International Journal of Science and Research Archive

eISSN: 2582-8185 CODEN (USA): IJSRO2

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Estimation of Serum bilirubin and other biochemical parameters in patients with fatty liver disease

Zeyad Swadi Obaid Al-Isawi *

Lecturer of Medical chemistry, Basic Sciences Department, Faculty of Dentistry, University of Kufa, Iraq.

International Journal of Science and Research Archive, 2025, 14(01), 258-266

Publication history: Received on 01 December 2024; revised on 08 January 2025; accepted on 10 January 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.14.1.0068

Abstract

Background/Goals: Serum bilirubin has cytoprotective and antioxidant properties. Furthermore, a lower risk of cardiovascular and metabolic disorders is linked to higher serum bilirubin levels. Few studies, meanwhile, have examined the potential link between blood bilirubin as well as NAFLD, or Fatty liver disease without alcohol consumption, which is strongly linked to additional.metabolic conditions. Clarifying the relationship between serum total bilirubin levels and NAFLD was the goal of this investigation.

Methods: A cross-sectional study was carried out on 17,348 participants who were getting regular checkups. Participants with a history of hepatitis or who tested positive for the hepatitis B or hepatitis C viruses were not included. Normal ultrasound findings and a daily alcohol consumption of less than 20 g were used to diagnose NAFLD.

Results: 9,076 (52.3%) of the subjects were men, and their average age was 49. In both men and women, As blood bilirubin levels increased, the prevalence of NAFLD gradually decreased (P<0.001 for both). Blood bilirubin levels and the prevalence of NAFLD were found to be negatively correlated by multivariate regression research that controlled for other metabolic risk factors [odds ratio (OR)=0.88, 95% confidence interval (CI)=0.80-0.97]. Additionally, NAFLD and serum total bilirubin levels were inversely/dose-dependently related (OR=0.83, 95% CI=0.75-0.93 in the third quartile; OR=0.80, 95% CI=0.71-0.90 in the fourth quartile vs. lowest quartile, P for trend <0.001).

Conclusions: The prevalence of NAFLD was found to be inversely correlated with serum bilirubin levels, regardless of recognized metabolic risk factors. For NAFLD, serum bilirubin may serve as a protective indicator.

Keywords: Bilirubin; Fatty liver disease; Metabolic risk factors; NAFLD

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent liver condition, which is thought to affect 20–30% of people.1,2 NAFLD includes cirrhosis, hepatocellular cancer, nonalcoholic steatohepatitis (NASH), and simple steatosis, among other hepatic dysfunctions.3. Coronary artery disease and type 2 diabetes, obesity, dyslipidemia, and insulin resistance are also strongly associated with non-alcoholic fatty liver disease (NAFLD).4. As a result, it is regarded as a metabolic syndrome hepatic presentation.5, 6 One etiopathogenic factor for NAFLD has been suggested to be oxidative stress, despite the fact that the pathogenic mechanisms responsible for the development of NAFLD are yet unknown.7-9. According to earlier research, giving mice or rats the azo chemical that produces free radicals caused the liver to swell with fat by raising triacylglycerol and lowering phospholipid.10–12 Additionally, the concurrent administration of antioxidants that scavenge free radicals inhibited the buildup of fat in the liver.11. As a result, a number of antioxidants, including vitamin E, have been proposed as a viable therapeutic approach.13.

* Corresponding author: Zeyad Swadi Obaid Al-Isawi

The final byproduct of heme catabolism, serum bilirubin, possesses potent cytoprotective and antioxidant properties.14, 15 Bilirubin scavenges reactive nitrogen species, hydroxyl radicals, and peroxyl radicals at the molecular level.14 It stops intracellular lipids from oxidizing in this way.16 Bilirubin's advantageous cytoprotective properties are well supported by a number of clinical lines of evidence. Diabetes, insulin resistance, and higher bilirubin levels are all inversely correlated.17, 18 A lower risk of cardiovascular conditions, such as peripheral vessel disease, coronary artery disease, and stroke, is also linked to elevated bilirubin levels.19, 20.

