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Comparison of Insulin Resistance between Nonalcoholic Fatty Liver Disease Patients with and those without *Helicobacter pylori* Infection

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Abstract

Aim/background: Insulin resistance [IR] is the key pathophysiological mechanism for nonalcoholic fatty liver disease [NAFLD] and nonalcoholic steatohepatitis [NASH]. Recent studies have disclosed the relationship between *Helicobacter pylori* (*H. pylori*) infection and IR. This study aimed to evaluate the differences in IR between NAFLD patients with and those without *H. pylori* infection.

Patients and method: From January 2011 to December 2012, patients with pathologic proof of NAFLD were included. All patients received a C^{14} urea breath test for *H. pylori* infection evaluation. Serum samples were taken for homeostasis model assessment of insulin resistance [HOMA-IR], lipid profile, inflammatory cytokine and adipokine studies. Patients with *H. pylori* infection received standard triple eradication. Serum samples were then subjected to HOMA-IR three and six months after eradication therapy.

Results: Eleven patients with both NAFLD and *H. pylori* infection and 18 patients with only NAFLD were included into this study. There were no significant differences between the two groups in demographic, laboratory or histological data. The only difference was mean HDL value. Patients with *H. pylori* infection had higher mean HDL values than patients without *H. pylori* infection [50.1 ± 8.8 vs. 38.8 ± 9.7 mg/dL, p=0.004]. The serial values of HOMA-IR from patients with *H. pylori* infection following *H. pylori* eradication 0, 3 or 6 months later did not reveal an increasing or decreasing trend following *H. pylori* eradication treatment.

Conclusion: There were no significant differences in HOMA-IR values between NAFLD patients regardless of whether they had *H. pylori* infection. For patients with NAFLD and *H. pylori* infection, the HOMA-IR value was not affected by *H. pylori* eradication treatment.

Key Words: *Helicobacter pylori*; Insulin resistance; Leptin; Steatohepatitis; Fatty liver

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Introduction

Insulin resistance [IR] is the key pathophysiological mechanism for nonalcoholic fatty liver disease [NAFLD] and nonalcoholic steatohepatitis [NASH] [1-4]. Abnormal adipokine (adiponectin, leptin) [5-7] and inflammatory cytokines [8-10] are the "second hit" for the pathogenesis of NASH. The liver may be exposed to the metabolic hepatotoxic byproducts generated by the gut microbiome, including acetaldehyde, ammonia, and phenols. Gut microbiota may be involved in the pathogenesis of NASH [11]. *Helicobacter pylori* (*H. pylori*) infection induces chronic inflammation in the human stomach. This inflammatory response may induce local gastritis and systemic immune responses. Recent studies have shown evidence for possible relationships between *H. pylori* infection, IR [11-13], leptin [14, 15] and metabolic syndrome [16-18]. Although IR was reported to be related to both *H. pylori* infection and NASH, few studies have investigated the influence of *H. pylori* infection on IR among NASH patients [19-21]. This study aimed to evaluate any difference in homeostasis model assessment of IR [HOMA-IR] values between NAFLD patients with and those without *H. pylori* infection. For patients with NAFLD and *H. pylori* infection, the HOMA-IR values were compared before and after *H. pylori* eradication treatment.

