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Effects of gender and age on prevalence of cholelithiasis in patients with chronic HCV infection

A community-based cross-sectional study in an HCV-hyperendemic area

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Abstract

This study investigated the effects of age and gender on the prevalence of cholelithiasis in patients with chronic HCV infection.

Demographic and clinical data of 8489 subjects (3671 males, 4818 females; mean age 47.5 years) receiving township-wide health examinations between September 2012 and August 2013 were analyzed. The main endpoint was prevalence of cholelithiasis. Risk factors (age, gender, body mass index, concomitant diseases, lifestyle, laboratory parameters, and HCV status) were evaluated. Univariate and multivariate logistic regression analyses were performed to identify associations between cholelithiasis and variables.

Cholelithiasis was more prevalent among HCV subjects than non-HCV subjects (females: 8.1% vs 4.2%; males: 9.1% vs 3.9%; both $P < .001$); rates ranged from 5.6% to 8.3% in females and 4.7% to 10.6% in males. HCV status and age were associated with cholelithiasis occurrence (OR = 2.17 for HCV vs non-HCV; OR = 2.44, 3.54 for age 45–55, and >55 vs <45 years; all $P < .05$). Multivariate analysis showed a significant association between cholelithiasis and age/sex interaction terms (OR = 0.517 for age >55 vs <45 for sex; $P = .011$). Cholelithiasis prevalence was significantly associated between age and sex interaction terms but not anymore if considering positive HCV status. All noninvasive tests for liver fibrosis were associated with cholelithiasis but only fibrosis-4 index was significantly associated (OR = 1.28, $P = .019$).

Age, gender, and HCV infection are associated with increased risk and prevalence of cholelithiasis. After age of 55 years, cholelithiasis is more prevalent among HCV-positive males than females. Females of age 55 and more may be protected against cholelithiasis as sex hormones decrease.

Abbreviations: ALT = alanine aminotransferase, APRI = AST to platelet ratio index, AST = aspartate aminotransferase, BMI = body mass index, CHC = chronic hepatitis C, CI = confidence interval, DM = diabetes mellitus, FIB-4 = fibrosis-4 index, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HDL = high-density lipoprotein, HGB = hemoglobin, LDL = low-density lipoprotein, NPV = negative predictive value, OR = odds ratio, PPV = positive predictive value, TG = triglyceride.

Keywords: age, cholelithiasis, chronic hepatitis C infection, gender, Taiwan

1. Introduction

Hepatitis C virus (HCV) is a blood-borne hepatotropic RNA virus that results in progressive liver damage. HCV transmission occurs through unsafe injection drug use, unsterile medical procedures in countries with high prevalence of HCV,[1] and through transfusions of blood or blood products received before

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HCV testing became routine for blood donors.[2] In transfusion-acquired HCV infection, disease progression is sequential, from acute to chronic infection, then to cirrhosis, and finally hepatocellular carcinoma (HCC).[3] While individuals with posttransfusion HCV infection often die from liver failure or HCC,[2] the viral load may be an independent risk factor for HCC development but not necessarily for liver-related mortality.[4] An estimated 30 million people worldwide have chronic HCV infection, accounting for 27% of cirrhosis cases and 25% of liver cancer.[5] However, life expectancy can still be relatively long due to the slow progression from fibrosis to cirrhosis (about 40% at 5 years) and subsequent HCC. [6] However, risk factors such as age, alcohol consumption, and male gender are more strongly associated with fibrosis progression in HCV infection than virological factors.[7] In particular, health-risk behaviors such as smoking, alcohol consumption, unhealthy diets, and drug use are associated with higher mortality risk in HCV patients.[8] Occupational exposure to toxic chemicals is another risk factor for HCC development in individuals whose liver function is already compromised with HCV.[9] Additionally, risk of early recurrence of HCC is exceptionally high in patients with HCV-cirrhosis and successfully treated previous HCC and who had received subsequent direct-acting antiviral drugs (DAAs).[10] Besides these multiple associated risks, HCV infection is also increasing. Prevalence of chronic HCV infection was 1% at the start of the 21st century and had increased to 2.2% by 2007.[5] Prevalence of chronic HCV appears to be age-specific in hyperendemic areas of Japan, China, and Taiwan, where individuals of age over 50 years account for a 20-fold greater prevalence.[11,12]

