Prevalence of *Helicobacter pylori* Infection in Nondiabetic NAFLD and its Association in the Severity of Fibrosis

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of cirrhosis worldwide. A considerable amount of patients without DM still has NASH and advanced fibrosis. *Helicobacter pylori* (*H. pylori*) infection contribute to the increase in levels of pro-inflammatory cytokines which have different metabolic effects and associated with insulin resistance (IR). Therefore, *H. pylori* may possibly have an effect on fibrosis progression in NAFLD in nondiabetic patients.

Objectives: Our study aims to investigate the prevalence of *H. pylori* infection in non-diabetic NAFLD patient and its association in advance fibrosis.

Method: Nondiabetic patients who were diagnosed NAFLD by abdominal ultrasonography and/or controlled attenuation parameter (CAP) from FibroScan® were recruited for *H. pylori* infection testing by a 14C-urea breath test. All participants were evaluated liver fibrosis with transient elastography. They were collected standard biochemical test (e.g. FPG, LDL, liver function test) and interleukin-6 to evaluate the inflammatory response. To minimized the confounding effect, we excluded patients who had BMI > 28 kg/m^2 or received steatogenic drugs. The outcome was the prevalence of *H. pylori* infection in NAFLD patients. We also investigated factors which independently associated with advanced fibrosis.

Result: A total of 117 NAFLD patients were enrolled. We found overall *H. pylori* infection in 54 patients (46%). In patients with advanced fibrosis (defined as fibrosis stage ≥ 3 from transient elastography), the prevalence of advanced hepatic fibrosis in NAFLD patients with *H. pylori* infection is higher (22/54, 40.7%) than in non-infected group (18/63, 28.6%). Obesity was the variable most associated with advanced fibrosis (OR 3.18 [1.25 - 8.09], p-value 0.02) in univariate analysis. *H. pylori* infection had a marginal effect to advanced fibrosis (OR 1.72 [0.8 - 3.71], p-value 0.17) but no statistically significant in multivariate analysis. Other metabolic variables and interleukin-6 were not significantly associated with advanced fibrosis.

Conclusion: Prevalence of *H. pylori* infection in nondiabetic NAFLD patient similar to the general Thai population. Our results could not demonstrate the risk of *H. pylori* infection to advanced fibrosis. However, NAFLD patients with *H. pylori* infection tend to have a higher prevalence of advance hepatic fibrosis.

Keywords: *Nonalcoholic Fatty Liver Disease (NAFLD); Nonalcoholic Fatty Liver (NAFL); Nonalcoholic Steatohepatitis (NASH); Liver Cirrhosis (LC); Helicobacter pylori (H. pylori)*

Background

Nonalcoholic fatty liver disease is one of the most common causes of cirrhosis worldwide. The significance of NAFLD is currently considered to be the most common liver disorder in western countries, affecting up to 25%-30% of individuals among the general population and almost 70% among obese subjects [1,2]. NAFLD has a board spectrum range from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH), which may progress to liver cirrhosis (LC).

NAFLD is now regarded as the liver manifestation of the metabolic syndrome. It is strongly associated with obesity, diabetes, cardiovascular disease (CVD), dyslipidemia and insulin resistance (IR) [1]. A considerable amount of patients without DM still had NASH (64%) and advanced fibrosis (17%) [3].

Approximately one-third of NAFLD patients progress in the fibrosis stage during average 4 to 5 year of follow-up, some of whom have a more rapid course. Advanced fibrosis (stage 3 - 4) was an independent predictor of overall and disease-specific mortality of NAFLD [4]. In previous analysis showed old aged and inflammation from liver biopsy is an independent factor associated with progression to liver fibrosis [4].

Helicobacter pylori (*H. pylori*) infection is one of the most common gastrointestinal infections in humans. The chronic, low-grade inflammation (CLGI) of *H. pylori* contributes to the increase in levels of pro-inflammatory cytokines secreted into circulation which has different metabolic effects [5]. In view of this effect on metabolic variables, *H. pylori* is associated with insulin resistance (IR). Therefore, *H. pylori* could predispose patients to NAFLD because of chronic inflammatory state and associated metabolic derangement [1].