Thus, it is possible to hypothesize that elevated serum bilirubin can lower inflammation, oxidative stress, and the danger of fatty liver disease that is not caused by alcohol. The relationship between serum bilirubin levels and NAFLD, however, has only been the subject of a small number of investigations.21–23 In order to assess the relationship between NAFLD and serum bilirubin levels in a population that appeared to be in good health, we carried out a large-scale cross-sectional investigation.

2. Material and methods

2.1. Examine populations

21,130 patients in all came to the hospital for a standard physical examination. 21,128 patients over the age of 18 who had abdominal ultrasonography and a blood bilirubin level test were first chosen from the total number of subjects. We then eliminated participants who had other possible Hepatitis B virus (as indicated by the presence of hepatitis B surface antigen), hepatitis C virus (as indicated by the presence of hepatitis C antibody), excessive alcohol consumption (20 g/day), or any other history of hepatitis as identified by a questionnaire and a comprehensive medical history taking (Hemochromatosis, autoimmune hepatitis, Wilson's disease, and primary biliary cirrhosis) are reasons for chronic liver disease. Furthermore, we eliminated those who had consumed substances known to induce fatty liver during the previous 12 months. Additionally, subjects with cancer and long-term illnesses like coronary artery disease were not included. Ultimately, 17,348 individuals were included in the analysis.

2.2. Laboratory and clinical evaluations

On the same day, each participant finished laboratory testing, an anthropometric evaluation, and a questionnaire. A fasting blood glucose level of 126 mg/dL or higher, or the use of anti-diabetic medication, were considered indicators of diabetes mellitus. Having blood pressure readings more than 90 mmHg at the diastolic or 140 mmHg at the systolic, or being on antihypertensive medication, was considered hypertension. Those who had smoked at least one cigarette a day over the past 12 months were considered current smokers. A digital scale was used to measure the respondents' height and weight while they were dressed in light clothing. The formula used to determine Weight (kg) divided by height squared (m2) yielded the body mass index (BMI). A skilled examiner measured the waist circumference at the midpoint between the iliac crest and the lower costal margin, measuring to the nearest millimeter with a tape measure. On the same day, two measurements of the systolic and diastolic blood pressure were made, and the mean values were used for analysis. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum bilirubin, hepatitis B surface antigen, total cholesterol, Laboratory markers included an antibody to the hepatitis C virus and fasting hyperglycemia. Each participant's blood was drawn after a 12-hour overnight fast, before 10 AM. Using standard operating procedures, every biochemical analysis was performed in the same laboratory. A person is diagnosed with metabolic syndrome if they satisfy three or more of the following criteria, according to the updated NCEP criteria24: 1) waist circumference, as defined according to the Asian-Pacific International Obesity Task Force Standards population25, greater than 90 cm for men and greater than 80 cm for women; 2) 3) HDL cholesterol less than 40 mg/dL for men and less than 50 mg/dL in females or those taking medication; 4) triglycerides 150 mg/dL or medication usage; 5) fasting glucose <100 mg/dL or medication use (insulin or oral agents); and antihypertensive medication use or blood pressure of 130/85 mmHg.

2.3. Serum bilirubin

The Architect ci8200 Integrated System is an automated system manufactured by Abbott Laboratories in Abbott Park, USA. was used to measure the total bilirubin in fasting serum using the vanadate oxidation method reference standards, >1.2 mg/dL of total bilirubin in fasting blood was considered elevated. Additionally, both men's and women's serum bilirubin levels were separated into quartiles. Serum bilirubin values of 0.8 mg/dL were included in total bilirubin quartile 1 for men, 0.9-1.0 mg/dL for quartile 2, 1.1-1.4 mg/dL for quartile 3, and Ā1.5 mg/dL for quartile 4. Serum bilirubin levels in women fell into four quartiles: 0.6 mg/dL in quartile 1, 0.7-0.8 mg/dL in quartile 2, 0.9-1.0 mg/dL in quartile 3, and 1.1 mg/dL in quartile 4.