Patients and Method

From January 2011 to December 2012, patients with abnormal serum alanine aminotransferase [ALT] values [more than normal upper limit], abdominal ultrasonographic evidence of fatty liver and ultrasound-guided fine needle liver biopsy results characteristic of NAFLD were included into this study. Exclusion criteria included daily consumption of >10 g alcohol or other compatible volume beer or wine; taking oral hypoglycemia agent; and the presence of chronic inflammation disease such as autoimmune disease or chronic viral hepatitis B or C. A detailed history of alcohol consumption was obtained by two physicians independently and consultation with family members to confirm negligible alcohol consumption [less than 10 g of ethanol per day]. Because exercise, diet control and body weight reduction are important for improving insulin resistance, patients should be asked to take an appropriate exercise and diet control before entering the study. Otherwise, the effect of insulin resistance [HOMA-IR change] could not be attributed to *H pylori* eradication if the patients also had body weight reduction and adequate exercise. In the first 3 months following inclusion into this study, patients were persuaded to participate in frequent [at least twice per week] appropriate exercises and to maintain a low-fat diet. To prevent interferences in IR test, patients who were diagnosed to have a disease of Diabetic mellius and used antidiabetic agents, such as biguanides [metformin], insulin, thiazolindinediones [pioglitazone] etc, were excluded in the study. All patients received a C^{14} urea breath test for *H. pylori* infection evaluation. Serum samples were taken for ALT, cholesterol, triglyceride, fasting glucose/insulin level [for IR evaluation by HOMA-IR], inflammatory cytokines (tumor necrosis factor alfa (TNF-α) and high sensitive C reactive protein (HS-CRP) and adipokine (adiponectin, leptin) studies. Demographic information including age, sex, body weight, body height, and waist circumferences were also collected. Patients with *H. pylori* infection would receive standard triple eradication with esomeprazole 40 mg, clarithromycin 500 mg and amoxicillin 1 g twice daily for a total of 7 days. For a patient with a history of amoxicillin allergy, the eradication regiments would change amoxicillin into metronidazole. The dose of metronidazole was 500 mg twice per day. After eradication therapy, patients received the same serum tests three and six months later. Follow-up UBT was performed three months after *H. pylori* eradication **(Figure 1)**.

Homeostasis Model Assessment of Insulin Resistance [HOMA-IR]

The estimate of IR by HOMA score was calculated by the formula: [fasting plasma insulin [mU/L] × fasting plasma glucose [mmol/L]]/22.5. A high HOMA-IR score denotes low insulin sensitivity and IR [22].

Pathologic criteria for NASH

Liver biopsy specimens were reviewed by an experienced pathologist (Dr. Chang LC) and scored by the NAFLD activity score (NAS: steatosis 0-3, lobular inflammation 0-2, hepatocellular ballooning 0-2, and fibrosis 0-4). The diagnosis of NASH was most possible when the NAS score was more than 5 and the diagnosis was not likely when the NAS score was less than 3 [23].

Immunohistochemical stain [IHC] for *H. pylori* detection

Imunohistochemistry for *H. pylori (*polyclone, Zytomed Systems GmbH) was performed on formalin-fixed paraffin-embedded tissue from hepatic biopsy specimens. A single representative block from each specimen was sectioned at 3 µm onto positively charged slides. Slides were then stained using the Bond-Max autostainer (Leica Biosystems). Briefly this involved slides being dewaxed in Bond Dewax solution [Leica Biosystems] and hydrated in Bond Wash solution (Leica Biosystems). Antigen retrieval was performed at acidic pH using Epitope Retrieval 1 solution [Leica Biosystems] for 10 min at 100 °C. Slides were then incubated with the primary antibody at a concentration of 1:800 for 30 min at room temperature. The detection kit used was the Bond Polymer Refine Detection kit [DS9800]. The protocol included incubation with post primary antibodies for 8 min, polymerization for 8 min, DAB for 5 min and Haematoxylin for 5 min.

Statistical analysis

The paired-t test was used for comparison of continuous data if the sample size was greater than 30 in each group. However, the Mann-Whitney test was used when the sample size was less than 30. Categorical data were analyzed with the Chi-square test or Fisher's exact test, where appropriate. All statistical tests were 2-tailed. $P<0.05$ was considered to represent statistical significance. Statistical analyses were performed using the statistical package SPSS for window [Version 14.0, Chicago, IL, USA].

This research was approved by the Institutional Review Board of the Chang-Gung Memorial Hospital IRB No 99-3075C.