Chronic HCV infection is also associated with gallstone formation, especially in patients of both genders who have already progressed to liver cirrhosis.[13] Cholelithiasis, or gallstone disease, develops through a complex interaction of genetic and environmental factors and is associated with aging, hyperlipidemia, and obesity.[14,15] In patients with chronic liver disease, the presence of gallstones is associated with the degree of liver dysfunction, with gallstone formation occurring more readily in liver cirrhosis compared to normal biochemistry or chronic hepatitis without cirrhosis.[16] Gallstones are found in chronically infected HCV patients at a younger age than in those without liver disease and are associated with central obesity and liver steatosis, but not inherited gallstone disease.[17] Although the complex relationship between HCV infection and gallstone disease is not explained precisely, biliary lithogenesis is suggested as a related factor to HCV infection. A known histological characteristic for chronic HCV infection is bile duct damage and HCV core protein may play a role in the malignant transformation of human biliary epithelial cells. Such evidence suggests that HCV infection damages the gallbladder mucosa, which may lead to gallstone formation.[18]

Gender differences have been seen in the prevalence of gallstones among patients with chronic HCV infection evaluated in population-based studies conducted in Taiwan and the United States.[19,20] The male-dominant prevalence of cholelithiasis in chronic HCV-infected individuals may be attributable to age over 50 years and more progressive underlying liver pathology in males compared to females; anti-HCV was only associated with males, and not with females.[16] Nevertheless, women of all ages are still twice as likely as men to develop cholesterol gallstones from puberty through childbearing years before tapering off, highlighting the possible influence of female sex hormones.[21,22]

To the best of our knowledge, the influence of female sex hormones such as estrogen on the prevalence of cholelithiasis has not been studied in patients with chronic HCV infection. We hypothesized that the male-dominant prevalence of cholelithiasis in patients with chronic HCV may change in different age groups in response to the effects of female sex hormones. Therefore, this study investigated the effects of age and gender on the prevalence of cholelithiasis in patients with chronic HCV infection.

2. Patients and methods

2.1. Study design

Between September 2012 and August 2013, township-wide community health screening of the residents of Mailiao Township, Yunlin County, Taiwan, was conducted. All township residents were invited by mail, telephone, and the media to undergo a comprehensive health examination. A total of 12,348 participants responded and received this examination. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review board of Chang Gung Memorial Hospital. All enrolled subjects provided signed informed consent to participate in this study.

2.2. Study population

The data of 12,348 participants were screened from the community-wide database. A total of 3739 subjects who were <20 years of age, missing data for abdominal ultrasound examination and AST/ALT data, had invalid serum lipid profiles, were new residents of the designated township, had a history of chewing betel nut, alcohol drinking, or smoking status, were excluded. In addition, 120 patients who had received cholecystectomy previously were also excluded. Finally, the data of 8489 subjects (3671 males and 4818 females) with a mean age of 47.5 years (range: 20−102 years) were included for analysis (Fig. 1).

2.3. Study variables

The main endpoint of the present study was the prevalence of cholelithiasis, or gallstones. Different risk factors for the formation of gallstones were analyzed. Data obtained for each participant included demographics (age, gender), body mass index (BMI), disease associations (self-reported medical conditions such as hypertension and diabetes), lifestyle or behavioral factors (betel nuts chewing, smoking history, alcohol use, etc), and laboratory examinations (HCV antibodies, triglycerides [TGs], total cholesterol, HDL, LDL, hemoglobin [HGB], total bilirubin, direct bilirubin, and ALT/AST ratio, APRI and FIB-4 for noninvasive assessment of liver fibrosis).