2.4. Ultrasonographic examinations

NAFLD was identified by ultrasonography (US) as fatty liver when none of the following conditions were present: (1) excessive consumption of alcohol (20 g/day), (2) the presence of an antibody to the hepatitis C virus or the hepatitis B surface antigen, and (3) established liver etiologies, illness, and (4) drugs that are known to cause fatty liver. At the time of the procedure, skilled radiologists performing hepatic US evaluations were blinded to the subjects' clinical and laboratory information. Using previously established defined criteria, US (Siemens, Acuson, Sequoia 512, Mountain View, CA) made the diagnosis of fatty liver.4.

2.5. Statistical analysis

Using a student's t-test or ANOVA for continuous variables and a chi-square test for categorical variables, the variables were compared between patients based on the Serum bilirubin levels in the relevant quartiles and between participants with and without NAFLD. The examination of multivariate logistic regression includes variables having a P value <0.05 in the univariate studies and established risk factors. SPSS 19 was used for all statistical analyses (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as a two-tailed P value <0.05.

3. Results

The mean ages of the 17, 348 individuals were 50.1 ± 11.6 years for the 9, 076 men and 8, 272 women, respectively. Of them, 5, 768

Table 1 Clinical, anthropometric, and biochemical parameters of NAFLD subjects and controls stratified according to sex

Men				**Women**		
Control (n=4,828) NAFLD (n=4,248) P value				**Control (n=6,752) NAFLD (n=1,520) P value**		
Age (yr)	49.9±12.3	50.2±10.6	0.337	47.1±11.2	55.9±9.8	<0.001
BMI (kg/m2)	23.3±2.4	25.7±2.5	<0.001	21.5±2.5	24.8±3.0	<0.001
Waist circumference (cm)	84.8±6.8	91.1±6.4	<0.001	79.5±7.4	88.9±7.8	<0.001
SBP (mm Hg)	118.2±14.8	122.1±14.8	<0.001	110.8±15.7	121.8±16.9	<0.001
DBP (mm Hg)	77.5±10.8	80.9±11.0	<0.001	69.7±11.2	75.9±11.2	<0.001
AST (IU/L)	22.9±9.2	27.2±12.6	<0.001	20.5±9.3	24.4±11.1	<0.001
ALT (IU/L)	23.8±14.7	36.7±24.6	<0.001	16.9±12.2	25.8±16.1	<0.001
Total bilirubin (mg/dL)	1.20±0.44	1.16±0.43	<0.001	0.94±0.34	0.89±0.33	<0.001
Cholesterol (mg/dL)	186.8±31.0	197.8±32.8	<0.001	186.5±32.7	202.9±34.9	<0.001
Fasting blood glucose (mg/dL)	98.0 ±16.9	104.6±22.3	<0.001	91.3±10.6	101.5±19.8	<0.001
Diabetes mellitus (%)	255 (5.3)	353 (8.3)	<0.001	150 (2.2)	158 (10.4)	<0.001
Hypertension (%)	719 (14.9)	972 (22.9)	<0.001	572 (8.5)	422 (27.8)	<0.001
Smoking (%)	1,585 (32.8)	1,577 (37.1)	<0.001	335 (5.0)	37 (2.4)	<0.001
Metabolic syndrome (%)	648 (13.4)	1,695 (39.9)	<0.001	591 (8.8)	695 (45.7)	<0.001

Means ± standard deviation are used to present the data.
NAFLD stands for nonalcoholic fatty liver disease; AST stands for aspartate aminotransferase; ALT for alanine aminotransferase; and SBP for systolic and DBP for diastolic blood pressure.

