# Helicobacter Pylori Infection, Lipid Profile, and Insulin Resistance in Obese Patients with Non-Alcoholic Fatty Liver Disease

Ahmed Waheed Samir [1], Mohamed Sabry Aboelnasr[2], Heba Ahmed Mohammed Mourad [3], Waleed S. Mohamed [2]

[1] Internal Medicine Resident, Internal Medicine Department, Zefta General Hospital, Egypt.

[2] Lecturer of Internal Medicine, Internal Medicine Department, Faculty of Medicine, Tanta University, Egypt, mohamed.aboelnasr@med.tanta.edu.eg, ORCID: 0000-0002-2409-8773.

[2] Professor of Internal Medicine, Internal Medicine Department, Faculty of Medicine, Tanta University, Egypt, waleed.samy@med.tanta.edu.eg.

[3] Professor of Clinical Pathology, Clinical Pathology Department, Faculty of Medicine, Tanta University, Egypt, hebamourad@hotmail.com.

## Corresponding Author

Ahmed Waheed Samir, Resident of Internal Medicine, Internal Medicine Department, Faculty of Medicine, Tanta University, Egypt.

**Mobile:** +20 1010878400

**E-mail:** ahmed_w105@yahoo.com

**DOI: 10.21608/ajgh.2024.306471.1057.**

Submission date:22 July 2024.

Revision date: 10 August 2024.

Acceptance date: 12 August 2024.

First online: 17 August 2024.

**Abstract**

**Aim:** Nonalcoholic fatty liver disease (NAFLD) is characterized by the presence of hepatic steatosis without any secondary causes contributing to the accumulation of fat in the liver. The influence of gut microbiota, including Helicobacter pylori (H. pylori), on liver injury has not been thoroughly investigated.

**Methods:** This cross-sectional case-control study involved sixty patients with NAFLD (group 1) and sixty healthy individuals of matched age and sex as the control group (group 2). NAFLD patients were diagnosed by ultrasound, aged≥ 18 years old, and body mass index (BMI) ≥ 30 $kg/m^2$. Investigations included fasting insulin level, fasting blood glucose (FBG), cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), liver profile, H. pylori antigen in stools, H. pylori IgG antibodies (IgG Abs) and homeostatic model assessment for insulin resistance (HOMA-IR).

**Results:** Group 1 had significantly higher systolic and diastolic blood pressures ($p < 0.001$ and 0.015, respectively). Additionally, the NAFLD group exhibited significantly elevated levels of FBG ($p = 0.004$), $HbA_{1c}$ ($p < 0.001$), cholesterol ($p = 0.007$), LDL-C ($p = 0.001$), alanine transferase (ALT) ($p < 0.001$), and aspartate transferase (AST) (p: 0.025). Furthermore, the NAFLD group had a significantly higher number of patients with positive H. pylori antibodies ($p < 0.001$), although the antibody titers were not statistically different between the two groups (p: 0.516).

**Conclusion:** Obese patients with NAFLD had a significantly higher number of patients with positive H. pylori antibodies and showed increased insulin resistance and dyslipidemia compared to the control group.

***Keywords:*** *H. Pylori, Insulin Resistance, Metabolic Syndrome, Nonalcoholic Fatty Liver Disease, dyslipidemia, obesity, chronic liver disease, HOMA IR, gut microbiota, hepatic steatosis.*

## Introduction

Globally, non-alcoholic fatty liver disease (NAFLD) is a significant contributor to chronic liver diseases and is an essential indication for liver transplantation. [1] .Some estimates suggest that up to one-third of the population may be affected. [2]. NAFLD spectrum includes nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH), depending on the presence or absence of substantial inflammation [3].

Non-alcoholic fatty liver disease is defined by the accumulation of excessive fat inside hepatocytes, with steatosis affecting more than 5% of these cells in the absence of alcohol consumption. [4] NAFLD can manifest as cirrhosis, inflammation, fibrosis, or simple steatosis [3].

Multiple factors contribute to the development of NAFLD, including lipid buildup, environmental factors, and genetics [5]. Additionally, NAFLD is substantially linked to elements of metabolic syndrome, e.g., obesity, hypertension (HTN), and dyslipidemia. This suggests that insulin resistance (IR) is a significant contributing factor. [5, 6]

The gut microbiota is one of the additional variables that could influence the onset and course of the disease. There is evidence that Helicobacter pylori (H. pylori) infection may play a part in the onset and progression of NAFLD. [7]. It is estimated that H. pylori infection impacts at least 50% of the global population. [8] Most infections start in childhood, especially in underdeveloped countries [9].

