipidemia, especially hypertriglyceridemia, may

subsequently increase the transportation of TG and other fats into hepatocytes, resulting in hepatic steatosis [34].

As a retrospective analysis, this study has many limitations. First of all, the diagnosis of NAFLD is based on ultrasound examination, which cannot distinguish NASH from fibrosis. Secondly, because this is a cross-sectional study, we can't infer the direct cause and influence between PRL and NAFLD, and need further mechanical study to clarify their exact relationship. Thirdly, PRL secretion appears in pulse form, the best time to draw blood for PRL is from 9: 00 to 11: 00 a.m., and patients should avoid emotional excitement.Finally, due to the limited number of samples in this study, the effects of drugs for treating cardiovascular diseases, controlling blood lipid on PRL levels have not been meditated,which requires further layered analysis in the future work. Moreover, the small sample size can not reflect the large scale population based cross-sectional epidemiological study, so it is necessary to increase the sample size.

Conclusions

In a word, our research shows that serum PRL levels are related to NAFLD in T2DM population in physiological range, and are also connected to known metabolic risk factors. Our research results may help to predict the risk of developing NAFLD, so as to better understand the disease,importantly, and to formulate effective prevention strategies.

Abbreviations

NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus; PRL: Prolactin; Met S: Metabolic syndrome; NASH:Non-alcoholic steatohepatitis; SBP: Systolic pressure; DBP: Diastolic pressure; BMI: Body mass index; AST: Asparticacid aminotransferase; ALT: Alanine aminotransferase; GGT: Glutamyltransferase; FBG: Fasting bloodglucose; TG: Triglyceride; TC: Total cholesterol; HDL: High densitylipoprotein; LDL: Low density lipoprotein; Homa-IR (CP): Modified homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment for beta (C-peptide- diabetes mellitus); HbA1c: Glycosylated hemoglobin; SD: Standard deviation; OR: Odds ratio; CI: Confidence interval

Footnotes

Contributorship statement

YZ conceived the study, collected clinical data, analyzed and interpreted the data and wrote the manuscript. HL made a revised version. All authors read and agreed to the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Data Sharing Statement

All data generated or analyzed during this study are included in the article.The data that support this study are available from the corresponding author only upon reasonable request, once the study has been published.

Ethics approval and consent to participate

This study was a retrospective research, so it was exempted from informed consent with the approval of the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (2020MCZQ09).

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Running title:Prolactin and fatty liver disease

Cross-sectional correlations of prolactin levels and nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a retrospective analysis of patients from a single hospital in China

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Abstract:

Objective: This research aims to retrospectively assess the association between prolactin (PRL) and nonalcoholic fatty liver disease (NAFLD), specifically in patients with type 2 diabetes mellitus(T2DM).

Design and setting: A cross-sectional study was conducted in Anhui, China.

Participants: A total of 406 patients with T2DM (153 men, 110 women) were selected.

Primary and secondary outcome measures: P values for the independent T test, Mann–Whitney rank sum test, Spearman correlation analysis, and multiple logistic regression models were used to explore the association between PRL and NAFLD in patients with T2DM.

Results: The results indicated that in both men and women, the levels of PRL were significantly lower in the T2DM with NAFLD group than that in the T2DM without NAFLD group (men: 9.56 ng/mL vs. 10.36 ng/mL, women: 10.38 ng/mL vs. 12.97 ng/mL). In male subjects, the levels of PRL were negatively correlated with hip circumference, homeostasis model assessment for insulin resistance (C-peptide) and triglyceeide (TG), and inversely correlated with high density lipoprotein (HDL) (r=-0.141, p=0.032, r=-0.141, p=0.032, r=-0.252, p=0.000, r=0.147, p=0.025, respectively). In female subjects, PRL levels were negatively related with body mass index, diastolic blood pressure, waist circumference, hip circumference and TG (r=-0.192, p=0.011, r=-0.220, p=0.003, r=-0.152, p=0.044, r=-0.157, p=0.037,

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r=-0.258, p=0.001, respectively). Logistic regression analysis revealed a negative relationship between PRL and NAFLD (men: OR95% CI: 0.891 (0.803-0.989), p=0.031, women: OR95% CI: 0.874 (0.797-0.957), p= 0.004, respectively).

As PRL levels increased, NAFLD prevalence decreased in both sexes (men: p=0.012, women: p= 0.013, respectively).

Conclusion: Our results supported that a low levels of PRL in the physiological range was a markers of NAFLD in T2DM patients and suggested that prolactin within the biologically high range may play a protective role in the pathogenesis of NAFLD.

Keywords: Type 2 diabetes mellitus; Nonalcoholic fatty liver disease; Prolactin;

Strengths and limitations of this study

►Abdominal colour ultrasonography is a common and simple method for the clinical diagnosis of nonalcoholic fatty liver disease (NAFLD).

►The normal range of serum prolactin (PRL) levels differs by sex, so we conducted a sex stratification analysis of patients with type 2 diabetes mellitus (T2DM) and found that in both men and women, the levels of PRL were significantly lower in the T2DM with NAFLD group than that in without NAFLD group.

►The change of PRL levels promotes medical workers to pay attention to the occurrence of NAFLD in patients with T2DM.

►P values for independent T tests and multiple logistic regression models were used to assess the association between PRL and NAFLD in patients with T2DM.

►This was a cross-sectional study and cannot provide evidence of causal relationships.

Introduction

The liver is an important organ of glycolipid metabolism in the body. When triglyceride deposition in hepatocytes increases and exceeds 5% and other factors causing liver steatosis (such as alcohol consumption and viral hepatitis) are excluded, NAFLD can be diagnosed[1]. In China, with the gradual improvement of living standards, NAFLD has surpassed chronic viral hepatitis to become the primary cause of chronic liver diseases[2]. Currently, the global incidence of NAFLD is 25.2%[3], while the prevalence of NAFLD diagnosed by ultrasound in patients with T2DM is

73.7%[4]. T2DM is an important factor associated with the progression of NAFLD to NASH and fibrosis[1].

NAFLD is closely related to central obesity, hypertension, hyperlipidaemia, T2DM and metabolic syndrome (MetS)[5]. Among MetS-related diseases, only NAFLD is considered a strong predictor of MetS, and the incidence of MetS in fatty liver patients is more than 4 times that in nonfatty liver patients[6]. Therefore, NAFLD is considered the expression of MetS in the liver.

Prolactin (PRL) is a type of hormone that is mainly secreted by adenohypophysis. Its main physiological function is to stimulate breast development and milk secretion[7]. Its receptors are widely distributed in various tissues and organs of the body, including fat, liver, pancreas and so on [8]. PRL can increase the proliferation of β cells, stimulate insulin secretion and participate in the regulation of glucose metabolism [9]. PRL can also inhibit lipolysis and activate adipocyte differentiation by activating peroxisome proliferator-activated receptor γ[10]. Studies in China and abroad have found that the decrease in serum PRL at the physiological level is closely related to the occurrence of T2DM. Wang et al[11] discovered that the PRL levels of patients with T2DM and impaired glucose regulation were significantly lower than those of people with normal glucose metabolism. The researchers further pointed out that the decrease in physiological levels of PRL was related to an increased risk of T2DM[9]. Manshaei et al[12] also found that the serum PRL concentration of patients with T2DM was lower than that of healthy people. Because of the high incidence of NAFLD in patients with T2DM, T2DM is also an important factor in MetS. The relationship among PRL, NAFLD and MetS at the physiological level has not been explored. The goal of this research was to explore the relationship among PRL, NAFLD and MetS in patients with T2DM.

Methods

Participants

All participants in this study were recruited from a hospital located Anhui, China. This was a cross-sectional survey. A total of 656 patients with T2DM were investigated in this study, but 15 participants were excluded due to the use of that

affect PRL (metoclopramide, methyldopa, opiates, and imetidine). Thirty participants were excluded because their levels of thyroid-stimulating hormone,cortisol, oestradiol and testosterone were higher than the normal range. Four participants had pituitary diseases, five had hyperglycaemia, 56 exhibited excessive alcohol consumption (intake of alcohol exceeding 140 g/week for men and 70 g/week for women), 11 had cancer, 5 were pregnant, 7 had type 1 diabetes, 25 had acute complications of diabetes, 15 had acute cardiovascular events, 30 had severe hepatic and renal insufficiency, 8 had viral liver disease, 30 had alcoholic liver disease, 5 had drug-induced liver disease and 4 had autoimmune liver disease.Finally, 406 participants (230 men and 176 women) were included in this study. This study was a retrospective study, so it was exempted from the requirement of informed consent and was approved by the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine.