Table 2 Comparison of men's baseline traits with their total bilirubin quartile

	Quartile 1* (n=1,916)	**Quartile 2 (n=2,087)**	**Quartile 3 (n=3,218)**	**Quartile 4 (n=1,855)**	**P value**
Age (year).	52.1 ± 12.5	50.6 ± 11.6	50.0 ± 11.4	49.0 ± 11.1	< 0.001
BMI (kg/m2).	24.5±2.8	24.6 ±2.7	24.4 ±2.7	24.2±2.8	< 0.001
Waist.circumference (cm).	88.3 ± 7.7	88.3±7.1	87.5 ±7.1	87.0±7.3	< 0.001
SBP (mm Hg).	121.2 ± 15.5	121.4 ± 14.8	120.0 ± 14.7	120.1 ± 14.6	0.716
DBP (mm Hg).	77.9 ± 11.3	78.9 ± 11.1	78.9 ± 11.2	78.9 ± 10.8	0. 077
AST (IU/L).	23.9 ± 10.2	24.8 ± 9.8	24.9 ± 12.2	26.0 ± 11.8	0.001
ALT (IU/L).	28.8 ± 19.8	29.9 ± 19.5	28.9 ± 20.5	29.8 ±24.2	0. 601
Cholesterol(mg/dL).	191.0 ± 33.6	191.9 ± 32.1	191.8 ± 32.1	192.0 ± 31.5	0. 037
Fasting.glucose (mg/dL).	104.3 ± 23.6	101.4 ±19.1	100.5 ±19.2	98.4 ± 17.2	<0. 001
Diabetes.mellitus(%).	221(11.6)	143(6.7)	174(5.5)	74(3.8)	< 0.001
Hypertension (%).	408(21.3)	412(19.8)	582(18.2)	293(15.8)	< 0.001
Smoking (%).	807(42.3)	792(37.8)	1035(32.2)	527(28.6)	< 0.001
Metabolic.syndrome (%).	624(32.6)	587(28.3)	753(23.5)	378(20.5)	< 0.001
NAFLD.prevalence (%).	964 (50.5)	1,057(50.7)	1,448(45.1)	783(42.3)	< 0.001

Standard deviation (SD) ± means are used to present the data; *Quartiles 1 and 4 of total bilirubin are 0.8, 0.9, and 1.0 mg /dL, 1.1, and 1.4 mg /dL, respectively, and 1.5 mg /dL, respectively; Non-alcoholic fatty liver disease (NAFLD), systolic and alanine aminotransferase (ALT), aspartate aminotransferase (AST), and diastolic blood pressure.

Table 3 Comparison of the total bilirubin quartile with the women's baseline features

***Quartile 1(n= 1,424) Quartile. 2 (n= 2,578) Quartile 3 (n= 2,062) Quartile 4 (n= 2,208)**					**P value**
Age (years)	48.7 ± 11.7	49.8 ± 11.5	49.2 ± 11.4	47.4±11.4	< 0.001
BMI(kg/m2)	22.27 ± 2.91	22.35 ± 3.01	22.03 ± 2.82	21.67 ± 2.74	< 0.001
Waist circumference(cm)	81.9 ± 8.2	82.1 ± 8.5	81.1 ± 8.2	80.1 ± 8.2	< 0.001
SBP(mm Hg)	113.1 ± 16.7	113.8 ± 17.2	112.7 ± 16.6	111.7 ± 15.7	0.001
DBP(mm Hg)	70.5 ± 11.6	71.4 ± 11.7	70.7 ± 11.7	70.6 ± 11.3	0.056
AST(IU/L)	21.5 ± 10.6	21.3± 12.8	21.2± 6.9	21.3 ± 7.5	0.881
ALT(IU/L)	18.8 ± 12.4	18.8 ± 17.4	18.5 ± 10.1	18.5 ± 11.7	0.733
Cholesterol(mg/dL)	184.8 ± 32.1	190.7 ± 33.5	191.3 ± 33.7	189.7± 34.6	0.037
Fasting glucose(mg/dL).	94.0 ± 13.1	94.0 ± 13.6	94.1 ± 15.1	92.1± 11.8	< 0. 001
Diabetes mellitus(%).	59(4.2)	109(4.3)	68(3.3)	76 (3.5)	0.263
Hypertension(%).	173(12.2)	331(12.8)	254(12.4)	236(10.8)	0.147
Smoking(%).	79 (5.6)	121(4.6)	86(4.2)	88(4.1)	0.164
Metabolic syndrome(%).	281(19.8)	463(17.8)	291(14.2)	253(11.6)	< 0.001
NAFLD prevalence(%).	311(21.7)	508(19.8)	357(17.5)	342(15.6)	< 0.001