Results

Initially 451 patients were enrolled and 44 patients agreed to undergo echo-guided fine needle liver biopsy. Among the 44 patients, NAFLD was diagnosed in 35 patients based on pathologic NAS score and clinical presentation. Six patients were excluded because of taking oral hypoglycemia agents (2 patients) or loss to follow-up (4 patients). Finally, 29 patients (11 patients with *H. pylori* infection) were included into this study. Demographic data are presented in **Table 1**. Most patients were centrally obese with waist circumference > 90 cm, had insulin resistance (HOMA-IR value >2.5), body mass index [BMI] ≥ 27 kg/m^2 [obesity criteria for Asians] and hypertriglyceridemia (>150 mg/dL). The diagnosis of NASH was made in 10 patients [34.5%, 10/29] with NAS score more than 5. There were no significant differences in age, gender distribution, body weight, and body height or body mass index between the two groups. Laboratory data including fasting plasma glucose, insulin, HOMA-IR, lipid profile, adipokine, inflammatory cytokine, and liver biochemistry and histological findings are presented in **Tables 2 and 3**. HDL value was the only variable that differed significantly between the two groups. The mean HDL value in patients with *H. pylori* infection was higher than that in patients without *H. pylori* infection [50.1 ± 8.8 vs. 38.8 ± 9.7 mg/dL, p=0.004]. Multivariate linear regression analysis by backward selection revealed waist circumference and triglyceride value, but not *H. pylori* infection status were the predictors for HOMR-IR value (**Table 4**). Analysis from the serial values of HOMA-IR from patients 0, 3 or 6 months after eradication therapy revealed no increasing or decreasing trend of HOMA-IR values following *H. pylori* eradication treatment.

The *H. pylori* eradication success rate in this study was 81.8% [9/11]. For patients with or without gastric *H. pylori* infection, none of the hepatic specimens showed evidence of *H pylori* by IHC antibody stain (**Figure 2**).

Discussion

The hyperinsulinemic-euglycemic clamp technique, the

Table 1 Demographic data.

	H. pylori infection N=11	No *H. pylori* infection N=18	P value
Age	54.6 ± 11.0	49.5 ± 11.3	0.24
Gender (F/M)	4/7	3/15	0.38
Body height (cm)	164.2 ± 8.2	162.9 ± 8.5	0.69
Body weight (Kg)	72.3 ± 10.3	75.2 ± 14.6	0.57
BMI†	26.9 ± 4.2	28.2 ± 4.5	0.45
Waist circumference (cm)	93.5 ± 6.9	95.1 ± 11.0	0.67

†BMI (body mass index): body weight (kg)/ (body height (m)^2

Table 2 Laboratory data for 29 patients.

	H. pylori infection N=11	No *H. pylori* infection N=18	P value
Lipid profile			
Cholesterol (mg/dL)	184.9 ± 50.9	181.6 ± 28.9	0.83
Triglyceride (mg/dL)	157.5 ± 84.9	155.2 ± 74.4	0.94
HDL† (mg/dL)*	50.1 ± 8.8	38.8 ± 9.7	0.004
LDL† (mg/dL)	116.8 ± 23.1	113.4 ± 24.7	0.71
Adipokine			
Adiponectin (ug/mL)	6.7 ± 3.4	6.6 ± 3.9	0.95
Leptin (ng/mL)	8.2 ± 6.0	9.7 ± 5.1	0.48
Inflammatory cytokine			
HS-CRP‡ (mg/L)	2.1 ± 1.0	1.8 ± 1.8	0.61
TNF-α§ (pg/mL)	21.2 ± 23.4	17.7 ± 23.8	0.70
Blood glucose/insulin			
Fasting glucose (mg/dL)	113.0 ± 19.9	113.0 ± 28.3	0.99
Fasting insulin (mU/L)	12.6 ± 6.5	14.6 ± 8.5	0.51
HBA1C (%)	5.9 ± 0.5	6.0 ± 0.6	0.65
HOMR-IR¶	3.4 ± 1.6	4.5 ± 3.8	0.37

† High density lipid, low density lipid

‡ High sensitive C-reactive protein

§ Tumor necrosis factor alfa

¶ Homeostasis model assessment of insulin resistance

Table 3 Liver biochemistry and pathologic studies.