The association between *H. pylori* infection and NAFLD has been demonstrated in several clinical trials. In previous several studies, the prevalence of NAFLD was significantly higher in the patients with *H. pylori* infected than in those without [6-8]. There are also some studies demonstrating a higher prevalence of *H. pylori* infection in cirrhotic patients with chronic hepatitis C [9]. *H. pylori* may influence the fibrosis progression in chronic hepatitis C [10]. Extrapolation from this study, we hypothesize *H. pylori* possibly have an effect on fibrosis progression in NAFLD. Despite, there have been few reports considering the potential effect between *H. pylori* infection and the clinical of NAFLD but the majority of the patient group was diabetes which may be a confounding factor of disease severity. Interestingly, NAFLD patients without DM may have another hit to develop insulin resistance and also fibrosis progression other than diabetes. Therefore, we investigated the *H. pylori* status in nondiabetic NAFLD with significant liver fibrosis and evaluate the variables predicting in significant fibrosis in nondiabetic NAFLD.

Material and Methods

Patients

Non-diabetes patients with NAFLD were enrolled to our study. The diagnosis of NAFLD was based on the following criteria: [1] evidence of fatty liver from abdominal ultrasonography and/or transient elastography show controlled attenuation parameter (CAP) more than 5% steatosis (11); Stage 0 < 5% steatosis, Stage 1(≥ S1: 5 - 33% steatosis, stage 2 (≥ S2: 34% - 66% steatosis) and stage 3 (≥S3: > 66% steatosis) [2] exclusion of liver diseases of other etiology.

All of the patients have no history of consuming more than 10 g of alcohol in female and 20 g in male per day. Informed consent will be obtained from all patients. The exclusion criteria are [1] alcohol consumption excess the limit of NAFLD diagnosis. [2] pregnancy [3] the evidence of decompensated cirrhosis or HCC [4] patients who received steatogenic drugs; corticosteroids, methotrexate, amiodarone [5] patients who had BMI > 28 kg/m^2.

Laboratory parameters

Baseline demographic features will be measured in body weight (kg) and height (m). BMI will be calculated by using the following formula: weight in kg/ (height in m^2). Obesity defines as BMI over than 24.9 according to WHO classification of obesity in Asia.

Venous blood will be drawn from every patient. The laboratory evaluation measures of the following levels:

- Liver function test: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, albumin, bilirubin.
- Complete blood count (CBC), platelets count.
- Lipid profile: total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C).
- Fasting plasma glucose.
- Insulin level.
- Pro-inflammatory cytokines: Interleukin-6 (IL-6).

The HOMA-IR will be calculated on the basis of the fasting values of plasma glucose and insulin according to the HOMA model formula: HOMA-IR = fasting insulin level (mU/L) x Fasting plasma glucose (mg/dL)/405.

A high HOMA-IR score denotes low insulin sensitivity and insulin resistance.

NAFLD fibrosis score (NFS) will be use to predict disease severity.

Non-invasive fibrosis score; NAFLD fibrosis score will be calculated to evaluate the severity according to the following formula:

NAFLD Score = -1.675 + (0.037*age [years]) + (0.094*BMI [kg/m^2]) + (1.13*IFG/diabetes [yes = 1, no = 0]) + (0.99*AST/ALT ratio) - (0.013*platelet count [×10^9/L]) - (0.66*albumin [g/dl])

The AST-to-Platelet Ratio Index (APRI) was a good predictor for advanced fibrosis in NAFLD patients area under the ROC curve 0.8307 [13].

APRI index = {[(AST level/AST upper normal limit)]/platelet count x 10^9} x 100

All of the participants was evaluated the degree of liver steatosis and fibrosis by FibroScan® which represented in controlled attenuation parameter (CAP) and liver stiffness measurement (LSM). The standard M probe will be used in the patient with BMI < 28 kg/m^2. The advanced fibrosis is considered at ≥ F 3 stage.

A complete 13C-Urease breath test was used for the diagnosis of active *H. pylori* infection status.

We investigated the prevalence of *H. pylori* in NAFLD. Laboratory parameters and clinical data were investigated which independently associated with advanced fibrosis of the liver.

Statistical analysis

The results were presented as medians and interquartile ranges for quantitative data or as numbers with percentages for qualitative data. Significant differences in categorical data will be determined by using the Chi-square test or Fisher's exact test. The Mann-Whitney U test was used for comparison of continuous data. A multivariate analysis will be performed by logistic regression analysis to identify which variables associated with advanced fibrosis in nondiabetic NAFLD. Differences consider statistically significant at all p-values less than 0.05. Statistical analyses were performed using Stata/SE 14.0 for windows, 2015.