Data collection

We collected data on sex, age, menopausal history of women, height, weight, diabetes course, preadmission hypoglycaemic plan (including metformin, insulin and other hypoglycaemic drugs such as sulfonylureas, glinides, thiazolidinediones, α-glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl peptidase 4 inhibitors, sodium-glucose cotransporter protein 2 inhibitors), history of alcohol consumption, occurrence of cancer, history of other liver diseases, waist circumference, hip circumference, and blood pressure. All patients' morning venous blood samples were collected on the second day after admission, and all blood was extracted with a centrifuge. After separation of serum, fasting blood glucose (FBG), blood fat, liver and kidney function were measured using an automatic biochemical analyser (7600-020; Hitachi). Fasting C-peptide (FCP) was examined using an enzyme-linked immunosorbent assay (A2000 Plus; Autolumo). An automated chemiluminescent immunoassay (Siemens Immulite 2000, UK) was used to measure PRL. The coefficients of intra-assay and interassay variation ranged from 2.49-3.47% and 2.91-3.14%, respectively. PRL levels are affected by many conditions including

the use of various drugs, stress, and exercise, so we took blood samples at 9: 00 am after the patients were admitted to the hospital on the first day and the next morning. We took 2 ml blood samples each time. The patients fasted and rested in a sitting position for 30 minutes, and then the average value of two blood pressure readings was taken High-performance liquid chromatography was used to check glycosylated haemoglobin (HbA1c) (Variant II; Bio－Rad).

Definitions, counts and groups

The diagnosis of T2DM was based on the diagnostic criteria proposed by the World Health Organization (WHO) Diabetes Expert Committee in 1999. The physiological level of PRL is based on the normal reference range of our hospital, which is 2.78-29.20 ng/mL for premenopausal women, 1.79-20.28 ng/mL for menopausal women and 2.12-17.69 ng/mL for men.

NAFLD was diagnosed by ultrasound[13] by a senior technician. The ultrasonic diagnosis of fatty liver is as follows: near-field of liver permeate punctiform hyperecho, composition of the intrahepatic duct not clearly demonstrated by ultrasonography, and weak echo in the distal echo. Diagnosis of NAFLD is based on the following requirements: no history of alcohol consumption, no other types of liver diseases, and unexplained increase in serum alanine aminotransferase (ALT), aspartic acid aminotransferase (AST) or glutamyltransferase (GGT) over 6 months[14].

The diagnosis of MetS conformed to the standard put forward in the ninth edition of internal medicine in China [15], and the diagnostic standard included the following three or more items: ① central obesity and/or abdominal obesity: waist circumference is greater than 90 cm for men and 85 cm for women; ② hyperglycaemia: FBG > 6.1 mmol/L or 2-hour blood glucose >7.8 mmol/L and/or confirmation of diabetes diagnosis and treatment with hypoglycaemic therapy; ③ Hypertension: blood pressure exceeding 130/85 mmHg and/or diagnosis of hypertension and treatment with antihypertensive therapy; ④ fasting triglyceride (TG) level exceeding 1.7 mmol/l; and ⑤ fasting high-density lipoprotein (HDL) Level below 1.04 mmol/l. Body mass index (BMI) was computed by dividing the body weight (kg) by the square of the height (m^2). homeostasis model assessment of

insulin resistance (C-peptide) (HOMA-IR (CP)) was determined by FCP as a substitute for fasting insulin as follows: HOMA-IR (CP) =1.5+FBG (mmol/L)xFCP (pmol/L)/2800. HOMA-β (CP-DM) =0.27x FCP (pmol/L) (FBG (mmol/L) -3.5)[16].

In conformity with ultrasonic diagnosis, patients with T2DM who met the inclusion criteria were segmented into the without NAFLD group (77 men, 66 women) and the with NAFLD group (153 men, 110 women).

Patient and public involvement

Patients and the public were not involved in the design, conduction, reporting, or dissemination plans of our research.

Statistical analysis

SPSS 21.0 statistical software was used for the data analysis, and the Kolmogorov–Smirnov normality test was performed for all data. The measured data with a normal distribution are represented as the mean ± standard deviation (SD). Comparisons were conducted between two groups, and comparisons were performed using independent T tests. Measurement data with nonnormal distributions are expressed as medians (interquartile intervals). In this situation, two groups were compared by using the Mann–Whitney rank sum test. Categorical variables are shown as the number of cases, and the chi-square test was adopted to demonstrate the differences within two or more groups. Spearman correlation analysis compared the relationship between PRL levels and other variables. The relationships among PRL, NAFLD and MetS were analysed by logistic regression. $P<0.05$ or $P<0.01$ represented obvious significant differences.

Results

1.Comparison of general findings and laboratory test targetsin each group

The ultrasonic diagnostic rate of NAFLD was 263 cases (153 plus 110 cases) (64.8%) (Table 1). Men with NAFLD had a younger age and higher BMI, waist circumference, hip circumference, diastolic blood pressure (DBP), GGT, FBG, TG, total cholesterol (TC), low-density lipoprotein (LDL), HOMA-IR (CP)、, HbA1C, and MetS incidence. Women with NAFLD also had higher BMI, ALT, GGT, TG, HOMA-IR (CP), HbA1C, and MetS incidence. HDL and PRL were markedly reduced

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in the patients with NAFLD than in those without NAFLD in both sexes ($p<0.05$ or $p<0.01$). In terms of medication history, there was no significant difference between the two groups of male and female patients in hypoglycaemic programs, which could exclude the influence of hypoglycaemic drugs on the study.

Table 1 Comparison of general characteristics and biochemical indexes of each group

	Men			Women		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	77	153		66	110	
Age(years)	63(54-63)	54(48-62)	0.000	65(57-71)	61(55-69)	0.077
Metabolic syndrome(%)	64.9	85.6	0.000	59.100	80.000	0.003
Menopause(%)	NA	NA		99.100	83.600	0.117
Diabetes course(years)	10(3-15)	8(3-12)	0.280	10(5-20)	10(4-15)	0.070
BMI(kg/m^2)	24.90±2.97	27.18±2.94	0.000	24.54±3.35	26.33±3.55	0.000
Systolic pressure(mmHg)	130(125-146)	132(121-145)	0.880	130(124-151)	130(123-144)	0.233
Diastolic pressure(mmHg)	81.48±9.59	85.80±9.94	0.002	80.48±8.59	79.07±8.16	0.277
Waist circumference(cm)	90.71±8.02	96.29±8.45	0.000	89.02±9.07	91.38±9.41	0.103
Hip circumference(cm)	96.64±6.77	100.66±6.18	0.000	97.00±6.52	97.73±7.81	0.526
ALT(U/L)	19(13-28)	21(15-32)	0.082	15(12-21)	19(14-33)	0.000
AST(U/L)	18(15-23)	19(15-23)	0.881	17(15-20)	18(15-25)	0.094
GGT (U/L)	24(17-36)	35(23-56)	0.000	19(14-28)	25(19-35)	0.000
FBG (mmol/L)	6.81(5.41-9.49)	7.80(6.21-11.0)	0.002	6.61(5.48-9.34)	7.89(6.05-10.96)	0.050
TG (mmol/L)	1.20(0.79-1.75)	2.01(1.42-3.27)	0.000	1.23(0.93-1.50)	1.81(1.19-2.35)	0.000
TC(mmol/L)	4.32±0.92	4.83±1.10	0.001	4.77±1.24	5.02±1.11	0.158
HDL (mmol/L)	1.04(0.96-1.18)	0.97(0.82-1.11)	0.004	1.23(1.05-1.47)	1.10(0.99-1.28)	0.002
LDL (mmol/L)	2.45±0.78	2.78±0.85	0.004	2.84±1.05	2.98±0.88	0.373
HOMA-IR(CP)	2.90(2.46-3.97)	3.99(3.18-5.20)	0.000	2.97(2.54-3.68)	3.68(2.91-4.41)	0.001
HOMA-β(CP-DM)	46.94(25.29-88.92)	44.33(27.17-83.92)	0.686	38.55(22.52-80.19)	48.27(25.02-73.90)	0.553
HbA1C (%)	7.5(6.7-9.1)	8.3(7.0-9.7)	0.043	7.7(6.7-9.3)	8.5(7.4-9.9)	0.020
PRL(ng/mL)	10.36(9.35-14.72)	9.56(7.81-12.60)	0.001	12.97(10.03-16.58)	10.38(8.43-14.27)	0.001
Hypoglycaemic plan						
Metformin	26(33.8%)	62(40.5%)		17(25.8%)	43(39.1%)	
Other hypoglycaemic drugs	16(20.8%)	44(28.8%)	0.083	23(34.8%)	27(24.5%)	0.150
Insulin	35(45.4%)	47(30.7%)		26(39.4%)	40(36.4%)	