	H. pylori infection N=11	No *H. pylori* infection N=18	P value
AST (U/L)	56.5 ± 26.7	53.5 ± 40.2	0.83
ALT (U/L)	80.9 ± 45.7	63.5 ± 30.6	0.23
Alk-P (U/L)	67.6 ± 17.1	76.0 ± 16.3	0.71
rGT (U/L)	59.2 ± 39.6	51.1 ± 34.4	0.57
Bilirubin (T) (mg/dL)	1.0 ± 0.4	1.1 ± 0.5	0.58
Albumin	4.4 ± 0.4	4.5 ± 0.4	0.52
Fatty metamorphosis (%)	53.0 ± 28.0	48.0 ± 23.0	0.61
Fibrosis score	1.2 ± 1.2	1.0 ± 1.4	0.70
NAS† score	4.7 ± 1.3	5.1 ± 1.7	0.51

†Nonalcoholic fatty liver disease activity score

Table 4 Multivariate linear regression analysis for HOMR-IR value.

Factor	Exp (B)†	95% Confidence Interval (CI)		p value
Waist circumference	0.206	0.080	0.332	0.003
Triglyceride	0.014	0.001	0.027	0.036
H. pylori infection	-0.849	-2.897	1.198	0.398
rGT	0.009	-0.003	0.021	0.126
HR-CRP	-0.849	-1.703	0.005	0.051
Leptin	0.159	-0.087	0.405	0.193

†B: unstandardized coefficient in regression analysis

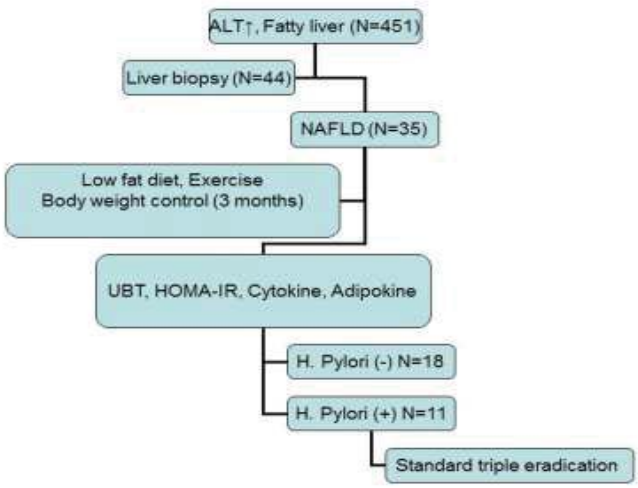

Figure 1 Patient selection and survey flow chart, N=patients number.

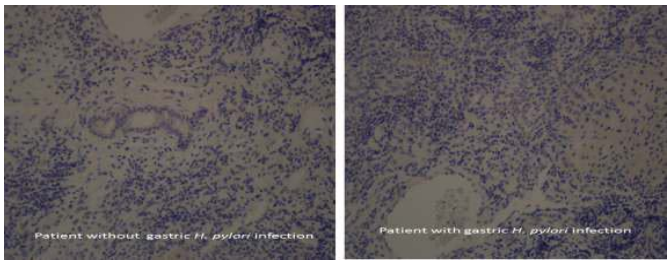

Figure 2 Immunohistochemical stain for *H. pylori* antibody detection from two patients' liver biopsy specimens (right from one patient without gastric *H. pylori* infection; left from another patient with gastric *H. pylori* infection, 400X). Both slides revealed negative finding for H. pylori antibody detection.

