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Cross-sectional association between prolactin levels and non-alcoholic 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 non-alcoholic 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) was 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), homoeostasis 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 (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 to 0.989, p=0.031; women: OR 0.874, 95% CI 0.797 to 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.

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 non-alcoholic 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 (TG) deposition in hepatocytes increases and exceeds 5%, and other factors causing liver steatosis (such as alcohol consumption and viral hepatitis) are excluded, non-alcoholic fatty liver disease (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 type 2 diabetes mellitus (T2DM) is 73.7%.[4] T2DM is an important factor associated with the progression of NAFLD to non-alcoholic steatohepatitis (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 patients with fatty liver is more than four times that in patients with non-fatty liver.[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, 5 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.

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 ELISA (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% to 3.47% and 2.91% to 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 09:00 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 min, 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 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 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 mm Hg and/or a diagnosis of hypertension and treatment with antihypertensive therapy; (4) A fasting 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 homoeostasis 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) × FCP (pmol/L)/2800. HOMA-β (CP-DM)=0.27× 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 with NAFLD group (153 men, 110 women).

Statistical analysis

SPSS V.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 SD. Comparisons were conducted between two groups, and comparisons were performed using independent t-tests. Measurement data with non-normal 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 χ^2 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. Values of $p<0.05$ or $p<0.01$ represented obvious significant differences.

Patient and public involvement

Neither the patients nor the public were involved in the design, conduct, 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 + 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 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 statistically 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.

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 $p<0.01$).

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.

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 (OR: 0.891, 95% CI 0.803 to 0.989, p=0.031, for men; OR: 0.874, 95% CI 0.797 to 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).

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 ng/mL (n=57 patients), 8.29 ng/mL ≤T2<9.93 ng/mL (n=58 patients), 9.93 ng/mL ≤T3<12.68 ng/mL (n=57 patients) and T4≥12.68 ng/mL (n=58 patients) for men (n=230 patients) and T1<8.95 ng/mL (n=44 patients), 8.95 ng/mL≤T2<11.32 ng/mL (n=44 patients), 11.32 ng/mL≤T3<14.95 ng/mL (n=44 patients) and T4≥14.95 ng/mL (n=44 patients) for women (n=176 patients). The χ^2 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

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 (mm Hg)	130 (125–146)	132 (121–145)	0.880	130 (124–151)	130 (123–144)	0.233
Diastolic pressure (mm Hg)	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%)	0.083	17 (25.8%)	43 (39.1%)	0.150
Other hypoglycaemic drugs	16 (20.8%)	44 (28.8%)		23 (34.8%)	27 (24.5%)	
Insulin	35 (45.4%)	47 (30.7%)		26 (39.4%)	40 (36.4%)	

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

ALT, alanine aminotransferase; AST, aspartic acid aminotransferase; BMI, body mass index; HOMA-IR (CP), homoeostasis model assessment for insulin resistance (C-peptide); FBG, fasting blood glucose; GGT, glutamyltransferase; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; HOMA-β (CP-DM), homoeostasis model assessment for beta (C-peptide-diabetes mellitus); LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; PRL, prolactin; TC, total cholesterol; T2DM, type 2 diabetes mellitus; TG, triglyceride.

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 patients without NAFLD, patients with NAFLD 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 hyperprolactinaemia) and

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 (mm Hg)	121.83±7.08	128.50±8.78	0.107	133(127-152)	131(122-145)	0.167
Diastolic pressure (mm Hg)	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

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

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 above-mentioned 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 TG 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 TGs 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 patients without NAFLD, the PRL value of patients with NAFLD was lower in both sexes. Age, BMI, TG, LDL, HOMA-IR (CP) and

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

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

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 1 SD, the risk among male patients with NAFLD 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 patients with NAFLD diagnosed by ultrasound were significantly lower than those of patients without NAFLD, whether they were male or female. In addition, with the increase in the 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 with 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 *et al*[26] 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

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 to 0.989)	0.010	−0.044	0.957 (0.912 to 1.004)	0.070
BMI	0.255	1.291 (1.122 to 1.484)	0.000	0.090	1.094 (0.97 to 1.224)	0.120
Menopause				0.213	1.237 (0.281 to 5.441)	0.778
TG	0.176	1.193 (0.959 to 1.483)	0.113	0.981	2.666 (1.404 to 5.064)	0.003
LDL	0.493	1.637 (1.046 to 2.561)	0.031	−0.121	0.886 (0.596 to 1.318)	0.550
HOMA-IR (CP)	0.360	1.134 (1.062 to 1.936)	0.019	0.215	1.240 (0.859 to 1.788)	0.250
HbA1c	0.057	1.059 (0.872 to 1.287)	0.564	0.047	1.048 (0.840 to 1.308)	0.676
PRL	−0.115	0.891 (0.803 to 0.989)	0.031	−0.135	0.874 (0.797 to 0.957)	0.004

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.
BMI, body mass index; HbA1c, glycosylated haemoglobin; HOMA-IR (CP), homoeostasis model assessment for insulin resistance (C-peptide); LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; PRL, prolactin; TG, triglyceride.

receptor failure. The injection of PRL could improve insulin sensitivity and prevent visceral adipocyte hypertrophy.[27] Clinical studies have found that low serum PRL levels in the physiological range are related to poor metabolic outcomes in patients with MetS and T2DM.[11] In overweight and obese men, serum PRL levels were lower.[27] Friedrich *et al*[28] 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.[29] A systematic retrospective analysis of 2052 patients with PCOS 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.[30] Arterial hypertension is a component of MetS. A prospective study of 874 postmenopausal women found that PRL levels increased by 1 SD during 8 years of follow-up, and the relative risk of hypertension was 1.31.[31] 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.[32] Dyslipidaemia, especially hypertriglyceridaemia, may subsequently increase the transportation of TGs and other fats into hepatocytes, resulting in hepatic steatosis.[33]

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, 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.

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

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