Much research hasn't been done on how the gut microbiota, particularly H. pylori, produce liver damage. Potential mechanisms include the production of certain toxins. [10] and increased gut permeability, thus facilitating the entry of bacterial endotoxins into the liver through the portal vein [11].

## Subjects and Methods

This cross-sectional case-control study included subjects attending outpatient clinics and wards of our hospital from June 2020 to May 2021. The study included sixty obese patients with NAFLD (group 1) and sixty healthy subjects of comparable age and sex as control (group 2). Patients in group 1 were further subdivided into three subgroups based on the severity of NAFLD by ultrasound (US): grade 1, grade 2, and grade 3 [12].

The diagnosis of NAFLD necessitates patients to exhibit evidence of steatosis through imaging or biopsy in the absence of significant alcohol consumption, other causes of steatosis, or concurrent chronic liver disease. [13]In our study, the US was the imaging modality of choice; no patients needed a biopsy. Patients in group 1 had an age $\geq 18$ and a body mass index (BMI) $\geq 30$ kg/m$^2$.

***Exclusion criteria***

Patients with the following diseases/conditions were excluded from the study:

- History of alcohol consumption [14].
- Active or past infection with hepatitis B or hepatitis C viral infection.
- Other causes of secondary liver diseases, e.g., Wilson disease.
- Long-term parenteral feeding.

- Receiving lipid-lowering drugs.
- Diabetes mellitus.
- Recent rapid weight loss.
- History of bariatric surgery.
- Recent H. pylori eradication therapy.
- Recent intake of hepatotoxic medications.

All participants underwent comprehensive clinical assessment, emphasizing blood pressure and anthropometric measurements, including BMI and waist-to-hip ratio (WHR). Obesity was defined as a BMI $\geq 30$ kg/m² [15]. A WHR was considered normal if $\leq 0.85$ for women and $\leq 0.9$ for men. [16]Dietary intake was evaluated using a 24-hour food recall (24h-FR) method, known for its ease of application, cost-effectiveness, and independence from the respondent's literacy level. [17]. Physical activity levels were evaluated using a self-report measure. [18].

***Abdominal ultrasound***

Abdominal ultrasound was conducted using consistent equipment and by the same operator. Increased liver echogenicity served as the primary indicator of steatosis in the ultrasound scans. Steatosis severity was classified as follows. [12]:

- Grade 0: echogenicity of the right liver lobe is average compared to the right renal cortex.
- Grade 1: a slight, diffuse increase in delicate echoes in the liver parenchyma, with normal diaphragm and intrahepatic vessel borders visualization.
- Grade 2: moderate, diffuse increase in delicate echoes and slightly impaired visualization of the diaphragm and intrahepatic vessel borders.
- Grade 3: marked increase in delicate echoes, accompanied by poor or non-visualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver.

***Laboratory investigations***

Investigations done included fasting insulin level, fasting blood glucose (FBG), cholesterol, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, bilirubin, prothrombin time (PT), prothrombin activity, international normalized ratio (INR), H. pylori antigen in stools and H. pylori IgG antibodies (IgG Abs). IR

was assessed using the homeostatic model assessment for insulin resistance (HOMA-IR); Fasting Insulin (µg/ml)]*[Fasting Glucose (mmol/l)]/22.5 [19].

***Statistical analysis***

The SPSS software version 22 (SPSS Inc., Chicago, IL, USA) was employed for data collection, presentation, and statistical analysis. Normally distributed data were presented as mean (M) (± standard deviation [SD]). Non-normally distributed data were analyzed using the Mann-Whitney test and Spearman's rank-order correlation and reported as the median and interquartile range (IQR), denoting the $25^{th}$ to $75^{th}$ percentiles. The Kolmogorov test for normality was applied to quantitative data. Pearson's Chi-square test was utilized to explore associations between two variables in qualitative data. A probability (p) value < 0.05 was considered significant for interpreting test results. [20].

## Results

This study included 60 obese NAFLD patients diagnosed by ultrasound and 60 healthy apparent controls. Patients were subdivided into three subgroups (Grade I, Grade II, and Grade III) according to the severity of NAFLD by the US. No statistically significant difference was found between both groups regarding age and sex (p: 0.060 and 0.464, respectively). Around one-fifth of group 1 (13/60) had HTN. The control group showed statistically significantly higher values regarding physical activity levels than the NAFLD group (p: 0.039). No statistically significant difference in smoking status was found between both groups (p: 0.055). The frequency of fast-food meals was significantly higher, while daily vegetable and fruit portions per week were significantly lower in the NAFLD group compared to the control.