Note: NAFLD, Nonalcoholic fatty liver disease; T2DM, Type 2 diabetes mellitus; BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartic acid aminotransferase; GGT, Glutamyltransferase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; HOMA-IR(CP), homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for

beta(C-peptide- diabetes mellitus);HbA1c, Glycosylated haemoglobin; PRL, prolactin. The measured data with a normal distribution are represented as the mean±SD. Measurement data for nonnormal distributions are expressed as medians (interquartile intervals).Normally distributed variables: BMI, diastolic blood pressure, waist circumference, hip circumference, TC, LDL; Nonnormally distributed variables: Age, diabetes course, systolic blood pressure, ALT, AST, GGT, FBG, TG, HDL, HOMA-IR (CP), HOMA-β (CP-DM), HbA1C, and PRL.

Because women's serum PRL is affected by menopause, we analysed the metabolic status and PRL levels of female patients with or without NAFLD before and after menopause (Table 2). Premenopausal women with NAFLD had higher BMI, FBG, TG, HbA1C and MetS incidence. Postmenopausal women with NAFLD had higher BMI, ALT, GGT, TG, HOMA-IR (CP) and MetS incidence, while HDL and PRL were markedly reduced in the patients with NAFLD compared with the levels in those without NAFLD ($p<0.05$ or $p<0.01$).

Table 2 Comparison of clinical data of women with and without NAFLD before and after menopause

	Premenopause			Postmenopause		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	6	18		60	92	
Age(years)	44.80±3.76	45.20±4.37	0.848	66.15±8.34	64.61±8.16	0.261
Metabolic syndrome(%)	0	77.8	0.001	65	80.4	0.033
Diabetes course(years)	8.180±6.69	4.78±4.12	0.149	12.86±9.02	10.69±6.88	0.116
BMI(kg/m^2)	22.80±3.87	26.70±3.43	0.029	24.71±3.28	26.26±3.59	0.008
Systolic pressure(mmHg)	121.83±7.08	128.50±8.78	0.107	133(127-152)	131(122-145)	0.167
Diastolic pressure(mmHg)	77.50±7.18	84.06±6.78	0.055	80.78±8.71	78.10±8.08	0.054
Waist circumference(cm)	80.33±12.36	89.06±7.92	0.055	89.88±8.32	91.84±9.65	0.200
Hip circumference(cm)	94.67±6.83	95.61±7.65	0.791	97.23±6.50	98.14±7.81	0.456
ALT(U/L)	13(11-16)	15(13-45)	0.121	15(12-22)	20(15-33)	0.000
AST(U/L)	16(15-18)	16(13-35)	1.000	17(15-20)	19(16-25）	0.073
GGT (U/L)	20.67±14.28	37.67±31.34	0.217	19(14-29)	26(18-35)	0.002
FBG (mmol/L)	6.61±1.59	10.99±3.10	0.003	7.81±2.97	8.08±2.78	0.566
TG (mmol/L)	1.09(0.63-1.30)	2.02(1.37-2.83)	0.003	1.24(0.93-1.56)	1.74(1.17-2.34)	0.000
TC(mmol/L)	4.77±0.84	5.16±1.46	0.534	4.77±1.28	4.99±1.04	0.229
HDL (mmol/L)	1.23±0.17	1.06±0.21	0.086	1.27±0.29	1.14±0.27	0.005
LDL (mmol/L)	2.92±0.83	2.84±0.86	0.838	2.84±1.08	3.00±0.89	0.301
HOMA-IR(CP)	2.30±0.57	4.87±2.98	0.051	3.28±1.00	3.73±1.43	0.036
HOMA-β(CP-DM)	25.07(19.86-28.67)	25.99(13.78-56.47)	0.689	47.00(22.63-85.05)	51.60(28.83-75.27)	0.505
HbA1C (%)	7.62±0.89	9.53±1.66	0.014	8.16±1.82	8.53±1.67	0.196

PRL(ng/mL)	18.92±8.57	14.54±4.64	0.122	13.16±3.79	10.88±3.77	0.000

Note: NAFLD, Nonalcoholic fatty liver disease; T2DM, Type 2 diabetes mellitus; BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartic acid aminotransferase; GGT, Glutamyltransferase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low density lipoprotein; HOMA-IR(CP), homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for beta(C-peptide- diabetes mellitus);HbA1c, Glycosylated haemoglobin; PRL, prolactin. The measurement data with a normal distribution are represented as the mean±SD. Measurement data with nonnormal distributions are expressed as medians (interquartile intervals).[1]

2. Relationship between PRL levels and MetS-related parameters

We further investigated the relationship between PRL levels and MetS-related parameters (Table 3). We found that in male subjects, the levels of PRL were negatively correlated with hip circumference, TG and HOMA-IR (CP) and positively associated with HDL. In female subjects, PRL levels were negatively correlated with BMI, DBP, waist circumference,

Table3 Relationship between PRL levels and MetS-related parameters

	Men		Women	
	r	P value	r	P value
BMI	-0.092	0.166	-0.192	0.011
Systolic pressure	0.046	0.492	-0.045	0.552
Diastolic pressure	-0.125	0.059	-0.220	0.003
Waist circumference	-0.056	0.398	-0.152	0.044
Hip circumference	-0.141	0.032	-0.157	0.037
FBG	-0.109	0.098	-0.034	0.654
TG	-0.252	0.000	-0.258	0.001
TC	-0.096	0.146	-0.061	0.421
HDL	0.147	0.025	0.065	0.390
LDL	-0.042	0.528	-0.110	0.146
HOMA-IR(CP)	-0.141	0.032	-0.049	0.519
HOMA-β(CP-DM)	0.019	0.772	-0.044	0.562
HbA1C	-0.091	0.168	0.057	0.450

Note: Met S, metabolic syndrome; BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus); HbA1c, glycosylated haemoglobin; PRL, prolactin.; PRL, prolactin.

3.Multiple-factor logistic regression analysis of serum PRL levels and NAFLD risk

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The impact index for NAFLD was assessed using multiple logistic regression analysis, which included age, BMI, menopause, TG, LDL, HOMA-IR (CP), HbA1C and PRL as variables. We found that PRL levels were independently negatively associated with NAFLD in both men and women (odds ratio (OR): 0.891, 95% confidence interval (CI): 0.803-0.989, p=0.031, for men; OR: 0.874, 95% (CI): 0.797-0.957, p=0.004, for women). Other risk factors included age, BMI, LDL and HOMA-IR (CP) for men and TG for women(Table 4).

Table4 Multivariate logistic regression analysis of serum PRL levels and NAFLD risk

	Men			Women		
	β	OR(95% CI)	p value	β	OR(95% CI)	p value
Age	-0.045	0.956(0.924-0.989)	0.010	-0.044	0.957(0.912-1.004)	0.070
BMI	0.255	1.291(1.122-1.484)	0.000	0.090	1.094(0.97-1.224)	0.120
Menopause				0.213	1.237(0.281-5.441)	0.778
TG	0.176	1.193(0.959-1.483)	0.113	0.981	2.666(1.404-5.064)	0.003
LDL	0.493	1.637(1.046-2.561)	0.031	-0.121	0.886(0.596-1.318)	0.550
HOMA-IR(CP)	0.360	1.134(1.062-1.936)	0.019	0.215	1.240(0.859-1.788)	0.250
HbA1C	0.057	1.059(0.872-1.287)	0.564	0.047	1.048(0.840-1.308)	0.676
PRL	-0.115	0.891(0.803-0.989)	0.031	-0.135	0.874(0.797-0.957)	0.004

Note: The risk factors for NAFLD were assessed using multiple logistic regression analysis in men and women. The ORs with corresponding 95% CIs were adjusted for age, BMI, menopause, TG, LDL and HOMA-IR (CP), HbA1C and PRL levels as variables. NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HbA1c, glycosylated haemoglobin; PRL, prolactin.; PRL, prolactin. OR: Odds ratio; CI: Confidence interval

