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A Study of Helicobacter Pylori Infection in Patients with Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Background: Helicobacter pylori (H. Pylori) infection has been notified to enhance the progression of distinct extra-digestive manifestations, embraced type 2 diabetes, liver, and cardiovascular diseases. Currently, the association between H. pylori infection and nonalcoholic fatty liver disease (NAFLD) was suggested. Conversely, evidence from various studies was doubtful.

Aim of the study: This study was performed to assess the prevalence of H. Pylori infection and the possible predictors of H. Pylori infection among NAFLD patients.

Patients and Methods: This prospective observational case-control study included a total of 60 patients with NAFLD and 20 apparently healthy subjects served as control, attending at the Outpatient Clinic and Internal Medicine Department, Sayed Galal; Al-Azhar University Hospitals. This study was conducted between February 2018 to March 2019. All participants were submitted to full history taking, complete physical examination, and routine laboratory investigations. H. pylori Ag in stool test was tested to reveal the existence of infection along with abdominal ultrasonography using Hamaguchi scoring system for assessment of NAFLD.

Results: An overall 80 participants were included in the current investigation. Based on the presence of NAFLD, participants were assorted into healthy control group (20 candidates) and NAFLD group (60 patients) with a mean age of 35.65±10.73 and 39.72±11.14, respectively. An overall 48 patients were infected with H. Pylori. Of them, 6 (30%) were among the healthy control group, whereby 42 (70%) were among NAFLD group (P<0.001). Based on the multivariate logistic regression model, the male gender and the levels of high density lipo-proteins (HDL-C) were statistically significant predictors of H. Pylori infection among NAFLD patients.

Conclusions: The burden of H. Pylori infection among NAFLD patients was noticeably high. Additionally, obese males with decreased levels of HDL-C were more expected to be H. Pylori positive infection.

Keywords: H. Pylori, Nonalcoholic fatty-liver disease, High density lipoprotein cholesterol.

INTRODUCTION

Helicobacter pylori (H. pylori) infection attributes to a various array of gastric diseases**,** principally peptic ulcer, chronic gastritis**,** and gastric carcinoma **(1).** Throughout the recent era, the evidence has implicated H. pylori infection in extra-gastrointestinal disorders likewise neurological disorders, cardiovascular diseases**,** and metabolic disturbances **(1).** H. pylori colonize the stomach of approximately half the world's population and are pivotal components of the human microbiome **(2).** Infection is usually acquired early in life and persists throughout the host's life if untreated **(3).** H. pylori infection is not only affecting the stomach**,** but also is linked to a numerous extra-gastric disease**,** indicating that H. pylori may cause disease by a different biologic process far from the initial site of infection **(4).** Some studies showed the relationship between H. pylori infection and T2DM and metabolic syndrome **(5,6,7,8,9).** Non-alcoholic fatty liver disease (NAFLD) is the condition, whereby excessive fat infiltrate liver in the obscurity of considerable alcohol intake or secondary causes for steatosis **(10).** The clinical consequence of NAFLD is not limited to liver related mortality and morbidity but is also related to cardiovascular impairment**,** T2DM and metabolic syndrome **(11).** The epidemics of NAFLD are rapidly increasing**,** with huge clinical and economic burdens **(12).** Therefore, identifying the potential predictors along with therapeutic implications is a crucial factor in the management of NAFLD**.** The occurrence of NAFLD is a sophisticated process which comprises genetic susceptibility along with environmental factors **(13).** H. pylori infection may be the cornerstone in the development of NAFLD or even it may be a possible cause for NAFLD**.**

The present study was conducted to reveal the burden of H. Pylori infection and the possible predictors of H. Pylori infection among NAFLD patients.

PATIENTS AND METHODS

This prospective observational case-control study included a total of 60 patients with NAFLD, which established clinically and confirmed radiologically, and 20 apparently healthy subjects served as control, attending at the Outpatient Clinic and Internal Medicine Department, Sayed Galal; Al-Azhar University Hospitals. This study was conducted between February 2018 to March 2019.