Standard deviation (SD) ± means are used to present the data; *The quartiles for total bilirubin are 0.6 mg/dL for quartile 1, 0.7-0.8 mg/dL for quartile 2, 0.9-1.0 mg/dL for quartile 3, and 1.1 mg/dL for quartile 4; Aspartate aminotransferase (AST), alanine aminotransferase (ALT), systolic blood pressure (SBP), diastolic blood pressure (DBP), and non-alcoholic fatty liver disease (NAFLD).

Results in: Increased increased AST, ALT, total cholesterol, fasting glucose, and blood pressure, larger waist circumference, higher BMI, and the existence of metabolic syndrome, hypertension, and diabetes (P<0.001, respectively). Additionally, the NAFLD group had a significantly reduced serum total bilirubin level P<0.001. Tables 2 and 3 display features based on the male and female serum bilirubin level quartiles, respectively. As anticipated, a lower prevalence of metabolic syndrome, lower BMI, A less severe metabolic profile, lower fasting glucose levels, a smaller waist circumference, and lower serum cholesterol were all linked to higher serum total bilirubin levels (P<0.05, in both men and women). Additionally, a declining incidence increased blood A significantly increased risk of NAFLD was linked to total bilirubin levels (P for trend, respectively, <0.001, Fig.1). Additionally, NAFLD and the grade of total serum bilirubin levels were found to be dose-dependently related by multivariate regression analysis [odds ratio (OR) 0.80, 95% CI, 0.71-0.90 in the fourth quartile; OR 0.83, 95% CI, 0.75-0.93 in the third quartile vs. the first quartile, P for trend <0.001, Table 4[The prevalence of NAFLD was considerably lower in those Having blood bilirubin levels that are higher than normal (>1.2 mg/dL) than those that are normal (₮1.2 mg/dL).