Results

A total of 117 participants was included in this study. Demographic and summaries of the clinical and laboratory data of the population were presented in table 1. There were no significant differences in age, gender distribution, body mass index between *H. pylori* infected
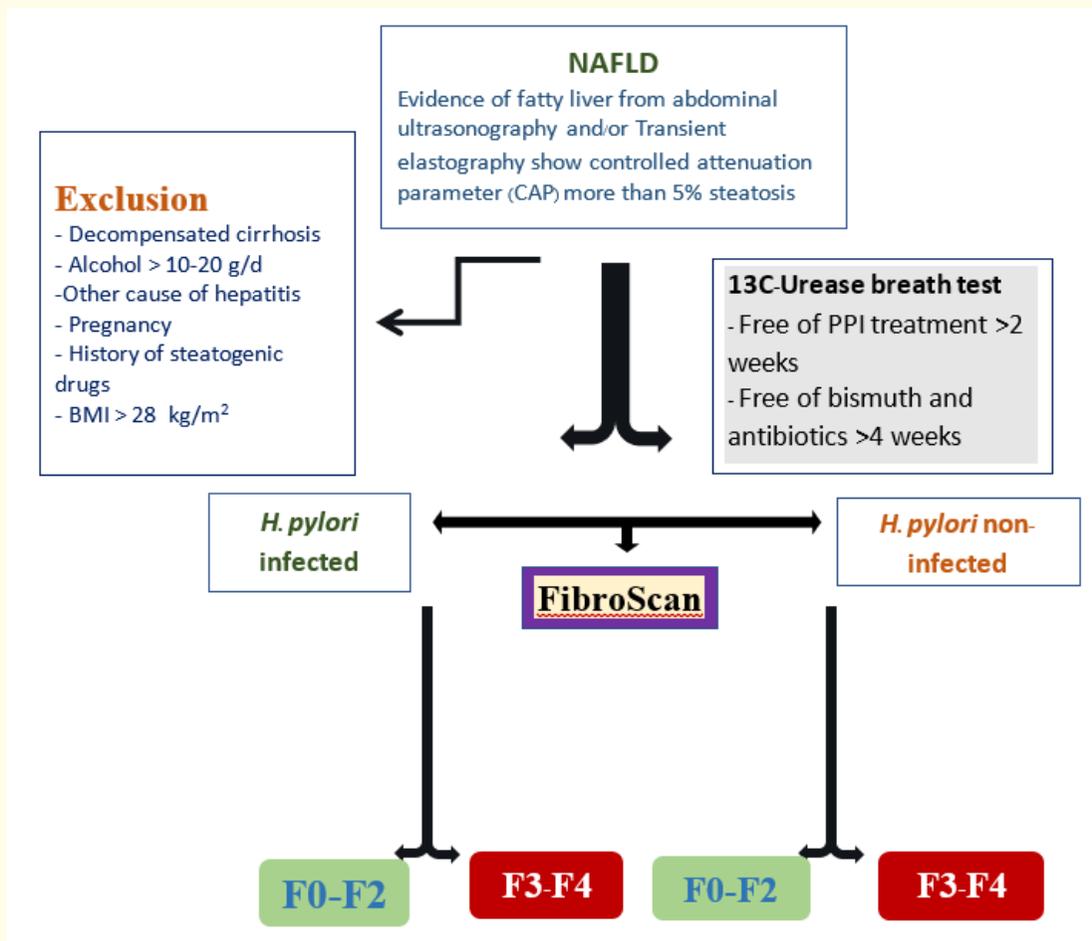
Figure 1: *Flow diagram of participants.*

and non-infected group. The 70% of participants were obese (BMI over than 24.9), had insulin resistance and impaired fasting glucose. According to the controlled attenuation parameter (CAP) from FibroScan®, level of liver steatosis in *H. pylori* infected group was lower than another (Figure 2). Other clinical parameters were similar between the two groups (Table 1). Interleukin-6, pro-inflammatory cytokines also was not different in both *H. pylori* infected or non-infected group (Figure 3). Prevalence of overall *H. pylori* infection is 46%. But in patients with advanced fibrosis (defined as fibrosis stage ≥ 3 from transient elastography). The prevalence of advanced hepatic fibrosis in NAFLD patients with *H. pylori* infection is higher (22/54, 40.7%) than in non-infected group (18/63, 28.6%) (Figure 4). The non-invasive scoring system, NAFLD fibrosis score and APRI score was no difference between two group (p = 0.46).