Ethical approval

The current investigation was executed with respecting the guidelines of the Ethical Research Board of the Faculty of Medicine, Al-Azhar

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University, Cairo coupled with hospital ethics committee roles. Written consents were gained from all participants before study execution after clear illustration of all study steps. Subsequently, all clinical procedures were executed based on the roles of the Declaration of Helsinki.

Inclusion Criteria

Patients with NAFLD, which established clinically and confirmed radiologically regardless their sex or age. Besides, participants without NAFLD were also included as a control healthy group.

Exclusion Criteria

Patients with history of malignancy, viral hepatitis, autoimmune hepatitis or other genre of chronic liver diseases (also advanced liver diseases for any causes), Patients with history of alcohol consumption, Patients on drugs which lead to hepatic steatosis (as estrogens, corticosteroids, amiodarone and valproate; currently or within the last 2 years), Patients on other chronic diseases e.g., DM, Obesity with BMI≥ 40 kg/m^2, SLE, etc. were excluded.

Clinical assessment

All participated subjects were subordinated to meticulous history taking with special emphasis on age, sex, history of alcohol and drug use. Subsequently, clinical examination was done encompass complete physical examination with special emphasis on general examination including height, weight, body mass index (BMI) calculation and abdominal examinations.

Laboratory assessment

Laboratory investigations including CBC, serum creatinine, fasting plasma glucose (FPG), aspartate transaminase (AST), Gamma glutamyl transferase (GGT), alanine transaminase (ALT), prothrombin time (PT) & INR, serum albumin, activated Partial Thromboplastin Time (APTT), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), serum bilirubin (Total and direct), and viral markers were done. Test for H. pylori antigen in stools was implemented for appreciation of H. pylori infection.

Radiological evaluation

Abdominal ultrasonography for assessment of NAFLD was implemented using Hamaguchi scoring system [(14)].

Statistical analysis

Continuous normally distributed variables were explicated using mean, and standard deviation (SD), and its groups were contrasted using student t-test. Categorical variables were expressed using number, and percentage and its confidential groups were compared using Pearson's chi-square test with Fisher's exact test. Furthermore, correlation analysis was conducted based on Spearman's rank correlation coefficient for categorical variables. Multi-variate logistic regression model was accomplished to elucidate the independent predictors of H. Pylori infection among the NAFLD patients using t-test analysis. The significance was elucidated based on two tails *P-value* < 0.05. Statistical analysis was executed using SPSS software version 23 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients demographic characteristics

An overall 80 participants were included in the current investigation. Having NAFLD, participants were assorted into healthy control group (20 candidates) and NAFLD case group (60 patients) with a mean age of 35.65 ± 10.73 and 39.72 ± 11.14, respectively. There were 13 (65%) males in the healthy group whereas there were 43 (71.7%) males in the case group. There was a statistically significant difference between both groups (p<0.001) regarding BMI, TC, TG, HDL-C, and LDL-C. **Table.1**

Among NAFLD group, the patients were furtherly categorized regarding the presence of H. Pylori infection into two groups; 18 (30%) patients with negative H. Pylori infection and 42 (70%) patients with positive H. Pylori infection. The mean age was 36.61 ± 10.09 years among positive infection group, whereas it was 13.90 ± 1.07 years among negative infection group. There was a statistically significant difference (p<0.001) between both groups regarding the gender whereas males were the predominant population among NAFLD (81.0%). Similar to that, patients among positive infection group showed statistically significant (p=0.038) higher means of LDL-C, in contrast with negative H. Pylori infection group. Subsequently, the mean values of HDL-C showed a significant difference between both groups (p=0.004), whereby the mean was 40.22 ± 10.72 and 32.02 ± 9.05 mg/dl among the positive and negative H. Pylori infection groups, subsets, respectively. **Table.2**