**Table 1. Comparison of demographic characteristics of the studied groups.**

| | | Group 1 (N=60) | Group 2 (N=60) | Test | p |
|---|---|---|---|---|---|
| **Age** | **M±SD** | 30.63± 4.89 | 30.40± 5.75 | t = 1.830 | 0.060 |
| **Gender [N (%)]** | **Female** | 26 (43.3%) | 30 (50%) | $X^2_{ChS}$ = 0.536 | 0.464 |
| | **Male** | 34 (56.7%) | 30 (50%) | | |
| **Co-morbidities [N (%)]** | **HTN** | 13 (21.7%) | 0 (0.0%) | $X^2_{ChS}$ = 14.579 | <0.001* |
| **Smoking [N (%)]** | | 19 (31.7%) | 10 (16.7%) | $X^2_{ChS}$ = 3.683 | 0.055 |
| **Physical activity** | | 11 (18.3%) | 21 (35.0%) | $X^2_{ChS}$ = 4.261 | 0.039* |

| | | | | | |
|---|---|---|---|---|---|
| **Frequency of fast-food meals /week** | **Median (IQR)** | 2 [0 - 3] | 1 [0 - 2] | Z = 2.357 | 0.018* |
| **Daily vegetable and fruit portions** | **Median (IQR)** | 1 [0 - 3] | 2 [1 - 3] | Z= 2.552 | 0.011* |

*** Significant at p-value <0.05**

The most common clinical symptoms in the patient group were heartburn (76.7%), epigastric pain (75.0%), and reflux (70.0%).

**Table 2. Main clinical symptoms of the patient group.**

| Symptoms | N (%) |
|---|---|
| Heartburn | 46 (76.7%) |
| Epigastric pain | 45 (75.0%) |
| Reflux | 42 (70.0%) |
| Bloating | 37 (61.7%) |
| The metallic taste of the mouth | 36 (60.0%) |
| Dysphagia | 17 (28.3%) |
| Oropharyngeal candidiasis | 15 (25.0%) |
| Loss of appetite | 8 (13.3%) |
| Recurrent vomiting | 6 (10.0%) |
| Feeling tired than usual | 45 (75.0%) |
| Shortness of breathing | 31 (51.7%) |
| Intermittent claudication and tingling | 15 (25.0%) |
| Skin tags | 8 (13.3%) |
| Acne | 2 (3.3%) |

*** Significant at p-value <0.05**

The patient group showed significantly higher BMI and WHR (p <0.001 for both) and considerably higher values for both SBP and DBP (p <0.001 and 0.015, respectively).

**Table 3. Comparison of the primary clinical data of the studied groups.**

| | | **Group 1 (N=60)** | **Group 2 (N=60)** | **Test** | **p** |
|---|---|---|---|---|---|
| **BMI** | **M±SD** | 32.60 ± 1.45 | 24.29 ± 1.64 | T = 29.469 | <0.001* |
| **WHR** | **M±SD** | 1.02 ± 0.07 | 0.86 ± 0.03 | T= 15.975 | <0.001* |
| **SBP** | **M±SD** | 123.83 ± 13.32 | 114.33 ± 11.33 | T = 4.208 | <0.001* |
| **DBP** | **M±SD** | 76.58 ± 8.61 | 72.83 ± 7.94 | T = 2.480 | 0.015* |

*** Significant at p-value <0.05**

**** FE: Fisher's exact test, $X^2_{ChS}$: Pearson's Chi-square test for independence of observations,**

Regarding laboratory parameters, group 1 shows statistically significantly higher values for FBG (p: 0.004), $HbA_{1c}$ (p <0.001), cholesterol (p: 0.007), LDL-C (p: 0.001), ALT (p <0.001*) and AST (p: 0.025), while group 2 shows statistically significant higher values for albumin level (p: 0.035). There was no statistically significant difference between the studied

groups regarding insulin level (p: 0.055), HOMA-IR (p: 0.057), TG (p: 0.064), HDL-C (p: 0.631), INR (p:0.746) and bilirubin (p: 0.823).