4.Relationship between PRL levels and the prevalence of NAFLD and MetS

According to the quartiles of PRL levels, the subjects were divided into four groups: T1<8.29 (n= 57 cases), 8.29≤T2<9.93 (n= 58 cases), 9.93≤T3<12.68 (n= 57 cases), T4≥12.68 (n= 58 cases) ng/mL in men (n= 230 cases) and T1<8.95 (n= 44 cases), 8.95≤T2<11.32 (n= 44 cases), 11.32≤T3<14.95 (n= 44 cases), T4≥14.95 (n= 44 cases) ng/mL in women (n= 176 cases). The chi-square test was used to compare the prevalence and composition ratio among different groups. The prevalence of NAFLD exhibited a decreasing trend with the rise of the quartile of PRL in both sexes (T1: 84.2%, T2: 63.8%, T3: 59.6%, T4: 58.6%, p=0.012 in men; T1: 79.5%, T2: 65.9%, T3: 54.5%, T4: 50%, p= 0.013 in women). However, the prevalence rates of MetS were T1: 86%, T2: 79.3%, T3: 77.2%, T4: 72.4% (p= 0.354) in men and T1:

84.1%, T2: 70.5%, T3: 77.3%, T4: 56.8% (p= 0.031) in women. Therefore, in female subjects, the prevalence rate of Met S in the fourth quartile of PRL was significantly lower than those in the first, second and third quartiles

Discussion

At present, due to the rapid increase in the incidence of obesity and obesity-related diseases, NAFLD has become an important public health problem[17]. NAFLD is considered the manifestation of MetS in the liver, especially in patients with T2DM[18]. In this study, it was found that the incidence of NAFLD diagnosed by abdominal liver colour Doppler ultrasound was 64.8%. Compared with non-NAFLD patients, NAFLD patients had higher BMI, TG, GGT, HOMA-IR (CP), HbA1C, and MetS incidence and lower HDL in both sexes. Zhang et al[19] obtained similar results. BMI, TG and HDL are components of MetS. Therefore, T2DM complicated with NAFLD promotes abnormalities in metabolic indexes.

PRL is a hormone closely related to metabolism[20]. Recent findings have shown that there is a close association between PRL and T2DM. A cross-sectional study included 2377 adults from the community population (excluding those with hyperprolactinemia) and found that individuals with impaired glucose regulation and T2DM had lower PRL levels. Researchers controlled for age, sex, BMI and other confounding factors and still discovered that the risk in the abovementioned people with high serum PRL was significantly reduced [11]. Further follow-up of 3.7 years revealed that female patients had a lower risk of T2DM in the highest quartile of PRL, with a risk ratio of 0.48[9]. Another cross-sectional study also found that the risk of MetS and T2DM in women with lower baseline PRL were increased[21]. A large meta-analysis indicated that higher serum PRL levels in the normal range were related to a low risk of T2DM[22]. Jha et al[23] also found that serum PRL had a significant correlation with liver disease and predicted mortality. In adipose tissue, PRL intervention can reduce the production of malonyl coenzyme A in human primary adipocytes, thus inhibiting triglyceride synthesis[24]. The PRL receptor can also directly inhibit the expression of fatty acid synthetase and fatty acid synthesis in 3T3L1 cells[25]. PRL reduced the accumulation of triglycerides in the liver through

the PRL receptor, thus improving liver steatosis [10]. These results indicate that higher PRL levels had a positive protective effect on glucose and lipid metabolism.

Considering that PRL secretion may differ according to sex, we studied male and female subjects separately. We found that compared with non-NAFLD patients, the PRL value of NAFLD patients was lower in both sexes. Age, BMI, TG, LDL, HOMA-IR (CP), and HbA1C were adjusted; additionally, among female subjects, menopausal factors were adjusted, and the study suggested that PRL levels had a negative relationship with the risk of NAFLD. In line with the quartile of PRL, the incidence of NAFLD showed a general decrease with the increase in PRL levels in both sexes. Zhang et al[26] noted that when PRL increased by one standard deviation the risk among male NAFLD patients decreased by 12.3%, and that among female patients decreased by 21.4%. PRL was proven to be a protective factor, that affected the existence and progression of NAFLD.In another study, Zhang et al[19] also found that the PRL levels of NAFLD patients diagnosed by ultrasound were significantly lower than those of non-NAFLD patients, whether male or female. In addition, with the increase in PRL quartile, the incidence of NAFLD decreased. All analyses were corrected for age, sex, BMI, insulin resistance, HbA1C, diabetes and other factors. The results showed that PRL an inverse association with NAFLD. We took into consideration that PRL levels are affected by many conditions including, various drugs, stress, and exercise. We ruled out the following cases: the use of drugs that affect PRL (metoclopramide, methyl-dopa, opiates, and cimetidine) and levels of thyroid-stimulating hormone, cortisol, oestradiol and testosterone that were higher than the normal range. In terms of medication history, there was no significant difference between the two groups of male and female patients in regard to hypoglycaemic programs, which could exclude the influence of hypoglycaemic drugs on the study.

In addition, the secretion of PRL may be affected by menopausal status. This paper analysed menopausal and nonmenopausal women and found that postmenopausal women with NAFLD had lower PRL levels. In addition, Zhang Zhuzi et al[27] divided the included women into a premenopausal group and a postmenopausal group

and found that in both groups, the PRL of patients with NAFLD was lower than that of patients without NAFLD, and the decrease in PRL levels in postmenopausal women with NAFLD was more significant. It was suggested that the decrease in the PRL of patients with NAFLD was affected by menopausal factors.

Studies have shown a correlation between PRL levels and the components of Met S, which could explain the role of PRL in NAFLD. According to basic studies, in an obese mouse model induced by a high-fat diet, severe metabolic changes would occur in mice with PRL receptor failure. Injection of PRL could improve insulin sensitivity and prevent visceral adipocyte hypertrophy [28]. Clinical studies have found that low serum PRL levels in the physiological range are related to poor metabolic outcomes of MetS and T2DM[11]. In overweight and obese men, serum PRL levels were lower [28]. Friedrich et al[29] found that PRL levels were negatively correlated with waist circumference in 1857 healthy women aged 20-79 years. The endocrine characteristics of MetS and polycystic ovary syndrome (PCOS) have a relatively high similarity rate [30]. A systematic retrospective analysis of 2052 PCOS patients revealed that the lower the serum PRL was, the higher the BMI. PRL had the opposite relationship with TG, TC and LDL-C [31]. Arterial hypertension is a component of MetS. A prospective study of 874 postmenopausal women found that PRL levels increased by 1 standard deviation during 8 years of follow-up, and the relative risk of hypertension was 1.31[32]. Our study found that in male subjects, the levels of PRL were negatively correlated with hip circumference, TG and HOMA-IR (CP) and positively associated with HDL. In female subjects, PRL levels were negatively correlated with BMI, DBP, waist circumference, hip circumference, and TG. In female subjects, the prevalence rates of MetS in the fourth quartile of PRL were significantly lower than those in the first, second and third quartiles. Furthermore, premenopausal and postmenopausal women with NAFLD had higher BMI, TG and MetS incidence. NAFLD is very common in obese and dyslipidaemic patients. Obese individuals produce relatively excessive proinflammatory factors, some of which inhibit the treatment of liver fat and promote the accumulation of lipids in hepatocytes [33]. Dyslipidaemia, especially hypertriglyceridaemia, may

subsequently increase the transportation of TG and other fats into hepatocytes, resulting in hepatic steatosis [34].

As a retrospective analysis, this study has many limitations. First, the diagnosis of NAFLD was based on ultrasound examination, which cannot distinguish NASH from fibrosis. Second, because this was a cross-sectional study, we cannot infer the direct cause and effect relationship between PRL and NAFLD and need further mechanical studies to clarify the exact relationship. Third, PRL secretion appears in pulse form, the best time to draw blood for PRL is from 9:00 to 11:00 a.m., and patients should avoid emotional excitement around this time. Finally, due to the limited number of participants in this study, the effects of drugs for treating cardiovascular diseases and controlling blood lipids on PRL levels have not been investigated, which requires further layered analysis in future work. Moreover, the small sample size cannot replace a large-scale population-based cross-sectional epidemiological study, so it is necessary to increase the sample size.

Conclusions

In summary, our research shows that serum PRL levels are related to NAFLD in the T2DM population in the physiological range and are also connected to known metabolic indicators. Our research results may help to predict the risk of developing NAFLD to better understand the disease and to formulate effective prevention. strategies.