The association of *H. pylori* and advanced fibrosis in NAFLD was examined (Table 2). Univariate analysis revealed that obesity (BMI > 25 kg/m^2) was only an independent variable associated with advanced fibrosis in NAFLD (OR 3.18 [1.25 - 8.09], p-value 0.02). *H. pylori* infection tend to affect severity of fibrosis in NAFLD (OR 1.72 [0.8 - 3.71], p-value 0.17). However, multivariate analysis revealed no independent variable associated with fibrosis.

Variables	H. pylori Negative (n = 63)	H. pylori Positive (n = 54)	All (n = 117)	p-value
Age	57.86 (±11.5)	56.44 (±11.2)	57.20 (±11.3)	0.50
Sex				
Male	23 (36.5%)	24 (44.4%)	47 (40.2%)	0.76
Female	40 (63.5%)	30 (55.6%)	70 (59.8%)	
BMI (kg/m^2)	26.12 (±3.0)	26.97 (±3.2)	26.51 (±3.1)	0.14
Hypertension				
Yes	19 (30.2%)	14 (25.9%)	33 (28.2%)	0.25
No	44 (69.8%)	40 (74.1%)	84 (71.8%)	
FBS (mg/dl)	109 (±14.1)	104 (±11.9)	107 (±13.3)	0.04
HBA1C	5.96 (±0.56)	5.8 (±0.49)	5.93 (±0.53)	0.45
LDL-C (mg/dl)	129.25 (±39.8)	123.46 (±35.5)	126.58 (±37.6)	0.41
HDL-C (mg/dl)	52.31 (±11.7)	52.81 (±10.2)	52.54 (±11.0)	0.81
Triglycerides	132.34 (±61.1)	114 (±48.4)	124 (±56.1)	0.07
AST (U/l)	35.25 (±15.5)	33.20 (±19.5)	34.30 (±17.4)	0.53
ALT (U/l)	52.04 (±24)	55.20 (±47.1)	53.50 (±36.6)	0.64
HOMA-IR	5.23 (0.82-32.1)	4.88 (0.75-23.3)	5.07 (0.75-32.1)	0.70
IL-6	3.48 (±2.9)	3.39 (±2.65)	3.45 (±2.78)	0.86
NAFLD fibrosis score	-1.30 (±1.40)	-1.55 (±1.33)	-1.41 (±1.37)	0.74
APRI score	0.44 (± 0.27)	0.39 (± 0.31)	0.42 (±0.291)	0.46
Fibrosis stage				
0	16 (25.4%)	11 (20.4%)	27 (23.1%)	0.60
1	22 (34.9%)	18 (33.3%)	40 (34.2%)	
2	7 (11.1%)	3 (5.6%)	10 (8.6%)	
3	9 (14.3%)	12 (22.2%)	21 (17.9%)	
4	9 (14.3%)	10 (18.5%)	19 (16.2%)	

Table 1: Characteristics of study participants at baseline.
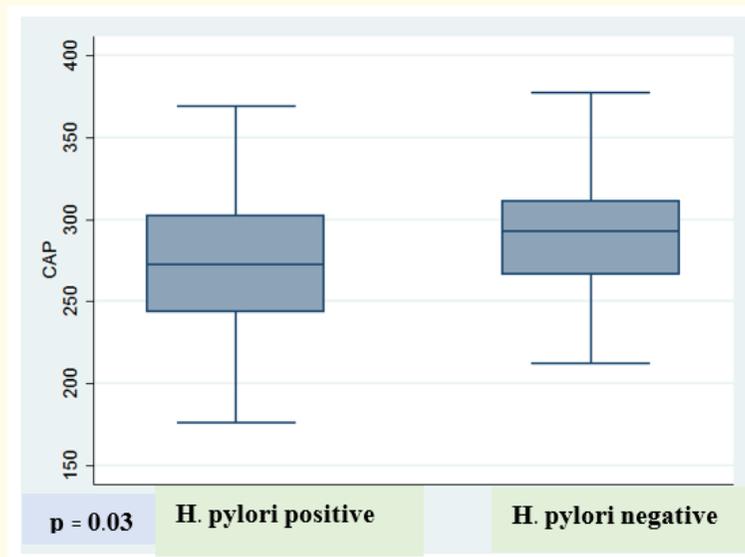

