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Original Article

Internal Medicine Section

Helicobacter pylori Infection in Patients with Non Alcoholic Fatty Liver Disease: A Cross-sectional Study

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ABSTRACT

Introduction: *Helicobacter pylori* (*H. pylori*) infection is postulated to predispose Non Alcoholic Fatty Liver Disease (NAFLD) through alteration of lipid profile, reduction of adiponectin, insulin resistance, etc. In the setting of increased incidence of NAFLD, the possible therapeutic and preventive implications of an association of *H. pylori* infection with NAFLD holds interest.

Aim: To determine the prevalence of *H. pylori* infection among NAFLD patients and its association with severity of NAFLD.

Materials and Methods: This hospital-based cross-sectional study was conducted in a tertiary care hospital in Imphal, Manipur, India, during July 2019 to December 2021 among 197 patients diagnosed with NAFLD. *H. pylori* infection was diagnosed by *H. pylori* specific anti-Immunoglobulin M (IgM) and anti-IgG antibody tests. Descriptive statistics like proportions, mean and Standard Deviation (SD) were used. Chi-square test was used to check for associations of disease severity with *H. pylori* infection.

Results: The study population included 90 females and 107 males. A total of 121 (61.4%) subjects had grade 1 fatty liver, while 46 (23.4%) had grade 2 fatty liver and 30 (15.2%) had grade 3 fatty liver. A total of 125 (63.45%) were *H. pylori* IgG positive. *H. pylori* infection positively associated with disease grade, Chronic Liver Disease (CLD) and decompensation (p-value <0.001).

Conclusion: The prevalence of *H. pylori* infection in patients with NAFLD was 63.45% and *H. pylori* seropositivity was significantly associated with disease severity.

Keywords: Chronic liver disease, Disease severity, Prevalence

INTRODUCTION

More than half population of the world is infected with *H. pylori* with the incidence being higher in developing countries compared to developed countries [1]. *H. pylori* cause alteration of lipid profile, reduction of adiponectin etc., but the most important is thought to be insulin resistance in predisposing to NAFLD [2]. Insulin resistance causes hyperinsulinaemia which in turn causes deposition of free fatty acids in liver, thereby causing steatosis by decreasing mitochondrial beta oxidation of fatty acids [2]. Studies have shown that patients with NAFLD were detected with increased anti-*H. pylori* IgG, insulin, Homeostatic Model of Assessment Insulin Resistance (HOMA-IR), Tumour Necrosis Factor (TNF)-alpha, and lesser total and high molecular weight adiponectin [3].

In the past two decades, NAFLD has become the most common liver disorder in western countries, affecting up to 25-30% individuals [4,5]. NAFLD is now regarded as the liver manifestation of metabolic syndrome and is strongly associated with obesity, cardiovascular diseases, diabetes and dyslipidaemia, because they all have a common denominator which is insulin resistance [6-8]. *H. pylori* infection is considered to be a major factor in increasing insulin resistance and subsequently leading to NAFLD [9]. Therefore, *H. pylori* infection among NAFLD needs to be investigated further to develop a novel approach towards management and prevention of NAFLD.

The association of NAFLD with abnormal intestinal flora, especially with H. pylori has been reported in various studies [10-12]. Another study reported about the relationship between NAFLD and intestinal microbes [13]. In several studies of "gut-liver axis", it has been mentioned about further association of several liver diseases including NAFLD with gastrointestinal microorganisms [14].

In the setting of increased incidence of NAFLD and the possible therapeutic and preventive implications of association of *H. pylori* infection with NAFLD, this study was undertaken to determine the prevalence of *H. pylori* infection in patients with NAFLD and its association with disease severity.

MATERIALS AND METHODS

It was a hospital-based cross-sectional study conducted in a tertiary hospital in Imphal, Manipur, India, during July 2019 and December 2021 after obtaining approval from Research Ethics Board, RIMS (Ref No. A/206/REB-Comm(SP)/RIMS/2015/557/35/2019).

Inclusion criteria: Patients older than 18 years, diagnosed as Non Alcoholic Fatty Liver (NAFL), Non Alcoholic Steatohepatitis (NASH) and NAFLD related Chronic Liver Disease (CLD) were recruited consecutively as study participants from the Medicine Outpatient Department (OPD) and wards. The diagnosis of NAFLD was based on abdominal ultrasonography.