Abbreviations

NAFLD: nonalcoholic fatty liver disease; T2DM: type 2 diabetes mellitus; PRL: prolactin; Met S: metabolic syndrome; NASH: nonalcoholic steatohepatitis; SBP: systolic pressure; DBP: diastolic pressure; BMI: body mass index; AST: aspartic acid aminotransferase; ALT: alanine aminotransferase; GGT: glutamyltransferase; FBG: fasting blood glucose; TG: triglyceride; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR (CP): Modified homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus); HbA1c: glycosylated haemoglobin; SD: standard deviation; OR: odds ratio; CI: confidence interval

Footnotes

Contributorship statement

YZ conceived the study, collected clinical data, analysed and interpreted the data

and wrote the manuscript. HL made a revised version. All authors read and agreed to the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Data availability statement

The data that support this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was a retrospective study, so it was exempted from the requirement of informed consent and the approval of the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (2020MCZQ09).

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Section/Topic	Item	Recommendation	Reported on page
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3–4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(a) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4–6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4–5
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	3–4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	n/a
		(d) If applicable, describe analytical methods taking account of sampling strategy	n/a
		(e) Describe any sensitivity analyses	n/a
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	n/a
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	n/a
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	6–11
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6–11
		(b) Report category boundaries when continuous variables were categorized	n/a
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6–11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11–13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13–14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Cross-sectional association between prolactin levels and nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a retrospective analysis of patients from a single hospital in China

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Abstract

Objective: This study aimed to retrospectively assess the association between prolactin (PRL) and nonalcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes mellitus (T2DM).

Design and setting: A retrospective, cross-sectional study was conducted at a single hospital in Anhui, China.

Participants: A total of 406 patients with T2DM (230 men and 176 women) were included.

Outcome measures: P values for the independent T test, the Mann-Whitney rank sum test, the Spearman correlation analysis, and multiple logistic regression models were used to explore the association between PRL and NAFLD in patients with T2DM.

Results: The results indicated that in both men and women, the levels of PRL were significantly lower in the T2DM with NAFLD group than in the T2DM without NAFLD group (men: 9.56 ng/mL vs. 10.36 ng/mL, women: 10.38 ng/mL vs. 12.97 ng/mL). In male patients, the levels of PRL were negatively correlated with hip circumference (r=-0.141, p=0.032), homeostasis model assessment for insulin resistance (C-peptide) (r=-0.141, p=0.032) and triglyceride (TG) (r=-0.252, p=0.000) values and inversely correlated with high-density lipoprotein (HDL) (r=0.147,

p=0.025) levels. In female patients, PRL levels were negatively related to body mass index (r=-0.192, p=0.011), diastolic blood pressure (r=-0.220, p=0.003), waist circumference (r=-0.152, p=0.044), hip circumference (r=-0.157, p=0.037) and TG (r=-0.258, p=0.001) values. Logistic regression analysis revealed a negative relationship between PRL and NAFLD (men: OR 0.891, 95% CI 0.803-0.989, p=0.031; women: OR 0.874, 0.797-0.957, p= 0.004). As PRL levels increased, NAFLD prevalence decreased in both sexes (men: p=0.012, women: p= 0.013).

Conclusion: Our results suggest that low levels of PRL in the physiological range were markers of NAFLD in patients with T2DM and that PRL within the biologically high range may play a protective role in the pathogenesis of NAFLD.

Keywords: Type 2 diabetes mellitus; Nonalcoholic fatty liver disease; Prolactin.

Strengths and limitations of this study

- ▸Abdominal colour ultrasonography, as used in the study, is a common and simple method for the clinical diagnosis of nonalcoholic fatty liver disease (NAFLD).
- ▸The normal range of serum prolactin (PRL) levels differs by sex, so we conducted a sex-stratified analysis of patients with type 2 diabetes mellitus (T2DM).
- ▸P values for independent T tests and multiple logistic regression models were used to assess the association between PRL and NAFLD in patients with T2DM.
- ▸This was a cross-sectional study that cannot provide evidence of causal relationships.

Introduction

The liver is an important organ for glycolipid metabolism in the body. When triglyceride deposition in hepatocytes increases and exceeds 5%, and other factors causing liver steatosis (such as alcohol consumption and viral hepatitis) are excluded, NAFLD can be diagnosed[1]. In China, with the gradual improvement of living standards, NAFLD has surpassed chronic viral hepatitis to become the primary cause of chronic liver diseases[2]. Currently, the global incidence of NAFLD is 25.2%[3],

while the prevalence of NAFLD diagnosed by ultrasound in patients with T2DM is 73.7%[4]. T2DM is an important factor associated with the progression of NAFLD to NASH and fibrosis[1].

NAFLD is closely related to central obesity, hypertension, hyperlipidaemia, T2DM and metabolic syndrome (MetS)[5]. Among MetS-related diseases, only NAFLD is considered a strong predictor of MetS, and the incidence of MetS in fatty liver patients is more than 4 times that in nonfatty liver patients[6]. Therefore, NAFLD is considered the expression of MetS in the liver.

Prolactin (PRL) is a type of hormone that is mainly secreted by the adenohypophysis. Its main physiological function is to stimulate breast development and milk secretion[7]. Its receptors are widely distributed in various tissues and organs of the body, including in fat, the liver, and the pancreas [8]. PRL can increase the proliferation of β cells, stimulate insulin secretion and participate in the regulation of glucose metabolism [9]. PRL can also inhibit lipolysis and activate adipocyte differentiation by activating peroxisome proliferator-activated receptor γ[10]. Studies in China and abroad have found that a decrease in serum PRL at the physiological level is closely related to the occurrence of T2DM. Wang et al.[11] discovered that the PRL levels of patients with T2DM and impaired glucose regulation were significantly lower than those of people with normal glucose metabolism. The researchers further pointed out that a decrease in physiological levels of PRL was related to an increased risk of T2DM[9]. Manshaei et al.[12] also found that the serum PRL concentration of patients with T2DM was lower than that of healthy people. Because of the high incidence of NAFLD in patients with T2DM, T2DM is also an important factor in MetS. The relationship among PRL, NAFLD and MetS at the physiological level has not been explored. The goal of this research was to explore the relationship among PRL, NAFLD and MetS in patients with T2DM.

Methods

Participants

All participants in this study were recruited from a hospital located in Anhui, China.

This was a cross-sectional survey. A total of 656 patients with T2DM were investigated in this study, but 15 participants were excluded due to the use of medications that affect PRL levels (metoclopramide, methyldopa, opiates, and cimetidine). Thirty participants were excluded because their levels of thyroid-stimulating hormone, cortisol, oestradiol and testosterone were higher than the normal range. Four participants had pituitary diseases, five had hyperglycaemia, 56 exhibited excessive alcohol consumption (intake of alcohol exceeding 140 g/week for men and 70 g/week for women), 11 had cancer, 5 were pregnant, 7 had type 1 diabetes, 25 had acute complications of diabetes, 15 had acute cardiovascular events, 30 had severe hepatic and renal insufficiency, 8 had viral liver disease, 30 had alcoholic liver disease, 5 had drug-induced liver disease and 4 had autoimmune liver disease. Ultimately, 406 participants (230 men and 176 women) were included in this study. This study was a retrospective study, so it was exempted from the requirement of informed consent and was approved by the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine.

Data collection

We collected data on sex, age, menopausal history of women, height, weight, diabetes course, preadmission hypoglycaemic plan (including metformin, insulin and other hypoglycaemic drugs such as sulfonylureas, glinides, thiazolidinediones, α-glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl peptidase 4 inhibitors, and sodium-glucose cotransporter protein 2 inhibitors), history of alcohol consumption, occurrence of cancer, history of other liver diseases, waist circumference, hip circumference, and blood pressure. Venous blood samples were collected in the morning on the second day after admission, and all blood was extracted with a centrifuge. After the separation of serum, fasting blood glucose (FBG), blood fat, liver and kidney function were measured using an automatic biochemical analyser (7600-020; Hitachi). Fasting C-peptide (FCP) levels were examined using an enzyme-linked immunosorbent assay (A2000 Plus; Autolumo). An automated chemiluminescent immunoassay (Siemens Immulite 2000, UK) was used

to measure PRL levels. The coefficients of intra-assay and interassay variation ranged from 2.49-3.47% and 2.91-3.14%, respectively. PRL levels are affected by many conditions, including the use of various drugs, stress, and exercise, so we took blood samples at 9:00 am on the first day after the patients were admitted to the hospital and the next morning. We took 2 ml blood samples each time. The patients fasted and rested in a sitting position for 30 minutes, and then the average value of two blood pressure readings was taken. High-performance liquid chromatography was used to check glycosylated haemoglobin (HbA1c) (Variant II; Bio-Rad).