**Table 4. Comparison of laboratory parameters of the studied groups.**

| | | Group 1 (N= 60) | Group 2 (N=60) | Test | p |
|---|---|---|---|---|---|
| FBG (mg/dl) | M±SD | 89.30± 7.84 | 84.22±10.86 | t = 2.936 | 0.004* |
| $HbA_{1c}$ (%) | M±SD | 5.89±.32 | 5.09±.36 | t=12.956 | <0.001* |
| Insulin level (miu/ml) | M±SD | 11.16±5.84 | 9.36±4.22 | t=1.937 | 0.055 |
| HOMA-IR | Median (IQR) | 2.20 (1.35–3.45) | 2.05 (1.20–2.65) | Z=1.905 | 0.057 |
| Cholesterol (mg/dl) | M±SD | 183.42± 23.98 | 169.91±29.87 | t=2.731 | 0.007* |
| TG (mg/dl) | M±SD | 128.03±30.81 | 116.62±35.85 | t=1.870 | 0.064 |
| HDL-C (mg/dl) | M±SD | 50.35±8.86 | 49.42±12.03 | t= 0.482 | 0.631 |
| LDL-C (mg/dl) | M±SD | 111.20± 25.24 | 96.34 ± 22.11 | t=3.430 | 0.001* |
| ALT (u/l) | M±SD | 30.42± 10.64 | 22.16±10.03 | t=4.373 | <0.001* |
| AST (u/l) | M±SD | 26.45±7.50 | 23.83 ± 4.81 | t=2.274 | 0.025* |
| Albumin (g/dl) | M±SD | 3.91±0.25 | 4.02±0.31 | r=2.131 | 0.035* |
| Bilirubin (mg/dl) | M±SD | 0.43±0.10 | 0.43±0.10 | t=0.224 | 0.823 |
| INR | M±SD | 1.19±0.09 | 1.18±0.09 | t=0.325 | 0.746 |
| PT (seconds) | M±SD | 12.97±0.60 | 12.97±0.60 | t=0.052 | 0.959 |
| Positive H. pylori Ab. N (%) | | 39(65.0%) | 19(31.7%) | $X^2_{ChS}$= 13.348 | <0.001* |
| H. pylori Ab. level (>1.1 u/ml) | M±SD | 3.06±1.20 | 2.85± 91 | t=0.653 | 0.516 |
| Positive H. pylori Ag in stools | | 24(40.0%) | 35 (58.3%) | $X^2_{ChS}$= 4.034 | 0.045* |

*** Significant at p-value <0.05**

Regarding H. pylori status, group 1 showed a significantly higher number of patients with positive H pylori antibodies (p <0.001). However, both groups had no statistical difference in antibody levels (p: 0.516). Group 2 revealed a considerably higher value of h pylori infection (positive H pylori Ag in stool) (p: 0.045).

Patients in group 1 were classified into three subgroups based on US evidence of the severity of steatosis: thirty-two patients had grade 1 NAFLD, twenty-five patients had grade 2 NAFLD, and three patients had grade 3 NAFLD. No statistically significant difference was found among the three groups regarding age, sex, smoking status, or physical activity levels (p: 0.587, 0.830, 0.590, 0.193, respectively).