Table 1. Comparison between the two studied groups regarding demographic data				
		Healthy control group	**NAFLD**	**P-value**
		No. = 20	**No. = 60**	
Age (years)	**Mean±SD**	**35.65 ± 10.73**	**39.72 ± 11.14**	**0.158**
Sex	**Male**	**13 (65.0%)**	**43 (71.7%)**	**0.573**
	Female	**7 (35.0%)**	**17 (28.3%)**	
BMI (kg/m^2)	**Mean±SD**	**24.56 ± 3.53**	**29.44 ± 3.66**	**0.000**
Hemoglobin (g/dl)	**Mean±SD**	**14.24 ± 0.87**	**14.06 ± 1.22**	**0.553**
S.Creatinine (mg/dl)	**Mean±SD**	**0.95 ± 0.04**	**1.06 ± 0.04**	**0.299**
TC (mg/dl)	**Mean±SD**	**174.38 ± 15.41**	**207.76 ± 27.48**	**0.000**
TG (mg/dl)	**Mean±SD**	**125.90 ± 25.26**	**210.32 ± 44.27**	**0.000**
LDL-C (mg/dl)	**Mean±SD**	**99.80 ± 14.51**	**131.22 ± 24.73**	**0.000**
HDL-C(mg/dl)	**Mean±SD**	**49.40 ± 5.22**	**34.48 ± 1.22**	**0.000**
Abbreviations; BMI: Body mass index, **TG:** Triglycerides, **TC:** total cholesterol, **LDL-C:** Low density lipoprotein cholesterol, **HDL-C:** High density lipoprotein cholesterol.				

Table.2 Comparison between the H. Pylori positive and negative groups among NAFLD group regarding demographic data				
		Negative H. pylori	**Positive H. pylori**	**P-value**
		No. = 18	**No. = 42**	
Age (years)	**Mean±SD**	36.61 ± 10.09	41.05 ± 11.41	0.159
Sex	**Male**	9 (50.0%)	34 (81.0%)	0.015
	Female	9 (50.0%)	8(19.0%)	
BMI(kg/m^2)	**Mean±SD**	28.70 ± 3.19	29.75 ± 3.83	1.312
Hemoglobin (g/dl)	**Mean±SD**	14.43 ± 1.49	13.90 ± 1.07	0.127
S.Creatinine (mg/dl)	**Mean±SD**	1.19 ± 0.04	1.00 ± 0.03	0.098
TC (mg/dl)	**Mean±SD**	202.58 ± 25.22	209.99 ± 28.39	0.343
TG(mg/dl)	**Mean±SD**	205.294 ± 44.87	212.19 ± 44.42	0.621
LDL-C (mg/dl)	**Mean±SD**	121.17 ± 23.58	135.52 ± 24.21	0.038
HDL-C (mg/dl)	**Mean±SD**	40.22 ± 10.72	32.02 ± 9.05	0.004
Abbreviations; BMI: Body mass index, **TC:** total cholesterol, **TG:** Triglycerides, **LDL-C:** Low density lipoprotein cholesterol, **HDL-C:** High density lipoprotein cholesterol.				

Prevalence of H. pylori infection

An overall 48 patients were infected with H. Pylori. Of them, 6 (30%) were among the healthy control group, whereby 42 (70%) were among NAFLD group (P<0.001). **Figure.1**

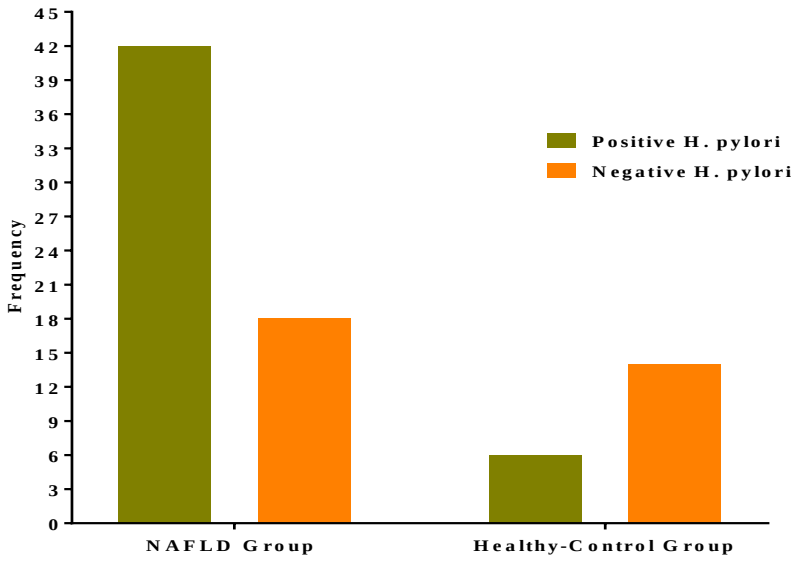

Figure.1 Bar chart showed the pattern of H. Pylori infection among the studied groups.