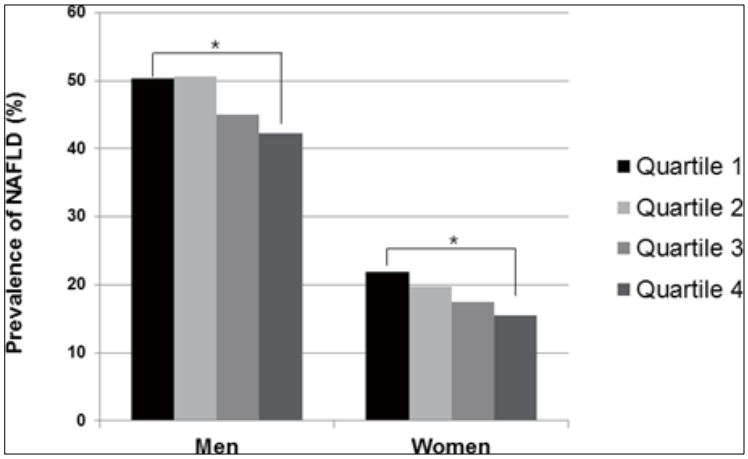

Figure 1 Men's and women's NAFLD prevalence based on the blood total bilirubin level's quartiles. Serum bilirubin levels across participants in quartiles 1-4 were ≤0.6, 0.7-0.8, 0.9-1.0, and ≥1.1 mg/dL for women and ≤0.8, 0.9-1.0, 1.1-1.4, and ≥1.5 mg/dL for males. *P for both sex groups' trend <0.001 bilirubin (P<0.001, respectively, Fig. 2). patients with unusually high blood bilirubin levels were 13% less likely to have non-alcoholic fatty liver disease (NAFLD) than patients with normal serum bilirubin levels in the multivariate logistic model (OR 0.87, 95% CI, 0.80-0.95, P = 0.002). Additionally, The prevalence of NAFLD and serum total bilirubin level were consistently inversely correlated in the fully adjusted model when examined as a continuous variable (OR 0.88, 95% CI, 0.80-0.97). A 12% reduction in the risk of NAFLD was linked to an increase of 1 mg/dL in bilirubin

4. Discussion

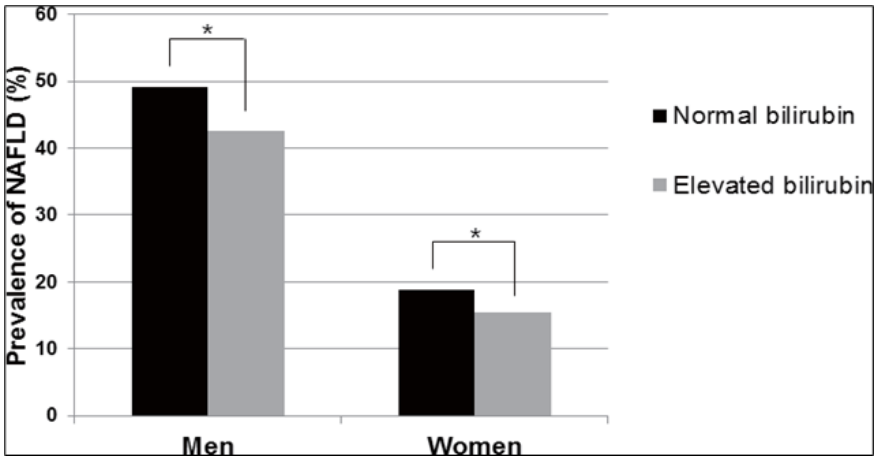

Figure 2 Men's and women's blood total bilirubin levels (normal ≤ 1.2 mg /dL; elevated ≥ 1.2 mg /dL) show the concentration of NAFLD, or nonalcoholic fatty liver disease. *P<0.001 for participants with increased bilirubin compared to those with normal bilirubin

An inverse relationship between the prevalence of NAFLD and the blood bilirubin level was the primary finding of this extensive investigation. Subjects with increased serum bilirubin had a significantly decreased frequency of NAFLD levels. Furthermore, it was discovered that, independent of established metabolic risk factors, the blood total bilirubin level was inversely and dose-dependently connected with NAFLD.

Table 4 ORs of risk variables for NAFLD occurrence, both univariate and multivariate, in relation to the overall bilirubin level range

Variable	**Univariate**		**Age- and sex-adjusted**				**Multivariate model***		
Total bilirubin quartiles	**OR**	**95% CI**	**P value**	**OR†**	**95% CI.**	**P value**	**OR†**	**95% CI.**	**P value**
Quartile1	1		<0.001†	1		<0.001†	1		<0.001†
Quartile2	0. 82	0.75- 0.90	<0.001	0.97	0.88-1.07	0.516	0.92	0.82-1.03	0.146
Quartile3	0. 84	0.77- 0.92	<0.001	0.81	0.74-0.89	<0.001	0.83	0.75-0.93	0.001
Quartile4	0. 62	0.56- 0.69	<0.001	0.74	0.66-0.82	<0.001	0.80	0.71-0.90	<0.001

The multivariate model was modified to account for smoking, total cholesterol, diabetes, hypertension, age, sex, body mass index, and waist circumference. The odds trend test's P-value.

NAFLD stands for nonalcoholic fatty liver disease; OR stands for odds ratio; and CI is for confidence interval.