**Table 5. Comparison of demographic and laboratory parameters among NAFLD subgroups**

| | Grade I NAFLD (N=32) | Grade II NAFLD (N=25) | Grade III NAFLD (N=3) | Test | p |
|---|---|---|---|---|---|
| • **Clinical parameters:** | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Age (years)** | **M±SD** | 29.41±4.38 | 30.12±4.60 | 32.00±5.57 | F = 0.538 | 0.587 |
| **Sex** | **Female** | 13 (40.6%) | 12(48.0%) | 1 (33.3%) | 0.558 | 0.830 |
| | **Male** | 19 (59.4%) | 13(52.0%) | 2 (66.7%) | | |
| **Co-morbidities** | **HTN** | 4 (12.5%) | 7(28.0%) | 2 (66.7%) | 5.386 | 0.049* |
| **Smoking** | | 12 (37.5%) | 6(24.0%) | 1 (33.3%) | 1.368 | 0.590 |
| **Physical activity level** | | 8 (25.0%) | 2(8.0%) | 1 (33.3%) | 3.612 | 0.193 |
| • **Laboratory parameters:** | | | | | | |
| FBG (mg/dl) | M±SD | 88.00 ± 7.66 | 90.28 ± 7.96 | 95.00 ± 7.55 | F= 1.449 | 0.243 |
| $HbA_{1c}$ (%) | M±SD | 5.92 ± 0.31 | 5.84 ± 0.33 | 6.00 ± 0.20 | F= 0.649 | 0.526 |
| Insulin level (miu/ml) | Median [IQR] | 9.02 [7.10-14.23] | 9.05 [5.47-16.30] | 13.76 [10.33- 20.42] | Z= 1.791 | 0.408 |
| HOMA-IR | Median [IQR] | 2.00 [1.60 - 3.05] | 2.20 [1.20 - 3.50] | 3.00 [2.40 - 5.10] | Z= 2.408 | 0.300 |
| Cholesterol (mg/dl) | M±SD | 182.94 ± 24.46 | 181.24± 22.40 | 206.67± 28.15 | F = 1.548 | 0.221 |
| TG. (mg/dl) | M±SD | 122.53± 28.85c | 128.92± 29.23c | 178.33 ± 21.39 a, b | F= 5.178 | 0.009* |
| HDL-C (mg/dl) | M±SD | 50.19±9.02 | 49.84±8.59 | 56.33±10.60 | F= 0.724 | 0.489 |
| LDL-C (mg/dl) | M±SD | 108.16±22.61 | 116.08± 26.20 | 103.00± 45.57 | F= 0.854 | 0.431 |
| ALT (u/l) | M±SD | 31.19±11.33c | 28.60± 10.11c | 37.33±0.58 a, b | F = 37.303 | <0.001* |
| AST (u/l) | Median [IQR] | 27.00 [24.00-33.50] | 24.00 [21.00- 28.00] | 22.00 [12.00- 38.00] | Z = 3.410 | 0.182 |
| PT (seconds) | M±SD | 12.99±0.68 | 12.91±0.46 | 13.33±0.81 | F= 0.697 | 0.502 |
| INR | M±SD | 1.20 ± 0.08 | 1.18±0.09 | 1.20 ± 0.04 | F= 0.240 | 0.788 |
| Albumin (g/dl) | M±SD | 3.93±0.23 | 3.87±0.18 | 3.97±0.15 | F= 0.633 | 0.535 |
| Bilirubin (mg/dl) | M±SD | 0.46±0.12 | 0.39±0.07 | 0.47±0.06 | F= 3.081 | 0.054 |
| Positive H. pylori Ab. N (%) | | 20 (62.5%) | 17(68.0%) | 2(66.7%) | $X^2_{FFH}$ = 0.374 | 0.901 |
| Positive H. pylori Ag in stool N (%) | | 12 (37.5%) | 11 (44.0%) | 1(33.3%) | 0.449 | 0.906 |

*** Significant at p-value <0.05.**
**$X^2_{FFH}$: Fisher-Freeman-Halton exact test**

There was no statistically significant difference between the three NAFLD subgroups regarding the number of patients with positive H. pylori Ag in the stool or the number of patients with positive H pylori Ab (p: 0.906 and 0.901, respectively). Laboratory parameters

among NAFLD sub-groups showed that group III had significantly higher values for TG and ALT (p: 0.009 and < 0.001, respectively). No statistically significant difference was found for other parameters. There was a statistically significant positive correlation between the severity of steatosis by US (grading) and BMI, ALT, and AST levels, with a non-significant correlation with other parameters).

**Table 6. Correlation of NAFLD grade (by US) with various parameters.**

| | NAFLD grading | |
|---|---|---|
| | **r** | **p** |
| **Age** | 0.128 | 0.329 |
| **BMI** | 0.628 | <0.001* |
| **WHR** | 0.172 | 0.190 |
| **Insulin level** | 0.038 | 0.770 |
| **FBG** | 0.200 | 0.125 |
| **HBA1c** | -0.067 | 0.609 |
| **HOMA-IR** | 0.053 | 0.686 |
| **Cholesterol** | 0.027 | 0.840 |
| **TG** | 0.093 | 0.478 |
| **HDL-C** | 0.069 | 0.602 |
| **LDL-C** | 0.169 | 0.198 |
| **H. pylori Ab** | 0.128 | 0.438 |
| **ALT** | 0.647 | <0.001* |
| **AST** | 0.704 | <0.001* |
| **PT** | -0.034 | 0.797 |
| **INR** | -0.113 | 0.392 |
| **S. albumin** | -0.104 | 0.431 |
| **T. bilirubin** | -0.185 | 0.157 |
| **SBP** | 0.191 | 0.143 |
| **DBP** | 0.073 | 0.581 |
| **Frequency of fast-food meals per week** | -0.170 | 0.193 |
| **Daily vegetable and fruit portion** | -0.011 | 0.934 |