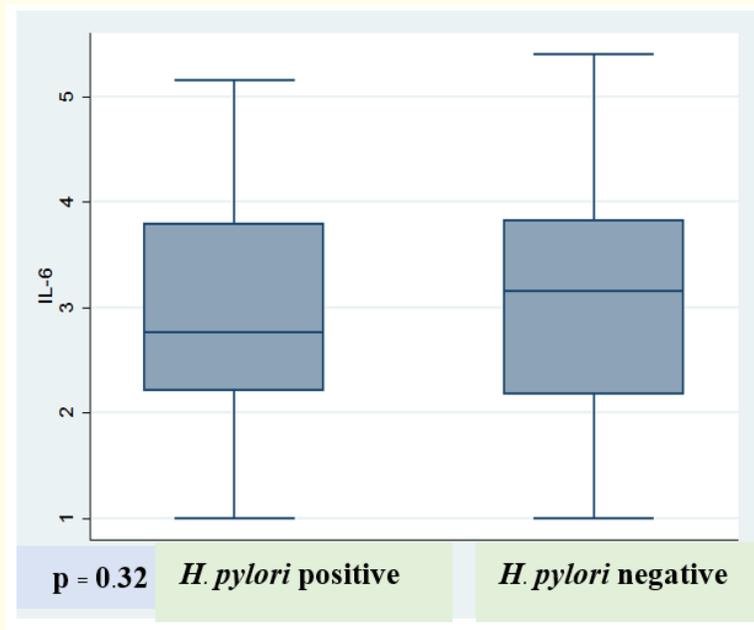
Figure 2: CAP in patients with and without H. pylori.

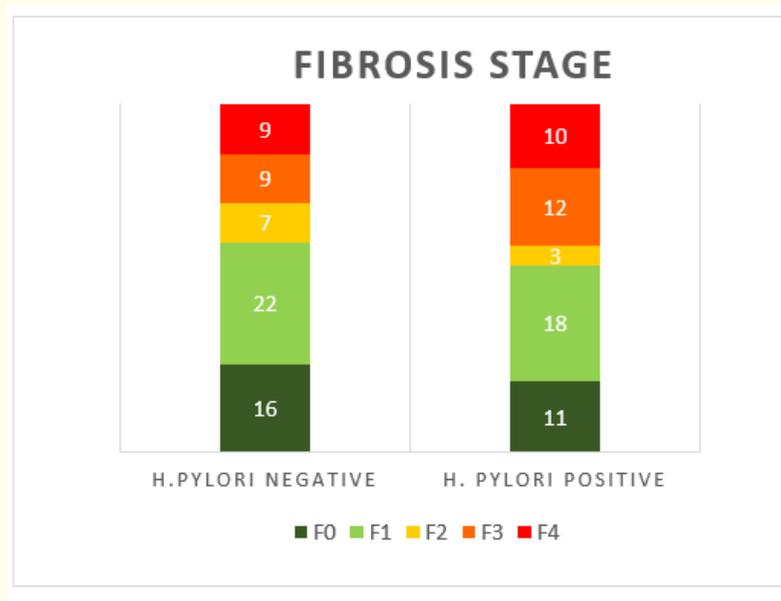***Figure 3:*** *Interleukin-6 in patients with and without H. pylori.*

Figure 4: *Fibrosis stage in patients with and without H. pylori.*

Variables	Univariate				Multivariate			
	OR	95%LCI	95%UCI	p-value	OR	95%LCI	95%UCI	p-value
H. pylori infection	1.72	0.80	3.71	0.17	1.30	0.62	2.82	0.46
Obesity (BMI>25)	3.18	1.25	8.09	0.02	1.57	0.67	3.70	0.30
IFG	1.25	0.57	2.77	0.58	1.54	0.69	3.41	0.29
DLP	0.68	0.30	1.51	0.34	0.74	0.33	1.66	0.47
HOMA-IR > 2.5	1.50	0.60	3.77	0.39	1.22	0.49	3.05	0.67

Table 2: *Univariate and multivariate analyses to identify independent variables associated with advance fibrosis.*

Discussion

In the previous study in Japan, the *H. pylori* status in NAFLD was 40% [8]. But almost of subjects in the study had diabetes and they evaluated *H. pylori* status by anti-*H. pylori* IgG level which had low accuracy due to the inability to differentiate between current and past infection [15]. This study is the first to show the prevalence of *H. pylori* in NAFLD who had no diabetes mellitus and we tested *H. pylori* status by urea breath tests which had high diagnostic accuracy. We found that the prevalence of *H. pylori* in non-diabetic NAFLD in our study (46%) was not different compared with the overall prevalence in Thailand nationwide (45.9%) [16]. From global prevalence, Asia was a moderate rate of infection area [17]. Some studies from highly endemic areas of *H. pylori* found no association between positive *H. pylori* serology and NAFLD [18,19].