2.3.1. Demographic data. Age and gender were recorded using questionnaires. Age was further grouped as those <45 years old, 45 to 55 years old, and >55 years old, mainly to evaluate the influence of pre-, peri-, and postmenopausal status of females on the development of cholelithiasis.

2.3.2. Anthropometric measures. Subjects were further categorized by weight using the World Health Organization (WHO) 1995 criteria for BMI of adults,[23] where BMI <18.5 kg/m^2 is underweight, BMI = 18.5−24.9 kg/m^2 is normal, BMI = 25−29.9 kg/m^2 is overweight, and BMI ≥30.0 kg/m^2 is obese.

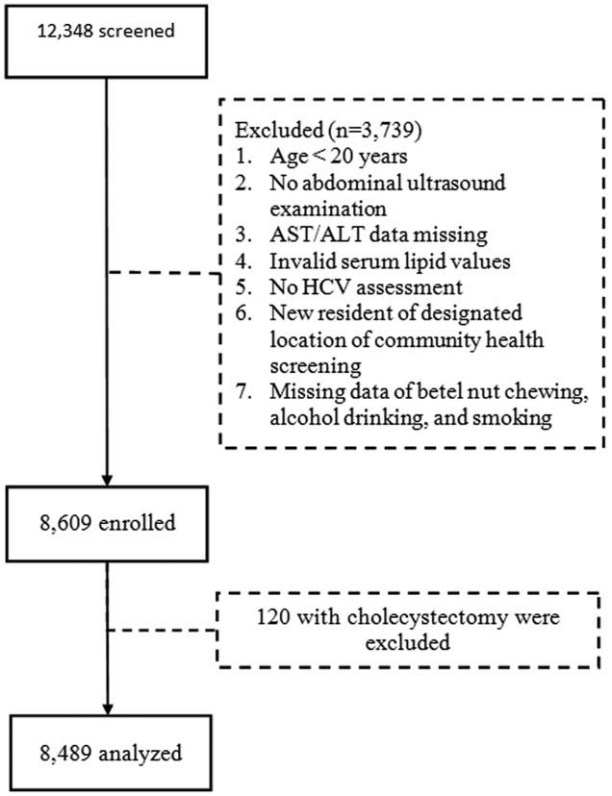

Figure 1. Flow chart of subjects enrolled.

2.3.3. Comorbid diseases. Comorbid diseases were self-reported using the questionnaires and they were included as variables. Relevant medical conditions (in addition to HCV status) included hypertension and diabetes mellitus (DM).

2.3.4. Lifestyle factors. Lifestyle measures, including betel nuts chewing, smoking tobacco, and alcohol consumption, were recorded using the questionnaires. Subjects were categorized as users or nonusers.

2.3.5. Laboratory examinations. Community health screening was sponsored by Chang Gung Memorial Hospital and all laboratory examinations were performed in the hospital laboratory as part of the health examinations. Tests included HCV antibody, TGs, total cholesterol, HDL, LDL, HGB, total bilirubin, and direct bilirubin. Subjects who tested HCV positive were further assessed by serum HCV-RNA within 3 months after the health examination.

2.3.6. Noninvasive assessment of liver fibrosis. Several noninvasive methods of assessing liver fibrosis have been used previously in clinical practice.[24] Three methods were used in the present study using available data from the community health screening database, including aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio index (ALT/AST ratio), AST to platelet ratio index (APRI), and fibrosis-4 index (FIB-4). Previous results[25] showed that an ALT/AST ratio of ≥1 demonstrated good specificity (although relatively low sensitivity) for detecting cirrhosis in patients with chronic hepatitis C (CHC) with reported positive and negative predictive values (PPV, NPV) ranging from 73.7% to 100% and 46.7% to 53.2%, respectively. APRI is calculated as (AST/ upper limit of normal range)/platelet count $(10^9\ L^{-1}) \times 100$. This test shows only moderate accuracy for diagnosing CHC-related fibrosis and is not used routinely. The FIB-4 score combines platelet count, ALT, AST, and age and was developed initially for use in HCV/HIV coinfection. FIB-4 provides good discrimination between severe fibrosis (AUROC 0.85) and cirrhosis (AUROC 0.91).