Definitions, counts and groups

The diagnosis of T2DM was based on the diagnostic criteria proposed by the World Health Organization (WHO) Diabetes Expert Committee in 1999. The physiological level of PRL was based on the normal reference range of our hospital, which is 2.78-29.20 ng/mL for premenopausal women, 1.79-20.28 ng/mL for menopausal women and 2.12-17.69 ng/mL for men.

NAFLD was diagnosed by ultrasound[13] by a senior technician. The ultrasonic diagnosis of fatty liver is as follows: the near-field of the liver permeates a punctiform hyperecho, the composition of the intrahepatic duct is not clearly demonstrated by ultrasonography, and a weak echo is present in the distal echo. The diagnosis of NAFLD is based on the following requirements: no history of alcohol consumption, no other types of liver diseases, and an unexplained increase in serum alanine aminotransferase (ALT), aspartic acid aminotransferase (AST) or glutamyltransferase (GGT) levels over 6 months[14].

The diagnosis of MetS conformed to the standard put forward in the ninth edition of internal medicine in China [15], and the diagnostic standard included three or more of the following items: ① central obesity and/or abdominal obesity: a waist circumference greater than 90 cm for men and 85 cm for women; ② hyperglycaemia: an FBG level > 6.1 mmol/L or a 2-hour blood glucose level >7.8 mmol/L and/or the confirmation of a diabetes diagnosis and treatment with hypoglycaemic therapy; ③ hypertension: a blood pressure exceeding 130/85 mmHg and/or a diagnosis of hypertension and treatment with antihypertensive therapy; ④ a fasting triglyceride

(TG) level exceeding 1.7 mmol/l; and ⑤ a fasting high-density lipoprotein (HDL) level below 1.04 mmol/l. Body mass index (BMI) was computed by dividing the body weight (kg) by the square of the height (m^2). The homeostasis model assessment of insulin resistance (C-peptide) (HOMA-IR (CP)) value was determined by the FCP level as a substitute for the fasting insulin level as follows: HOMA-IR (CP) =1.5+FBG (mmol/L)xFCP (pmol/L)/2800. HOMA-β (CP-DM) =0.27x FCP (pmol/L) (FBG (mmol/L) -3.5)[16].

In conformity with ultrasonic diagnosis, patients with T2DM who met the inclusion criteria were divided into the without NAFLD group (77 men, 66 women) and the with NAFLD group (153 men, 110 women).

Statistical analysis

SPSS 21.0 statistical software was used for the data analysis, and the Kolmogorov–Smirnov normality test was performed for all data. The measured data with a normal distribution are represented as the mean and standard deviation (SD). Comparisons were conducted between two groups, and comparisons were performed using independent T tests. Measurement data with nonnormal distributions are expressed as medians (interquartile intervals). In this situation, two groups were compared by using the Mann–Whitney rank sum test. Categorical variables are shown as the number of cases, and the chi-square test was adopted to demonstrate the differences within two or more groups. Spearman correlation analysis compared the relationship between PRL levels and the other variables. The relationships among PRL, NAFLD and MetS were analysed by logistic regression. $P<0.05$ or $P<0.01$ represented obvious significant differences.

Patient and public involvement

Neither the patients nor the public were not involved in the design, conduction, reporting, or dissemination plans of our research.

Results

Comparison of general findings and laboratory test targets in each group

The ultrasonic diagnostic rate of NAFLD was 263 patients (153 plus 110 patients)

(64.8%) (Table 1). Men with NAFLD were younger, had higher BMI, waist circumference, hip circumference, diastolic blood pressure (DBP), GGT, FBG, TG, total cholesterol (TC), low-density lipoprotein (LDL), HOMA-IR (CP), and HbA1C values, and had a higher MetS incidence. Women with NAFLD also had higher BMI, ALT, GGT, TG, HOMA-IR (CP), and HbA1C values and a higher MetS incidence. HDL and PRL levels were markedly reduced in the patients with NAFLD compared with those without NAFLD in both sexes ($p<0.05$ or $p<0.01$). In terms of medication history, there was no significant difference between the two groups of male and female patients in hypoglycaemic programmes, which could exclude the influence of hypoglycaemic drugs on the study.

Table 1. Comparison of the general characteristics and biochemical indices of each group

	Men			Women		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	77	153		66	110	
Age (years)	63(54-63)	54(48-62)	0.000	65(57-71)	61(55-69)	0.077
Metabolic syndrome (%)	64.9	85.6	0.000	59.100	80.000	0.003
Menopause (%)	NA	NA		99.100	83.600	0.117
Diabetes course(years)	10(3-15)	8(3-12)	0.280	10(5-20)	10(4-15)	0.070
BMI (kg/m^2)	24.90±2.97	27.18±2.94	0.000	24.54±3.35	26.33±3.55	0.000
Systolic pressure (mmHg)	130(125-146)	132(121-145)	0.880	130(124-151)	130(123-144)	0.233
Diastolic pressure (mmHg)	81.48±9.59	85.80±9.94	0.002	80.48±8.59	79.07±8.16	0.277
Waist circumference (cm)	90.71±8.02	96.29±8.45	0.000	89.02±9.07	91.38±9.41	0.103
Hip circumference (cm)	96.64±6.77	100.66±6.18	0.000	97.00±6.52	97.73±7.81	0.526
ALT (U/L)	19(13-28)	21(15-32)	0.082	15(12-21)	19(14-33)	0.000
AST (U/L)	18(15-23)	19(15-23)	0.881	17(15-20)	18(15-25)	0.094
GGT (U/L)	24(17-36)	35(23-56)	0.000	19(14-28)	25(19-35)	0.000
FBG (mmol/L)	6.81(5.41-9.49)	7.80(6.21-11.0)	0.002	6.61(5.48-9.34)	7.89(6.05-10.96)	0.050
TG (mmol/L)	1.20(0.79-1.75)	2.01(1.42-3.27)	0.000	1.23(0.93-1.50)	1.81(1.19-2.35)	0.000
TC (mmol/L)	4.32±0.92	4.83±1.10	0.001	4.77±1.24	5.02±1.11	0.158
HDL (mmol/L)	1.04(0.96-1.18)	0.97(0.82-1.11)	0.004	1.23(1.05-1.47)	1.10(0.99-1.28)	0.002
LDL (mmol/L)	2.45±0.78	2.78±0.85	0.004	2.84±1.05	2.98±0.88	0.373
HOMA-IR (CP)	2.90(2.46-3.97)	3.99(3.18-5.20)	0.000	2.97(2.54-3.68)	3.68(2.91-4.41)	0.001
HOMA-β (CP-DM)	46.94(25.29-88.92)	44.33(27.17-83.92)	0.686	38.55(22.52-80.19)	48.27(25.02-73.90)	0.553
HbA1C (%)	7.5(6.7-9.1)	8.3(7.0-9.7)	0.043	7.7(6.7-9.3)	8.5(7.4-9.9)	0.020

PRL (ng/mL)	10.36(9.35-14.72)	9.56(7.81-12.60)	0.001	12.97(10.03-16.58)	10.38(8.43-14.27)	0.001
Hypoglycaemic plan						
Metformin	26(33.8%)	62(40.5%)		17(25.8%)	43(39.1%)	
Other hypoglycaemic drugs	16(20.8%)	44(28.8%)	0.083	23(34.8%)	27(24.5%)	0.150
Insulin	35(45.4%)	47(30.7%)		26(39.4%)	40(36.4%)	

Note: NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartic acid aminotransferase; GGT, glutamyltransferase; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus); HbA1c, glycosylated haemoglobin; PRL, prolactin. The measured data with a normal distribution are represented as the mean±SD. Measurement data for nonnormal distributions are expressed as medians (interquartile intervals). Normally distributed variables: BMI, diastolic blood pressure, waist circumference, hip circumference, TC, LDL; Nonnormally distributed variables: Age, diabetes course, systolic blood pressure, ALT, AST, GGT, FBG, TG, HDL, HOMA-IR (CP), HOMA-β (CP-DM), HbA1C, and PRL

Because women's serum PRL levels are affected by menopause, we analysed the metabolic status and PRL levels of female patients with or without NAFLD before and after menopause (Table 2). Premenopausal women with NAFLD had higher BMI, FBG, TG, and HbA1C values and a higher MetS incidence. Postmenopausal women with NAFLD had higher BMI, ALT, GGT, TG, and HOMA-IR (CP) values and a higher MetS incidence, while HDL and PRL values were markedly reduced in the patients with NAFLD compared with those in patients without NAFLD ($p<0.05$ or $p<0.01$).