DM and obesity are the factors contributing to more severe NAFLD and accelerates the progression of hepatic fibrosis. Therefore, we excluded these specific groups to avoid the confounding effect. In advanced fibrosis NAFLD patients, the prevalence of *H. pylori* infection was higher than the non-infected group. This data may indicate an influence of *H. pylori* to hepatic fibrosis in NAFLD. Evidence of chronic *H. pylori* infection promoted hepatic fibrosis was shown in one animal model study [20]. Chronic infection stimulated profibrogenic cyto-kine (TGF-ß) contributing to the production of extracellular matrix by activation of hepatic stellate cells (HSCs). However, univariate and multivariate analysis showed no association of *H. pylori* and the severity of fibrosis. The result may be due to the small sample subjects.

Our results in the grading of liver steatosis represented by controlled attenuation parameter (CAP) show an average of CAP value in *H. pylori* infected group was significantly lower than the non-infected group. On the other hand, we found advance fibrosis participants who had *H. pylori* infected more than the non-infected group. This finding may because of CAP was inversely correlated with the grading of fibrosis. High CAP value imply a lower risk of fibrosis/cirrhosis [21]. In consistent with lipid profile, triglyceride and LDL in *H. pylori* non-infected group was higher than infected group. But this might because the participants in *H. pylori* took lipid lowering drugs more than non-infected group.

The non-invasive scoring system such as NAFLD fibrosis score and APRI index has high accuracy for advanced fibrosis exclusion in low cut off. Both of scoring system, we found no difference between NAFLD with and without *H. pylori* infection. Most of participants were classified into an intermediate group which needed other modality testings to define the advance fibrosis. FibroScan®, the other test to evaluate liver fibrosis, transient elastography with controlled attenuation parameter (CAP) has demonstrated a good accuracy in quan-tifying the levels of liver steatosis and fibrosis in patients with NAFLD [11,14]. Although only these scoring systems were not a sufficient tool to predict the advanced fibrosis, NFS found to have an acceptable sensitivity, specificity to predict advanced liver fibrosis [12]. Utility of non-invasive score can avoiding up to 75% of liver biopsies.

The variety of inflammatory cytokines involved in *H. pylori* infection. Increase in IL-6 in the serum and tissue level reflected low-grade chronic inflammation in human. Some study showed an association between serum anti-*H. pylori* IgG levels and serum IL-6 levels in *H.*

pylori-infected adults [22]. NAFLD also had an increase of pro-inflammatory cytokines by itself [23]. However, in our study IL-6 level showed no difference between *H. pylori* infected and non-infected group. Moreover, much of Interleukin studies have been performed *in vitro*, so that their *in vivo* importance is less clear [5].

A strength of our study, we tested *H. pylori* infection by urea breath tests which reflected current infective status. It differed from the previous study that mostly used anti-*H. pylori* IgG level. Our study has some limitations. First, this is a cross-sectional, preliminary in an only single center in Bangkok area. Second, our sample size was small number. At last, we were limited the standard of evaluation the degree of fibrosis by liver biopsy. Nowadays, a non-invasive method for diagnosis of NAFLD such as FibroScan® is widely used instead of liver biopsy. Because it provides a high rate of accuracy and has less complication. Further large case-control studies are needed to identify the association between *H. pylori* infection and fibrosis in NAFLD. A randomized controlled trials are also needed to determine whether *H. pylori* eradication therapy can improve NAFLD with advanced fibrosis.

Conclusion

The prevalence of *H. pylori* in NAFLD patients revealed no difference compared with the general population in Thailand. However, *H. pylori* infection may be another contributing factor in fibrosis progression in nondiabetics NAFLD patients.

Acknowledgement

Bibliography

1. Li M., *et al*. "Potential role of *Helicobacter pylori* infection in nonalcoholic fatty liver disease". *World journal of gastroenterology* 19.41 (2013): 7024-7031.