2.4. Statistical analysis

Categorical variables, including demographic and clinical characteristics, laboratory examinations, and lifestyle measures, are summarized as n (%) by HCV status for females and males. Differences between HCV and non-HCV subjects were compared using Pearson Chi-square test or Fisher exact test. Univariate and multivariate logistic regression model analyses were performed to identify associations between cholelithiasis and variables, including demographics, clinical characteristics, laboratory examination, noninvasive assessments of liver fibrosis, and lifestyles. The interaction terms between HCV, sex, and age were also evaluated. Results are presented as odds ratios (OR) with corresponding 95% confidence intervals (95% CI) and *P* values. All statistical assessments were 2-tailed and considered significant at $P < .05$. All statistical analyses were carried out with IBM SPSS statistical software version 22 for Windows (IBM Corp, Armonk, NY).

3. Results

Among 12,348 participants receiving community health examinations, 3739 subjects who were <20 years old, missing data for abdominal ultrasound examination and AST/ALT data, had invalid serum lipid profiles, were new residents of the township, or had a history of chewing betel nuts, drinking alcohol, and smoking were excluded. In addition, 120 patients who had received cholecystectomy previously were also excluded. Finally, the data of 8489 subjects (3671 males and 4818 females) with a mean age of 47.5 years (range: 20−102 years) were included for final analysis (Fig. 1).

Table 1 shows subjects' demographic and clinical characteristics by HCV and non-HCV for males and females. Females with HCV were associated with age, BMI, hypertension, DM, TG, HDL, total bilirubin, betel nuts chewing, and smoking (all $P < .05$); while males with HCV were associated with age, hypertension, DM, TG, cholesterol, HDL, LDL, direct bilirubin, total bilirubin, HGB level, and betel nuts chewing (all $P < .05$). The percentage of subjects with cholelithiasis was higher among HCV subjects than among non-HCV subjects (females: 8.1% vs 4.2%, respectively; $P < .0001$; males: 9.1% vs 3.9%, respectively; $P < .001$) (Table 1).

Results of univariate logistic regression analysis showed that HCV status and age were associated with the occurrence of cholelithiasis (OR = 2.17 for HCV vs non-HCV; OR = 2.44, 3.54 for age 45–55, and >55 vs <45 years; all $P < .05$). To document their interactions, multivariate analysis showed a significant association between cholelithiasis and age/sex interaction terms in females (OR = 0.517, age <45 given males vs <45 given females; $P = .011$) (Table 2). The prevalence of cholelithiasis was significantly associated with HCV subjects, but it was not statistically significant between age and sex interaction terms in HCV subjects. Figure 2 presents the prevalence of cholelithiasis as 2.6%, 6.1%, and 6.3% for age groups <45, 45 to 55, and >55 years, respectively, in non-HCV females and 1.7%, 4.3%, and

Table 1

Participants' demographics, clinical characteristics, laboratory examinations, and lifestyle measures by HCV status and gender.