Correlation and regression analysis

There was a statistically significant positive correlation between H. Pylori infection and patient sex (Males) (r=0.314, p= 0.014), whereby there was a statically significant negative correlation between H. Pylori infection and body weight (r=-0.259, p=0.045). **Table.3**

Table.3 Correlation between the clinical characteristics and the presence of H. Pylori infection among NAFLD patients

Variables	Correlation coefficient	P-Value
Age	-0.18	0.15
Sex (Males)	0.314	0.014
Weight	-0.259	0.045
Height	-0.203	0.11
BMI	-0.158	0.22

BMI; body mass index

Having laboratory variables, the results of the correlation analysis revealed that; the levels of LDL-C (r=-0.279, p=0.031) were negatively correlated with H. Pylori infection. On the contrary, the levels of HDL-C were positively correlated (r=0.35, p= 0.006) with H. Pylori infection. **Table 4**

Table.4 Correlation between the laboratory characteristics and the presence of H. Pylori infection among NAFLD patients

Variables	Correlation coefficient	P-Value
Hemoglobin	0.139	0.28
S. Creatinine	0.18	0.15
Total Cholesterol	-0.118	0.36
Total Glyceride	-0.079	0.54
LDL-C	-0.279	0.031
HDL-C	0.35	0.006
ALT	-0.118	0.36
AST	-0.142	0.27
GGTV	-0.112	0.39
Albumin	-0.062	0.636
Prothrombin Time	0.074	0.5
INR	0.082	0.5
APTT	0.1260	0.33
Total Bilirubin	-0.041	0.75
Direct Bilirubin	0.070	0.59
Prothrombin Concentration	-0.076	0.56

Abbreviations; **LDL-C:** Low density lipoprotein cholesterol, **HDL-C:** High density lipoprotein cholesterol, **AST:** Aspartate transferase, **ALT:** Alanine transferase, **GGT:** Gamma glutamyl transferase. **PT:** Prothrombin Time, **APTT:** activated Partial Thromboplastin Time, **INR:** International Normalized Ratio.

Based on multivariate logistic regression model, the male gender (B=-1.504, p=0.028) and the levels of HDL-C (B=0. 089, P=0.011) were statistically significant potential predictors of H. Pylori infection among NAFLD patients. **Table.5**

Table.5 predictors of H. Pylori infection among NAFLD patients

Variables	B	S.E.	Wald	df	Sig.	OR	95% C.I. for Odds ratio	
							Lower	Upper
Sex	-1.504	0.686	4.804	1	0.028	0.222	0.058	0.853
Weight	-.018	0.030	0.341	1	0.559	0.982	.926	1.043
HDL-C	.0890	0.035	6.489	1	.0110	1.093	1.021	1.170

Abbreviations; **HDL-C**= High density lipoprotein cholesterol

DISCUSSION

Nonalcoholic fatty liver disease (NAFLD) is a progressive liver disorder, whereby its histological spectrum varied from steatosis alone up to nonalcoholic steatohepatitis (NASH). In particular, the latter condition has an increased the risk for progression to liver cirrhosis [14]. NAFLD is a popular metabolic disorder which alters nearly 25% of the adult population worldwide [15]. Even though NAFLD is considerably common in all continents, the highest prevalence rates were notified from the Middle East (32%) and South America (31%), whereby the lowest prevalence was revealed from Africa (14%) [14]. Although its mechanism remains unclear, genetic, environmental and metabolic factors may be attributed to the underlying pathogenesis of NAFLD [14,15].

It was notified that gut dysbiosis is linked with NAFLD; on the other hand, there is some doubtful evidence regarding the nature of these changes. Of note, infection with *Helicobacter* species, principally *H. pylori*, has been related to rise of NAFLD risk [15]. Conversely, some studies have not succeeded to prove this result.