2. Wijarnpreecha K., *et al*. "*Helicobacter pylori* and Risk of Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis". *Journal of Clinical Gastroenterology* (2017).

3. Goh GB., *et al*. "Clinical spectrum of non-alcoholic fatty liver disease in diabetic and non-diabetic patients". *Biochimica et Biophysica Acta Clinical* 3 (2015): 141-145.

4. Stal P. "Liver fibrosis in non-alcoholic fatty liver disease - diagnostic challenge with prognostic significance". *World Journal of Gastroenterology* 21.39 (2015): 11077-11087.

5. Buzas GM. "Metabolic consequences of *Helicobacter pylori* infection and eradication". *World Journal of Gastroenterology* 20.18 (2014): 5226-5234.

6. Kim TJ., *et al*. "A cohort study on *Helicobacter pylori* infection associated with nonalcoholic fatty liver disease". *Journal of Gastroenterology* (2017).

7. Tang DM and Kumar S. "The Association Between *Helicobacter pylori* Infection and Nonalcoholic Fatty Liver Disease". *Current Gastroenterology Reports* 19.2 (2017): 5.

8. Sumida Y., *et al*. "*Helicobacter pylori* infection might have a potential role in hepatocyte ballooning in nonalcoholic fatty liver disease". *Journal of Gastroenterology* 50.9 (2015): 996-1004.

9. Ponzetto A., *et al*. "Helicobacter infection and cirrhosis in hepatitis C virus carriage: is it an innocent bystander or a troublemaker?". *Medical Hypotheses* 54.2 (2000): 275-277.

10. Esmat G., *et al*. "Role of *Helicobacter pylori* in patients with HCV-related chronic hepatitis and cirrhosis with or without hepatocellular carcinoma: possible association with disease progression". *Journal of Viral Hepatitis* 19.7 (2012): 473-479.

11. Wong VW., *et al*. "Validity criteria for the diagnosis of fatty liver by M probe-based controlled attenuation parameter". *Journal of Hepatology* 67.3 (2017): 577-584.

12. Treeprasertsuk S., *et al*. "NAFLD fibrosis score: a prognostic predictor for mortality and liver complications among NAFLD patients". *World Journal of Gastroenterology* 19.8 (2013): 1219-1229.

13. Peleg N., *et al*. "AST to Platelet Ratio Index and fibrosis 4 calculator scores for non-invasive assessment of hepatic fibrosis in patients with non-alcoholic fatty liver disease". *Digestive and Liver Disease* 49.10 (2017): 1133-1138.

14. Mikolasevic I., *et al*. "Transient elastography (FibroScan((R))) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand?". *World Journal of Gastroenterology* 22.32 (2016): 7236-7251.

15. Best LMJ TY., *et al*. "Non-invasive diagnostic tests for infection". *Cochrane Database of Systematic Reviews* 3 (2018).

16. Uchida T., *et al*. "*Helicobacter pylori* Infection in Thailand: A Nationwide Study of the CagA Phenotype". *PLoS One* 10.9 (2015): e0136775.

17. Hooi JKY., *et al*. "Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis". *Gastroenterology* 153.2 (2017): 420-429.

18. Kang SJ., *et al*. "Association between cagA negative *Helicobacter pylori* status and nonalcoholic fatty liver disease among adults in the United States". *PLoS One* 13.8 (2018): e0202325.

19. Okushin K., *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* 15 (2015): 25.

20. Goo MJ.,*et al*. "*Helicobacter pylori* promotes hepatic fibrosis in the animal model". Laboratory Investigation a *Journal of Technical Methods and Pathology* 89.11 (2009): 1291-303.

21. Karlas T., *et al*. "Impact of controlled attenuation parameter on detecting fibrosis using liver stiffness measurement". *Alimentary Pharmacology and Therapeutics* 47.7 (2018): 989-1000.

22. Nakagawa H., *et al*. "Significant association between serum interleukin-6 and *Helicobacter pylori* antibody levels among *H. pylori*-positive Japanese adults". *Mediators of Inflammation* 2013 (2013): 142358.

23. Das SK and Balakrishnan V. "Role of cytokines in the pathogenesis of non-alcoholic Fatty liver disease". *Indian Journal of Clinical Biochemistry* 26.2 (2011): 202-209.