quantitative insulin sensitivity check index [QUICKI] and HOMA-IR are all well validated methods for quantitatively assessing IR. Although the hyperinsulinemic-euglycemic clamp technique is considered the gold standard for the assessment of IR, HOMA-IR is the most common method for assessment of IR in clinical practice and epidemiologic studies [24, 25]. HOMA-IR is a minimally invasive index that requires only fasting serum samples. Although there is no widely accepted normal range for HOMA-IR, the upper cut-off value, which defines IR, has been proposed to be 2.5 [range 2.0 to 3.0] in most studies [18, 26, 27]. In this study, patients with NAFLD were included. The mean IR values were 3.4 from patients with *H. pylori* infection and 4.5 from patients without *H. pylori* infected group. Hence, the majority of patients in this study demonstrated insulin resistance. Although the data seem to indicate an association between *H. pylori* infection and IR, it is still unclear whether *H. pylori* infection plays a role in IR, especially in patients with NASH. It is unknown whether *H. pylori* triggers IR or whether chronic active *H pylori* infection promotes IR. We found that HOMA-IR values did not differ significantly between patients with *H. pylori* infection and those without this infection. Among the patients with *H. pylori* infection, the serial follow-up HOMA-IR values within the 6-month period following *H. pylori* eradication treatment didn't reveal any increasing or decreasing trend. Although some studies have reported an improvement in HOMA-IR following *H. pylori* eradication, other studies have shown no effect [20, 21]. Different follow-up interval

and multiple influencing factors of IR most likely contribute to the differences in results from the two studies. *H. pylori* infection is usually acquired early in life. It has been proposed that *H pylori* infection might precede *H. pylori* infection-IR interaction. The original hypothesis was that *H. pylori* induced chronic gastritis which resulted in IR. Chronic inflammation and alternations in counter-regulatory hormones are deemed responsible for IR pathogenesis. However, the inflammatory cytokine and *H. pylori* infection status did not reveal reciprocal change during *H. pylori* eradication treatment in this study. Neither IR nor abnormal ALT improved following *H. pylori* eradication. Hence, *H. pylori* eradication may play a minor role in improving IR in patients with NASH.

H. pylori infection can induce dyslipidemia, as it leads to elevated levels of total cholesterol, low-density lipoprotein cholesterol [LDL-C], triglyceride concentrations and decreased levels of high-density lipoprotein cholesterol [HDL-C] [17, 28, 29]. Increased HDL has been found in patients with duodenal ulcer following successful *H. pylori* eradication [28]. However, a higher mean HDL value was seen in patients with *H. pylori* infection in the current study, which was contrast to the results from some previous studies [17, 28, 29]. Because there was no statistical difference between the mean values of cholesterol, triglyceride, LDL between patients with *H. pylori* infection and those without *H. pylori* infection in this study, the reason of a higher mean HDL value in this study might be resulted from a type I error [small sample size]. Our latter community-based study with a larger sample size (more than 800 subjects) revealed no statistical difference of mean HDL value between subjects with *H pylori* and those without *H pylori* infection [30]. However, the result from a community-based study could not imply the same result from a subgroup, such as NAFLD patients. A further study with more NAFLD patients is warrant to elucidate the difference of mean HDL values between patients with and without *H pylori* infection.

A few studies have reported that the 16S recombinant RNA gene of *H. pylori* was present in liver samples from patients with NASH [31, 32]. If there is causality between *H. pylori* and NASH then *H. pylori* should be detected in hepatic biopsy specimens from patients with NASH [33]. Failure to detect or culture the bacterium in the liver could be considered an argument against the role of *H. pylori* in NASH [33]. Other explanations for being unable to detected *H pylori* by polyclonal antibody stain in this study may be because of low bacterial load and the adaptation of the bacteria to a specific environment, as reported in murine liver [34-36].

In the absence of definitive clinical or laboratory evidence of the above disorders, liver biopsy is the only way to confirm or exclude the diagnosis of NASH. The case number in this study was small because all eligible patients were required to undergo liver biopsy. A further study with a large number of patients is necessary to confirm the difference in HDL value between NAFLD patients with and those without *H. pylori*.

Conclusion

IR is a common condition in patients with NAFLD. We found that there was no significant difference in HOMA-IR value, adipokine (adiponectin, leptin), inflammatory cytokine [HS-CRP, TNF-α] or liver biochemistry between NAFLD patient with and those without *H. pylori* infection. The benefit of improving IR was not remarkable following *H. pylori* eradication for patients with NAFLD and *H. pylori* infection.

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Ethical Adherence

This research was approved by the Institutional Review Board of the Chang-Gung Memorial Hospital IRB No 99-3075C.

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