Exclusion criteria: Exclusion criteria were patients positive for Hepatitis B surface Antigen (HBs Ag), Hepatitis C virus (HCV) antibody and other viral, autoimmune, or toxin induced liver disease and genetic liver diseases like Wilsons disease and haemochromatosis, history of gastrectomy and significant alcohol intake (>30 g/day in males, >20 g/day in females).

Sample size calculation: The required sample size of 197 was calculated based on 41% prevalence of *H. pylori* infection among patients of NAFLD with 7% absolute error and 5% significance [15].

Study Procedure

Helicobacter pylori infection was diagnosed by *H. pylori* specific anti-IgM and anti-IgG antibody tests using Enzyme-linked Immunosorbent Assay (ELISA) kits. Biochemical and haematological investigations like liver function tests, lipid profile and blood sugar level were also evaluated. Hypertension was defined as blood pressure of >140/90 mmHg or patient was already on antihypertensive medication. Diabetes was defined as fasting blood sugar level more than 126 mg/dL or patient was already on antidiabetic medication. Dyslipidaemia was defined as Low-density Lipoprotein (LDL)

cholesterol level of >160 mg/dL or non High-density Lipoprotein (HDL) cholesterol level >190 mg/dL.

Ultrasonographic grading of fatty liver was done as: Grade 1-Increased hepatic echogenicity with visible periportal and diaphragmatic echogenicity, Grade 2-Increased hepatic echogenicity with imperceptible periportal echogenicity, without obscuration of diaphragm and Grade 3-Increased hepatic echogenicity with imperceptible periportal echogenicity and obscuration of diaphragm [16].

The CLD was defined as a progressive deterioration of liver functions for more than six months. Decompensated cirrhosis was defined as 'an acute deterioration in liver function in a patient with cirrhosis and characterised by jaundice, ascites, hepatic encephalopathy, hepatorenal syndrome or variceal haemorrhage'.

Due to the Coronavirus Disease-2019 (COVID-19) pandemic at the time of the data collection, endoscopy and biopsy (routine invasive maneuverers) were not done. So, the results were based on serum test.

STATISTICAL ANALYSIS

The data was tabulated in Microsoft excel and statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) software version 21.0. Descriptive statistics like mean, Standard Deviation (SD) and proportions were used. Chi-square test was used to check for associations of disease severity with *H. pylori* infection. The p-value less than 0.05 was taken as significant.

RESULTS

A total of 197 NAFLD patients were included in the study. The most common presenting feature was fatigue 108 (54.8%), followed by epigastric discomfort 106 (53.8%). The characteristics of the participants are shown in [Table/Fig-1]. Half of the participants 98 (49.7%) belonged to 41-60 years and 90 (45.7%) were females. Total 30 (15.2%) were in a stage of CLD and 27 (13.7%) had some form of decompensation. IgG seropositivity was detected in 125 participants, thus giving a prevalence of 63.45%. The mean levels of biochemical and haematological parameters are given in [Table/Fig-2].

Characteristic	No. of patients, n (%)
Age in years	
18-40	56 (28.4)
41-60	98 (49.7)
>60	43 (21.8)
Gender	
Male	107 (54.3)
Female	90 (45.7)
Body mass index (kg/m^2)	
Underweight (<18.5 kg/m^2)	16 (8.1)
Normal (18.5-24.9 kg/m^2)	99 (50.3)
Overweight (25-29.9 kg/m^2)	64 (32.5)
Obese (>30 kg/m^2)	18 (9.1)
Co-morbidities	
Dyslipidaemia	82 (41.6)
Hypertension	71 (36)
Diabetes	95 (48.2)
Grade of fatty liver [16]	
1	121 (61.4)
2	46 (23.4)
3	30 (15.2)
Chronic liver disease (CLD)	
Yes	30 (15.2)
No	167 (84.8)
Decompensation	
Yes	27 (13.7)
No	170 (86.3)
Helicobacter pylori	
IgG positive	125 (63.5)
IgG negative	72 (36.5)

[Table/Fig-1]: Characteristics of the participants (N=197).
IgG: Immunoglobulin G

Parameters	Mean±Standard deviation
Haemoglobin (g/dL)	11.70±0.09
Total leucocyte count (per microliter)	7005.11±175.47
Erythrocyte sendimentation rate (mm/hour)	35.23±1.85
Total bilirubin (mg/dL)	1.61±0.09
SGOT (IU/L)	67.92±3.92
SGPT (IU/L)	71.35±4.02
Albumin (g/dL)	3.51±0.03
Alkaline phosphate (IU/L)	147.74±2.65
GGT (U/L)	51.34±1.97
Random blood sugar (mg/dL)	160.32±4.53
Total cholesterol (mg/dL)	211.80±4.17
Triglycerides (mg/dL)	94.04±2.57
Low density lipoprotein (mg/dL)	156.79±4.06
High density lipoprotein (mg/dL)	37.46±3.09