In our study, the burden of H. Pylori infection was considerably high among NAFLD patients. In particular, male overweight patients with low levels of HDL-C were appeared to be more risk to experience NAFLD.

The association between *H. pylori* infection and NAFLD was firstly proposed when *H. pylori 16S rDNA* was discovered throughout liver biopsy from a NAFLD case [16]. Different investigations have revealed additional evidence about *H. pylori* infection and NAFLD [17].

On the contrary, evidence for the relationship between *H. pylori* infection and NAFLD remains constringed [18]. Subsequent to that, the possible mechanisms comprehend a relation between insulin resistance and *H. pylori* infection, inflammation and generation of proinflammatory cytokines, alterations in lipid metabolism, and enhancement of the intestinal permeability [18,19].

Several studies revealed a relation between *H. pylori* infection and NAFLD. In particular, ***Polyzos et al.***showed that *H. pylori* infection was frequently noticed in 28 NAFLD patients relative to 25 healthy controls [20]. In compliance with the current investigation, another investigation of 130 Japanese participants notified that the burden of NAFLD is noticeably higher in *H. pylori*-infected patients in comparison with non-infected candidates [19]. On the other hand, another Korean cross-sectional analysis of 3663 participants found that *H. pylori* are not a possible cause of NAFLD [13].

In order to clarify these conflicting points, it was planned in our study to appreciate the correlation between infection with *H. pylori* and NAFLD.

In our investigation, there was a significant difference between the healthy control group and NAFLD group regarding sex, whereby males were the most frequent gender. In addition, male was associated with more risk to experience H. Pylori infection among NAFLD patients. In this respect, kim ***et al.*** elucidated that male patients were more suseptible to be positive *H. pylori* (53.1% vs 49.5%)[19]. Similar to that, ***Fan et al.***showed that male patients were more vulnerable to be infected with *H. pylori* [11].

As regards lipid profile, the current study showed that; NAFLD patients with positive *H. pylori* had a noticeable rise in LDL-C values when compared to NAFLD patients with negative *H. pylori*. Also, there was a highly significant negative correlation between HDL-C values and positive *H. pylori* patients.

In compliance with our results ***Baeg et al.*** showed that *H. pylori*-positive patients had significantly higher LDL-C, and a lower HDL-C[4]. Similarly, ***Sumida et al.*** revealed higher serum LDL-C levels in patients with *H. pylori* infection relative to those without [21].

On the other hand, some investigations showed that there were no significant differences in serum HDL-C, LDL-C levels between *H. pylori*-seropositive and *H. pylori*-seronegative participants [21,22,23]. Subsequently, ***Kebapcilar et al.***demonstrated that *H. pylori* infection is considerably associated with lower HDL-C albeit they illustrated that the eradication of *H. pylori* had no impact on the lipid profile [18].

In our study, the mean values of TC and TG showed no obvious difference between sero-positive and seronegative H. Pylori patients. On the contrary, ***Baeg et al.***showed that *H. pylori*-positive group had noticeably higher TC concentration [4]. Similar to that, ***Fan et al.*** reported that among 28,171 subjects, there were 10,848 patients were infected with *H. pylori* (38.5%) [11]. This discrepancy might be attributed to the variety in the epidemiological distribution and methods used in the assessment of *H. pylori* infection [21].

The study of ***Fan et al.***revealed the burden rate of NAFLD was significantly increased in patients with *H. pylori* infection in contrast with those with sero-negative *H. pylori* infection in women (23.6% vs. 21.5%, $P < 0.05$), yet not in males (46.5% vs. 45.5%, $P > 0.05$) [11].

Recently, ***Abdel-Razik et al.*** showed that after therapy of *H. pylori* infection, there was a considerable amelioration in lipid profile, leptin, and

proinflammatory cytokines as well as, increasing HDL-C [(1)].

CONCLUSION

The burden of H. Pylori infection among NAFLD patients was noticeably high, in comparison with the healthy control group. Additionally, obese males with low levels of HDL-C were more expected to have H. Pylori infection. However, large randomized control trials are necessary to address the possible limitations of our study.

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