According to recent clinical research, a reduced frequency of diseases mediated by oxidative stress is linked to elevated serum bilirubin levels. It has been repeatedly shown that cardiovascular diseases, such as Ischemic stroke, peripheral atherosclerosis, and coronary artery disease, as well as their risk factors, such as obesity, diabetes mellitus, metabolic syndrome, and hypertension, are negatively connected with serum bilirubin.30 It is reasonable to believe that NAFLD has a negative correlation with serum bilirubin levels because it is intimately linked to metabolic risk factors and cardiovascular diseases4,31–33. It is challenging to get a consistent conclusion, nevertheless, because there have been few investigations on the relationship between blood bilirubin levels and NAFLD. While Kumar et al.23 showed that people with unconjugated hyperbilirubinemia had noticeably less severe liver disease, Hjelkrem et al.22 showed that patients with NASH had a much lower prevalence of unconjugated hyperbilirubinemia. Nevertheless, these studies' study populations were too tiny to make any inferences. Chang et al. have demonstrated a negative correlation between the incidence of NAFLD and the serum direct bilirubin level. But the only Korean guys in their cohort were middle-aged.21 As a result, extrapolating these findings to the broader male and female population is challenging. In a large population that appeared to be in good health, our study demonstrated an inverse relationship between serum bilirubin levels and US-diagnosed NAFLD.

Serum bilirubin possesses cytoprotective and antioxidant properties.14, 15 Earlier experimental research offers additional evidence for the function of bilirubin as a prophylactic measure for NAFLD. Pro-oxidant heme is broken down into biliverdin by the stress-responsive protein hemeoxygenase-1 (HO-1), which is subsequently transformed into the antioxidant bilirubin.34 By starting an antioxidant pathway, decreasing cytokine production, and altering fatty acid turnover, In both in vitro and in vivo settings, HO-1 can halt the progression of steatohepatitis.35 Thus, bilirubin, a HO-1 byproduct, is likewise thought to be a protective indicator of the development of NAFLD; nonetheless, more investigation is required to ascertain its exact role in this process. Males are more likely to be diagnosed with Gilbert's syndrome, the most prevalent hereditary bilirubin glucuronidation condition that manifests as unconjugated hyperbilirubinemia. The underlying process has been linked to a comparatively higher level of daily bilirubin generation in men and distinct effects of sex hormones on bilirubin metabolism.36 In line with the findings of earlier research, men were more likely than women to have increased serum bilirubin in this study (34.8%) compared to 13.8%. We examined the relationship between NAFLD and serum bilirubin levels in men and women independently in order to find gender differences. In both men and women, our study consistently found a negative correlation between blood Non-alcoholic fatty liver disease and bilirubin levels. There are several restrictions on this study. First, there was no evidence of a causal link between blood bilirubin levels and NAFLD due to the cross-sectional methodology. Second, in this investigation, we did not measure conjugated and unconjugated bilirubin independently. Given their distinct characteristics, these two forms of bilirubin might be associated with NAFLD in various ways. Third, we employed US to diagnose NAFLD without liver histology confirmation, which is considered the gold standard for NAFLD diagnosis. As a result, we were unable to distinguish between simple steatosis and NASH, where oxidative stress plays a larger role. Despite these drawbacks, this study has a number of benefits. First, even after splitting to examine sex differences,

our sample size is sizable. Second, according to a policy for health examinations, the research participants are thought to be typical of the overall community.

Abbreviations

- HO-1 stands for heme oxygenase-1;
- NASH stands for non-alcoholic steatohepatitis;
- OR stands for odds ratio;
- BMI (body mass index);
- CI (confidence interval);
- ALT (alanine aminotransferase);
- AST (aspartate aminotransferase);
- Ultrasonography in the US

5. Conclusion

Our study concludes that, in a large, healthy population, increased blood bilirubin levels are inversely correlated with the frequency of non-alcoholic fatty liver disease (NAFLD) independent of recognized metabolic risk variables. For NAFLD, serum total bilirubin may serve as a protective indicator.

Compliance with ethical standards

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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