Table 2. Comparison of the clinical data of women with and without NAFLD before and after menopause

	Premenopause			Postmenopause		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	6	18		60	92	
Age (years)	44.80±3.76	45.20±4.37	0.848	66.15±8.34	64.61±8.16	0.261
Metabolic syndrome (%)	0	77.8	0.001	65	80.4	0.033
Diabetes course (years)	8.180±6.69	4.78±4.12	0.149	12.86±9.02	10.69±6.88	0.116
BMI (kg/m^2)	22.80±3.87	26.70±3.43	0.029	24.71±3.28	26.26±3.59	0.008
Systolic pressure (mmHg)	121.83±7.08	128.50±8.78	0.107	133(127-152)	131(122-145)	0.167
Diastolic pressure (mmHg)	77.50±7.18	84.06±6.78	0.055	80.78±8.71	78.10±8.08	0.054

Waist circumference (cm)	80.33±12.36	89.06±7.92	0.055	89.88±8.32	91.84±9.65	0.200
Hip circumference (cm)	94.67±6.83	95.61±7.65	0.791	97.23±6.50	98.14±7.81	0.456
ALT (U/L)	13(11-16)	15(13-45)	0.121	15(12-22)	20(15-33)	0.000
AST (U/L)	16(15-18)	16(13-35)	1.000	17(15-20)	19(16-25）	0.073
GGT (U/L)	20.67±14.28	37.67±31.34	0.217	19(14-29)	26(18-35)	0.002
FBG (mmol/L)	6.61±1.59	10.99±3.10	0.003	7.81±2.97	8.08±2.78	0.566
TG (mmol/L)	1.09(0.63-1.30)	2.02(1.37-2.83)	0.003	1.24(0.93-1.56)	1.74(1.17-2.34)	0.000
TC (mmol/L)	4.77±0.84	5.16±1.46	0.534	4.77±1.28	4.99±1.04	0.229
HDL (mmol/L)	1.23±0.17	1.06±0.21	0.086	1.27±0.29	1.14±0.27	0.005
LDL (mmol/L)	2.92±0.83	2.84±0.86	0.838	2.84±1.08	3.00±0.89	0.301
HOMA-IR (CP)	2.30±0.57	4.87±2.98	0.051	3.28±1.00	3.73±1.43	0.036
HOMA-β (CP-DM)	25.07(19.86-28.67)	25.99(13.78-56.47)	0.689	47.00(22.63-85.05)	51.60(28.83-75.27)	0.505
HbA1C (%)	7.62±0.89	9.53±1.66	0.014	8.16±1.82	8.53±1.67	0.196
PRL (ng/mL)	18.92±8.57	14.54±4.64	0.122	13.16±3.79	10.88±3.77	0.000

Note: NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartic acid aminotransferase; GGT, glutamyltransferase; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus); HbA1c, glycosylated haemoglobin; PRL, prolactin. The measurement data with a normal distribution are represented as the mean±SD. Measurement data with nonnormal distributions are expressed as medians (interquartile intervals).[1]

Relationship between PRL levels and MetS-related parameters

We further investigated the relationship between PRL levels and MetS-related parameters (Table 3). We found that in male subjects, the levels of PRL were negatively correlated with hip circumference, TG and HOMA-IR (CP) values and positively associated with HDL levels. In female subjects, PRL levels were negatively correlated with BMI, DBP, and waist circumference values.

Table 3. Relationship between PRL levels and MetS-related parameters

	Men		Women	
	r	P value	r	P value
BMI	-0.092	0.166	-0.192	0.011
Systolic pressure	0.046	0.492	-0.045	0.552
Diastolic pressure	-0.125	0.059	-0.220	0.003
Waist circumference	-0.056	0.398	-0.152	0.044
Hip circumference	-0.141	0.032	-0.157	0.037
FBG	-0.109	0.098	-0.034	0.654

TG	-0.252	0.000	-0.258	0.001
TC	-0.096	0.146	-0.061	0.421
HDL	0.147	0.025	0.065	0.390
LDL	-0.042	0.528	-0.110	0.146
HOMA-IR(CP)	-0.141	0.032	-0.049	0.519
HOMA-β(CP-DM)	0.019	0.772	-0.044	0.562
HbA1C	-0.091	0.168	0.057	0.450

Note: Met S, metabolic syndrome; BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus); HbA1c, glycosylated haemoglobin; PRL, prolactin..

Multiple-factor logistic regression analysis of serum PRL levels and NAFLD risk

The impact index for NAFLD was assessed using multiple logistic regression analysis, which included age, BMI, menopause, TG, LDL, HOMA-IR (CP), HbA1C and PRL as variables. We found that PRL levels were independently negatively associated with NAFLD in both men and women (odds ratio (OR): 0.891, 95% confidence interval (CI): 0.803-0.989, p=0.031, for men; OR: 0.874, 95% (CI): 0.797-0.957, p=0.004, for women). Other risk factors included age, BMI, LDL and HOMA-IR (CP) for men and TG for women (Table 4).

Table 4 Multivariate logistic regression analysis of serum PRL levels and NAFLD risk

	Men			Women		
	β	OR(95% CI)	p value	β	OR(95% CI)	p value
Age	-0.045	0.956(0.924-0.989)	0.010	-0.044	0.957(0.912-1.004)	0.070
BMI	0.255	1.291(1.122-1.484)	0.000	0.090	1.094(0.97-1.224)	0.120
Menopause				0.213	1.237(0.281-5.441)	0.778
TG	0.176	1.193(0.959-1.483)	0.113	0.981	2.666(1.404-5.064)	0.003
LDL	0.493	1.637(1.046-2.561)	0.031	-0.121	0.886(0.596-1.318)	0.550
HOMA-IR(CP)	0.360	1.134(1.062-1.936)	0.019	0.215	1.240(0.859-1.788)	0.250
HbA1C	0.057	1.059(0.872-1.287)	0.564	0.047	1.048(0.840-1.308)	0.676
PRL	-0.115	0.891(0.803-0.989)	0.031	-0.135	0.874(0.797-0.957)	0.004

Note: The risk factors for NAFLD were assessed using multiple logistic regression analysis in men and women. The ORs with corresponding 95% CIs were adjusted for age, BMI, menopause, TG, LDL and HOMA-IR (CP), HbA1C and PRL levels as variables. NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HbA1c, glycosylated haemoglobin; PRL, prolactin.; PRL, prolactin. OR: Odds ratio; CI: Confidence interva

Relationship between PRL levels and the prevalence of NAFLD and MetS

According to the quartiles of PRL levels, the subjects were divided into four groups: T1<8.29 (n= 57 patients), 8.29≤T2<9.93 (n= 58 patients), 9.93≤T3<12.68 (n= 57 patients), and T4≥12.68 (n= 58 patients) ng/mL for men (n= 230 patients) and T1<8.95 (n= 44 patients), 8.95≤T2<11.32 (n= 44 patients), 11.32≤T3<14.95 (n= 44 patients), and T4≥14.95 (n= 44 patients) ng/mL for women (n= 176 patients). The chi-square test was used to compare the prevalence and composition ratio among different groups. The prevalence of NAFLD exhibited a decreasing trend with the rise in the PRL quartile in both sexes (T1: 84.2%, T2: 63.8%, T3: 59.6%, T4: 58.6%, p=0.012 in men; T1: 79.5%, T2: 65.9%, T3: 54.5%, T4: 50%, p= 0.013 in women). However, the prevalence rates of MetS were T1: 86%, T2: 79.3%, T3: 77.2%, and T4: 72.4% (p= 0.354) in men and T1: 84.1%, T2: 70.5%, T3: 77.3%, and T4: 56.8% (p= 0.031) in women. Therefore, in female subjects, the prevalence rate of MetS in the fourth quartile of PRL levels was significantly lower than those in the first, second and third quartiles.