*** Significant at p-value <0.05.**

****r: coefficient of Spearman's rank-order correlation. It indicates a direct correlation if positive (increased measurement with increased NAFLD grading) and an inverse correlation if negative (decreased measurement with increased NAFLD grading). Strength of correlation: r<0.3 is weak, r>0.3 and <0.7 is moderate, and r>0.7 is strong (regardless of the sign).**

## Discussion

In the present study, no significant differences were observed between the NAFLD and control groups concerning age, sex, or smoking status (p=0.060, 0.464, and 0.055, respectively) nor among NAFLD subgroups. The control group showed significantly higher values regarding physical activity (p=0.039). To ensure good health, some studies recommend that adults have at least 150 to 300 minutes per week of moderate-intensity exercise, 75 to 150 minutes per week of vigorous-intensity aerobic physical activity, or a combination of both. [21]The frequency of fast-food meals was statistically significantly higher in the NAFLD group. At the same time, the daily vegetable and fruit portion per week was statistically lower in the NAFLD group compared to the control. This was evaluated by asking about the daily intake of portions of vegetables and fruit per day and the frequency of fast-food meals per week for each group.

The most common clinical symptoms in the patient group were heartburn (76.7%), epigastric pain (75.0%), and reflux (70.0%). ***Werdmuller et al.*** [22] Almobarak et al. found similar results concerning gastrointestinal symptoms in H. pylori patients. Our study found a positive correlation between higher BMI, WHR, and the incidence of NAFLD**.** [23]***Okushin et al.*** found that BMI is linked to NAFLD, with a significant relationship ***(p= 0.05).*** [24] They concluded that BMI was linked to NAFLD (their study included 13,737 participants).

The NAFLD group exhibited higher mean SBP and DBP than the control group. Additionally, there was a notable discrepancy between NAFLD subgroups in terms of hypertension (HTN) prevalence, with an increasing incidence correlating with higher NAFLD grading. These results are consistent with those reported in a study conducted by ***Pardhe et al.*** [25]. Our study revealed a significant difference between the NAFLD and control groups regarding $HbA_{1c}$ and FBG levels. While HOMA-IR and fasting insulin values were elevated in NAFLD patients compared to controls, this disparity did not reach statistical significance. Other studies corroborated the observation of elevated HbA1c among NAFLD patients without diabetes mellitus. [26].

***Tanaka et al.*** [27] illustrated that the prevalence of NAFLD rose with glycemia, reaching an $HbA_{1c}$ level of 8.0%. However, our study observed no significant difference between the two groups concerning insulin levels and HOMA-IR. In contrast, in their research, ***Novakovic et al.*** [28] identified an essential difference between the NAFLD group and controls regarding fasting insulin levels and HOMA-IR. Additionally, he noted a statistically significant difference between both groups concerning fasting insulin levels. Our study found that serum

cholesterol and LDL-C were significantly higher in the NAFLD group (p= 0.007 and 0.001, respectively). At the same time, TG and HDL-C were not statistically different (p= 0.064 and 0.631, respectively). ***Cuenza et al.*** [29] found that mean LDL-C and cholesterol were higher in patients with NAFLD subjects. ***Pardhe et al. [25]*** found significant differences between the NAFLD group and the controls regarding serum cholesterol, TG, LDL-C, and HDL-C. Serum albumin had a significantly higher value in the control group than in the NAFLD group. Still, the two groups had no significant difference regarding bilirubin levels, PT, and INR.

ALT and AST were significantly higher in NAFLD patients than in the control group. Also, there was a significant difference between NAFLD subgroups as regards both ALT and TG ($p < 0.001$ and 0.009, respectively), with no significant differences regarding other laboratory parameters. ***Paschos et al*** **[30]** found that elevated serum AST and ALT values were the primary laboratory abnormalities in NAFLD patients. They also noticed that ALT levels are higher than AST levels. Elevated liver enzyme levels, particularly ALT, may serve as predictors for diabetes and NAFLD. [31].

H. pylori infection may contribute to the development of NAFLD. Our study observed that the NAFLD group had a significantly higher prevalence of H. pylori infection ($p<0.001$). However, a cross-sectional study conducted in Japan found no association between H. pylori infection and NAFLD. Notably, this study included asymptomatic individuals and utilized ultrasonography to define NAFLD [24].