[Table/Fig-2]: Biochemical parameters of the participants (N=197).
SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; GGT: Gamma-glutamyl transferase

The [Table/Fig-3] shows the association of *H. pylori* infection with fatty liver grading, chronicity and decompensation. *H. pylori* seropositivity was found to be positively associated with the grade of fatty liver, CLD and presence of decompensation and all were statistically significant.

Variables		*Helicobacter pylori*		p-value
		Negative	Positive	
Grade of fatty liver	Grade 1	60 (49.59%)	61 (50.41%)	0.0001
	Grade 2	8 (17.40%)	38 (82.60%)	
	Grade 3	4 (13.33%)	26 (86.67%)	
Chronic liver disease (CLD)	Yes	3 (10.00%)	27 (90.00%)	0.001
	No	69 (41.32%)	98 (58.68%)	
Decompensation	Yes	2 (7.41%)	25 (92.59%)	0.0001
	No	70 (41.18%)	100 (58.82%)	

[Table/Fig-3]: Association of *Helicobacter pylori* infection with grade of fatty liver, chronicity and decompensation (N=197).

DISCUSSION

Out of the 197 NAFLD patients enrolled in the study, 125 (63.45%) were *H. pylori* IgG positive. This finding is similar to the study by Abooali E and Salehi M, who also found a 63% prevalence of *H. pylori* infection in patients with NAFLD in Iran [17]. Khalil AE et al., and Mahbool MS et al., found a slightly higher prevalence of 70% and 67% respectively [18,19]. Thus, the prevalence of *H. pylori* infection in patients with NAFLD is high.

Out of the 121 patients with Grade 1 fatty liver, 61 (50.41%) were *H. pylori* positive, out of the 46 patients with Grade 2 fatty liver 38 (82.6%) were positive for *H. pylori*, out of the 30 patients with Grade 3 fatty liver 26 (86.67%) were *H. pylori* positive. Thus, a higher seropositivity rate was seen with higher grades of fatty liver. This is consistent with the findings of a previous study, where the *H. pylori* infection rate was significantly higher in patients with severe NAFLD [20]. A study conducted by Sumida Y et al., suggested that *H. pylori* infection may represent a contributing factor in the progression from

NAFLD to NASH [21]. This is also in line with the study conducted by Abo-Amer YEE et al., where *H. pylori* infection correlated with increased degree of steatosis [22]. This important observation that *H. pylori* infection in NAFLD speeds up the steatosis process may be due to the fact that *H. pylori* infection with altered so-called "Gut-liver" axis contributes to insulin resistance and altered lipid metabolism in liver. *H. pylori* seropositivity was also associated with CLD. Out of 27 decompensated patients, 23 (85.19%) were positive for *H. pylori* in comparison to 170 non decompensated patient where only 98 (57.65%) were positive for *H. pylori*. This suggests that NAFLD patients with *H. pylori* infection had a higher risk of progression to cirrhosis and decompensation. A possible explanation for this observation is the increased insulin resistance in these patients leading to more steatosis and hepatocyte damage which causes hepatocyte inflammation, death and later regeneration with excessive stimulation of stellate cell influenced fibrosis which ultimately leads to cirrhosis. However, this requires further intensive research and prospective studies, which may lead to *H. pylori* eradication being an effective method in delaying the progression of NAFLD to NASH/cirrhosis.

The strength of the present study is that, to the best of our knowledge, it is first study on *H. pylori* and NAFLD from North-East region of India.

Limitation(s)

The limitation of ultrasonography to determine the precise extent hepatocyte ballooning and severity of NAFLD is the major limitation of present study and further studies using liver biopsy or newer imaging modalities like Magnetic Resonance Imaging-Proton Density Fat Fraction (MRI-PDFF) or fibroscan for diagnosing and determining the severity of NAFLD can be conducted.

CONCLUSION(S)

The prevalence of *H. pylori* infection in patients with NAFLD is high with six out of every ten NAFLD patient positive for *H. pylori* infection. Also, *H. pylori* infection was significantly associated with higher grade of fatty liver, chronicity and decompensation of liver disease suggesting a potential role of *H. pylori* in disease progression.

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