Discussion

At present, due to the rapid increase in the incidence of obesity and obesity-related diseases, NAFLD has become an important public health problem[17]. NAFLD is considered the manifestation of MetS in the liver, especially in patients with T2DM[18]. In this study, it was found that the incidence of NAFLD diagnosed by abdominal liver colour Doppler ultrasound was 64.8%. Compared with non NAFLD patients, NAFLD patients had higher BMI, TG, GGT, HOMA-IR (CP), and HbA1C values, a higher MetS incidence and lower HDL levels in both sexes. Zhang et al.[19] obtained similar results. BMI, TG and HDL are components of MetS. Therefore, T2DM complicated with NAFLD promotes abnormalities in metabolic indices.

PRL is a hormone that is closely related to metabolism[20]. Recent findings have shown that there is a close association between PRL levels and T2DM. A

cross-sectional study included 2377 adults from the community population (excluding those with hyperprolactinemia) and found that individuals with impaired glucose regulation and T2DM had lower PRL levels. Researchers controlled for age, sex, BMI and other confounding factors and still discovered that the risk in the abovementioned people with high serum PRL levels was significantly reduced [11]. A further follow-up of 3.7 years revealed that female patients in the highest quartile of PRL levels had a lower risk of T2DM, with a risk ratio of 0.48[9]. Another cross-sectional study also found that the risk of MetS and T2DM in women with lower baseline PRL levels was increased[21]. A large meta-analysis indicated that higher serum PRL levels in the normal range were related to a low risk of T2DM[22]. Jha et al.[23] also found that serum PRL levels had a significant correlation with liver disease and predicted mortality. In adipose tissue, PRL intervention can reduce the production of malonyl coenzyme A in human primary adipocytes, thus inhibiting triglyceride synthesis[24]. The PRL receptor can also directly inhibit the expression of fatty acid synthetase and fatty acid synthesis in 3T3L1 cells[25]. PRL reduces the accumulation of triglycerides in the liver through the PRL receptor, thus improving liver steatosis [10]. These results indicate that higher PRL levels have a positive protective effect on glucose and lipid metabolism.

Considering that PRL secretion may differ according to sex, we studied male and female subjects separately. We found that compared with that of non-NAFLD patients, the PRL value of NAFLD patients was lower in both sexes. Age, BMI, TG, LDL, HOMA-IR (CP), and HbA1C were adjusted; additionally, menopausal factors were adjusted for among female subjects, and the study suggested that PRL levels had a negative relationship with the risk of NAFLD. In line with the quartile of PRL, the incidence of NAFLD showed a general decrease with the increase in PRL levels in both sexes. Zhang et al.[26] noted that when PRL increased by one standard deviation, the risk among male NAFLD patients decreased by 12.3% and that among female patients decreased by 21.4%. PRL was proven to be a protective factor that affected the existence and progression of NAFLD. In another study, Zhang et al.[19] also found that the PRL levels of NAFLD patients diagnosed by ultrasound were

significantly lower than those of non-NAFLD patients, whether they were male or female. In addition, with the increase in PRL quartile, the incidence of NAFLD decreased. All analyses were corrected for age, sex, BMI, insulin resistance, HbA1C, diabetes and other factors. The results showed that PRL levels had an inverse association with NAFLD. We considered that PRL levels are affected by many conditions, including various drugs, stress, and exercise. We excluded the following patients: patients with the use of drugs that affect PRL levels (metoclopramide, methyl-dopa, opiates, and cimetidine) and those with levels of thyroid-stimulating hormone, cortisol, oestradiol and testosterone that were higher than the normal range. In terms of medication history, there was no significant difference between the two groups of male and female patients in regard to hypoglycaemic programmes, which could exclude the influence of hypoglycaemic drugs on the study.

In addition, the secretion of PRL may be affected by menopausal status. This paper analysed menopausal and non-menopausal women and found that postmenopausal women with NAFLD had lower PRL levels. In addition, Zhang Zhuzi et al.[27] divided the included women into a premenopausal group and a postmenopausal group and found that in both groups, the PRL levels of patients with NAFLD were lower than those of patients without NAFLD, and the decrease in PRL levels in postmenopausal women with NAFLD was more significant. It was suggested that the decrease in the PRL levels of patients with NAFLD was affected by menopausal factors.

Studies have shown a correlation between PRL levels and the components of MetS, which could explain the role of PRL in NAFLD. According to basic studies, in a mouse model with obesity induced by a high-fat diet, severe metabolic changes would occur in mice with PRL receptor failure. The injection of PRL could improve insulin sensitivity and prevent visceral adipocyte hypertrophy[28]. Clinical studies have found that low serum PRL levels in the physiological range are related to poor metabolic outcomes in MetS and T2DM patients[11]. In overweight and obese men, serum PRL levels were lower [28]. Friedrich et al.[29] found that PRL levels were negatively correlated with waist circumference in 1857 healthy women aged 20-79

years. The endocrine characteristics of MetS and polycystic ovary syndrome (PCOS) have a relatively high similarity rate [30]. A systematic retrospective analysis of 2052 PCOS patients revealed that the lower the serum PRL level was, the higher the BMI. PRL levels had the opposite relationship with TG, TC and LDL-C levels [31]. Arterial hypertension is a component of MetS. A prospective study of 874 postmenopausal women found that PRL levels increased by 1 standard deviation during 8 years of follow-up, and the relative risk of hypertension was 1.31[32]. Our study found that in male subjects, the levels of PRL were negatively correlated with hip circumference, TG and HOMA-IR (CP) values and positively associated with HDL levels. In female subjects, PRL levels were negatively correlated with BMI, DBP, waist circumference, hip circumference, and TG values. In female subjects, the prevalence rates of MetS in the fourth quartile of PRL levels were significantly lower than those in the first, second and third quartiles. Furthermore, premenopausal and postmenopausal women with NAFLD had higher BMI and TG levels and a higher MetS incidence. NAFLD is very common in obese and dyslipidaemic patients. Obese individuals produce relatively excessive proinflammatory factors, some of which inhibit the treatment of liver fat and promote the accumulation of lipids in hepatocytes [33]. Dyslipidaemia, especially hypertriglyceridaemia, may subsequently increase the transportation of TGs and other fats into hepatocytes, resulting in hepatic steatosis [34].

As a retrospective analysis, this study has many limitations. First, the diagnosis of NAFLD was based on ultrasound examination, which cannot distinguish NASH from fibrosis. Second, because this was a cross-sectional study, we cannot infer the direct cause and effect relationship between PRL levels and NAFLD and further mechanical studies are needed to clarify the exact relationship. Third, PRL secretion appears in pulse form, the best time to draw blood for PRL measurement is from 9:00 to 11:00 a.m., and patients should avoid emotional excitement around this time. Finally, due to the limited number of participants in this study, the effects of drugs for treating cardiovascular diseases and controlling blood lipids on PRL levels have not been investigated, which requires further layered analysis in future work. Moreover, the small sample size cannot replace a large-scale population-based cross-sectional

epidemiological study, so it is necessary for future studies to increase the sample size.

Conclusions

In summary, our research shows that serum PRL levels in the physiological range are related to NAFLD in the T2DM population and are also connected to known metabolic indicators. Our research results may help to predict the risk of developing NAFLD to better understand the disease and to formulate effective prevention strategies.

Abbreviations

NAFLD: nonalcoholic fatty liver disease; T2DM: type 2 diabetes mellitus; PRL: prolactin; MetS: metabolic syndrome; NASH: nonalcoholic steatohepatitis; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; AST: aspartic acid aminotransferase; ALT: alanine aminotransferase; GGT: glutamyltransferase; FBG: fasting blood glucose; TG: triglyceride; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR (CP): modified homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus); HbA1c: glycosylated haemoglobin; SD: standard deviation; OR: odds ratio; CI: confidence interval.

Contributors

YZ conceived the study, collected the clinical data, analysed and interpreted the data and wrote the manuscript. HL revised the manuscript. All authors read and agreed to the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Data availability statement

The data that support this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was a retrospective study, so it was exempted from the requirement of informed consent with the approval of the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (2020MCZQ09).

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STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

Section/Topic	Item	Recommendation	Reported on page
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3–4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(a) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4–6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4–5
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	3–4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	n/a
		(d) If applicable, describe analytical methods taking account of sampling strategy	n/a
		(e) Describe any sensitivity analyses	n/a
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	n/a
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	n/a
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	6–11
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6–11
		(b) Report category boundaries when continuous variables were categorized	n/a
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6–11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11–13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13–14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.