A meta-analysis (which included over 80.000 middle-aged individuals of Asian descent) concluded that H. pylori infection is linked to a slightly elevated risk of both existing and new cases of NAFLD. [32]. The findings above were reaffirmed in another meta-analysis. [33]. In a cross-sectional study conducted in Japan, a notable association was found between H. pylori seropositivity and metabolic syndrome. Subsequent regression analysis unveiled a significant relationship between H. pylori seropositivity and elevated LDL cholesterol, decreased HDL cholesterol, and higher systolic blood pressure levels. [34].

A study found no association between H. pylori infection and NAFLD. [35]. The defined H. pylori infection is based on the positivity of both H. pylori Abs and H. pylori Ag in stool. In addition, the patient and control group had no gastrointestinal symptoms; in contrast to our cohort, the patient group had gastrointestinal symptoms. ***Baeg et al.*** **[26],** found that H. pylori infection is not a risk factor for NAFLD. NAFLD was diagnosed if hepatic steatosis index >

36 or ALD liver fat score > -0.640). There was no statistically significant difference among NAFLD subgroups regarding the number of patients with positive H pylori Ag in the stool or the number of patients with positive H pylori Abs. (p: 0.906 and 0.901, respectively). The study revealed a positive correlation between NAFLD severity and elevated BMI, ALT, and AST levels.

**Study limitations**

Further studies involving larger cohorts of patients and longer durations are necessary to establish the potential connections between NAFLD, H. pylori infection, dyslipidemia, and IR in NAFLD patients.

## Conclusion

In the current study, we observed that obese patients with NAFLD had a significantly higher prevalence of positive H. pylori Abs and a substantially lower prevalence of positive H. pylori antigen in stool compared to the control group. Additionally, they exhibited higher levels of $HbA_{1c}$ and HOMA-IR compared to the control group, along with a higher incidence of dyslipidemia, which suggests a potential link between dyslipidemia and NAFLD. Furthermore, the severity of steatosis detected by ultrasound was significantly and positively correlated with age, BMI, ALT, and AST levels.

## Footnotes.

Ahmed Fathy (Assistant professor of internal medicine, gastroenterology, and hepatology unit) and Sara Salem (lecturer of internal medicine, gastroenterology, and hepatology unit) were the peer reviewers.

**E- Editor:** Salem Youssef Mohamed, Osama Ahmed Khalil, Amany Mohammed.



Ethical considerations

Each patient's file was assigned a unique code number containing all investigations, ensuring the privacy of patient data. Before study inclusion, all subjects had written informed consent. The local ethics commission approved the study (approval code: 33913/6/29).

Consent for publication: the patients in this research gave written informed permission to publish the data in this study.

**Ethical approval:** All procedures involving human participants followed the institutional and national research committee's moral standards, the 1964 Helsinki Declaration, and its later amendments or comparable ethical standards. All authors declare that consent was obtained from the patient (or other approved parties) to publish this study.

**Data and materials availability:** The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

**Competing interests**: The authors declare that they have no competing interests.

**Funding**: This study had no funding from any resource.

This work was done according to the **STROBE** guidelines.

**Authors' contributions**

All listed authors have contributed substantially, directly, and intellectually to the work and have approved its publication.

**References**

1. Calzadilla Bertot L, Adams LA. The natural course of non-alcoholic fatty liver disease. International journal of molecular sciences. 2016;17(5):774.
2. Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. The Lancet gastroenterology & hepatology. 2022;7(9):851-61.
3. Harrison SA, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. The American journal of gastroenterology. 2003;98(9):2042-7.
4. Caldwell SH, Crespo DM. The spectrum expanded: cryptogenic cirrhosis and the natural history of non-alcoholic fatty liver diseasePowell EE, Cooksley WGE, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years [Hepatology 1990; 11: 74–80]. Journal of Hepatology. 2004;40(4):578-84.
5. Collaborators GO. Health effects of overweight and obesity in 195 countries over 25 years. New England journal of medicine. 2017;377(1):13-27.

6. Stefan N, Cusi K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. The lancet Diabetes & endocrinology. 2022;10(4):284-96.
7. Polyzos SA, Kountouras J, Zavos C, Deretzi G. The association between Helicobacter pylori infection and insulin resistance: a systematic review. Helicobacter. 2011;16(2):79-88.
8. Lehours P. Actual diagnosis of Helicobacter pylori infection. Minerva gastroenterologica e dietologica. 2018;64(3):267-79.
9. Perez-Perez GI, Rothenbacher D, Brenner H. Epidemiology of Helicobacter pylori infection. Helicobacter. 2004;9:1-6.
10. Taylor N, Fox J, Yan L. In-vitro hepatotoxic factor in Helicobacter hepaticus, H. pylori, and other Helicobacter species. Journal of medical microbiology. 1995;42(1):48-52.
11. Fukuda Y, Bamba H, Okui M, et al. Helicobacter pylori infection increases mucosal permeability of the stomach and intestine. Digestion. 2001;63(Suppl. 1):93-6.
12. Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology. 2002;123(3):745-50.
13. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328-57.
14. Marchesini G, Day CP, Dufour J-F, et al. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Journal of Hepatology. 2016;64(6):1388-402.
15. WHOP Status. The use and interpretation of anthropometry. WHO technical report series. 1995;854(9).
16. World Health Organization. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008. 2011.
17. Ribeiro AC, Sávio KEO, Rodrigues MdLCF, Costa THMd, Schmitz BdAS. Validation of a food frequency questionnaire for the adult population. Revista de Nutrição. 2006;19(5):553-62.
18. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. The American journal of clinical nutrition. 1982;36(5):936-42.
19. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412-9.
20. Dawson B, Trapp R. Basic and clinical biostatistics 3rd ed. In lange medical books: Oxford, London and Boston; 2001.
21. Piercy KL, Troiano RP, Ballard RM, et al. The physical activity guidelines for Americans. Jama. 2018;320(19):2020-8.
22. Werdmuller BF, Putten TBVD, Balk TG, Lamers CB, Loffeld RJ. Clinical presentation of Helicobacter pylori-positive and-negative functional dyspepsia. Journal of gastroenterology and hepatology. 2000;15(5):498-502.
23. Almobarak AO, Barakat S, Khalifa MH, et al. Non alcoholic fatty liver disease (NAFLD) in a Sudanese population: What is the prevalence and risk factors? Arab journal of gastroenterology. 2014;15(1):12-5.
24. Okushin K, Takahashi Y, Yamamichi N, et al. Helicobacter pylori infection is not associated with fatty liver disease including non-alcoholic fatty liver disease: a large-scale cross-sectional study in Japan. BMC gastroenterology. 2015;15(1):1-10.

25. Pardhe BD, Shakya S, Bhetwal A, et al. Metabolic syndrome and biochemical changes among non-alcoholic fatty liver disease patients attending a tertiary care hospital of Nepal. BMC gastroenterology. 2018;18:1-8.
26. Baeg MK, Yoon SK, Ko S-H, et al. Helicobacter pylori infection is not associated with nonalcoholic fatty liver disease. World J Gastroenterol. 2016;22(8):2592.
27. Tanaka K, Takahashi H, Hyogo H, et al. Epidemiological survey of hemoglobin A1c and liver fibrosis in a general population with non-alcoholic fatty liver disease. Hepatology Research. 2019;49(3):296-303.
28. Novakovic T, Mekic M, Smilic L, et al. Anthropometric and biochemical characteristics of patients with nonalcoholic fatty liver diagnosed by non-invasive diagnostic methods. Medical Archives. 2014;68(1):22-6.
29. Cuenza LR, Razon TLJ, Dayrit JC. Correlation between severity of ultrasonographic nonalcoholic fatty liver disease and cardiometabolic risk among Filipino wellness patients. Journal of cardiovascular and thoracic research. 2017;9(2):85.
30. Paschos P, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. Hippokratia. 2009;13(1):9.
31. Wong CA, Araneta MRG, Barrett-Connor E, et al. Probable NAFLD, by ALT levels, and diabetes among Filipino-American Women. Diabetes research and clinical practice. 2008;79(1):133-40.
32. Mantovani A, Turino T, Altomari A, et al. Association between Helicobacter pylori infection and risk of nonalcoholic fatty liver disease: an updated meta-analysis. Metabolism. 2019;96:56-65.
33. Wijarnpreecha K, Thongprayoon C, Panjawatanan P, et al. Helicobacter pylori and risk of nonalcoholic fatty liver disease: a systematic review and meta-analysis. Journal of clinical gastroenterology. 2018;52(5):386-91.
34. Gunji T, Matsuhashi N, Sato H, et al. Helicobacter pylori infection is significantly associated with metabolic syndrome in the Japanese population. Official journal of the American College of Gastroenterology| ACG. 2008;103(12):3005-10.
35. Mohammadifard M, Saremi Z, Rastgoo M, Akbari E. Relevance between helicobacter pylori infection and non-alcoholic fatty liver disease in Birjand, Iran. Journal of medicine and life. 2019;12(2):168.