		Female			Male		
		With HCV (n = 786)	Without HCV (n = 4032)	*P* value	With HCV (n = 497)	Without HCV (n = 3174)	*P* value
Age	<45 y	71 (9)	2213 (54.9)	<.0001*	85 (17.1)	1703 (53.7)	<.0001*
	45–55 y	132 (16.8)	742 (18.4)		84 (16.9)	600 (18.9)	
	>55 y	583 (74.2)	1077 (26.7)		328 (66)	871 (27.4)	
BMI	Fat	258 (33.3)	853 (20.9)	<.0001*	174 (35.5)	1070 (34)	.505
	Heavy	210 (27.1)	885 (22.1)		162 (33.1)	1005 (31.9)	
	Normal	306 (39.5)	2282 (57)		154 (31.4)	1074 (34.1)	
Hypertension	Yes	204 (26)	496 (12.3)	<.0001*	130 (26.2)	488 (15.4)	<.0001*
	No	582 (74)	3536 (87.7)		367 (73.8)	2686 (84.6)	
DM	Yes	121 (15.4)	188 (4.7)	<.0001*	74 (14.9)	222 (7)	<.0001*
	No	665 (84.6)	3844 (95.3)		423 (85.1)	2952 (93)	
TG	Abnormal	126 (16)	524 (13)	.023*	84 (16.9)	946 (29.8)	<.0001*
	Normal	660 (84)	3508 (87)		413 (83.1)	2228 (70.2)	
Cholesterol	Abnormal	266 (33.8)	1410 (35)	.544	110 (22.1)	1146 (36.1)	<.0001*
	Normal	520 (66.2)	2622 (65)		387 (77.9)	2028 (63.9)	
HDL	Abnormal	102 (13)	190 (4.7)	<.0001*	327 (65.8)	1805 (56.8)	<.0001*
	Normal	684 (87)	3842 (95.3)		170 (34.2)	1369 (43.1)	
LDL	Abnormal	225 (28.6)	1135 (28.1)	.786	130 (26.2)	1129 (35.6)	<.0001*
	Normal	561 (71.4)	2897 (71.9)		367 (73.8)	2045 (64.4)	
Bilirubin	Abnormal	12 (1.5)	65 (1.6)	.842	26 (5.3)	95 (3)	.008*
	Normal	772 (98.5)	3897 (98.4)		466 (94.7)	3070 (97)	
Total bilirubin	Abnormal	66 (8.4)	236 (5.9)	.007*	115 (23.1)	530 (16.7)	<.001*
	Normal	720 (91.6)	3796 (94.1)		382 (76.9)	2643 (83.3)	
HGB	Abnormal	82 (10.4)	478 (11.9)	.252	69 (13.9)	240 (7.6)	<.0001*
	Normal	704 (89.6)	3551 (88.1)		428 (86.1)	2933 (92.4)	
Chewing	Yes	13 (1.7)	25 (0.6)	.003*	144 (29)	657 (20.7)	<.0001*
	No	773 (98.3)	4007 (99.4)		353 (71)	2517 (79.3)	
Smoking	Yes	17 (2.2)	194 (4.8)	.001*	220 (44.3)	1285 (40.5)	.111
	No	769 (97.8)	3838 (95.2)		277 (55.7)	1889 (59.5)	
Drinking	Yes	21 (2.7)	118 (2.9)	.696	130 (26.2)	813 (25.6)	.797
	No	765 (97.3)	3914 (97.1)		367 (73.8)	2361 (74.4)	
Cholelithiasis	Yes	64 (8.1)	171 (4.2)	<.0001*	45 (9.1)	124 (3.9)	<.0001*
	No	722 (91.9)	3861 (95.8)		452 (90.9)	3050 (96.1)	

Data are summarized as n (%) by HCV status for females and males.

Differences between HCV and non-HCV subjects were compared using Pearson Chi-square test or Fisher exact test.

BMI = body mass index, DM = diabetes mellitus, HDL = high-density lipoprotein, HCV = hepatitis C virus, HGB = hemoglobin, LDL = low-density lipoprotein, TG = triglyceride.

* $P < 0.05$, indicates significant difference between HCV and non-HCV groups.

Table 2

Univariate and multivariate logistic regression analyses of associations between cholelithiasis and interactions between age, gender, and HCV status.

	Univariate		Multivariate					
			Model I		Model II		Model III	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
HCV (yes vs no)	2.175 (1.731, 2.733)	<0.001*	1.462 (1.146, 1.864)	.002*	2.388 (1.136, 5.018)	.022*	1.699 (1.181, 2.444)	.004*
Gender (female vs male)	1.063 (0.868, 1.301)	.557	0.772 (0.587, 1.017)	.066	1.033 (0.842, 1.268)	.755	1.104 (0.871, 1.400)	.413
Age								
45–55 vs 45	2.446 (1.816, 3.294)	<0.001*	1.262 (0.750, 2.126)	.381	2.460 (1.786, 3.387)	<.001*	2.353 (1.744, 3.174)	<.001*
>55 vs 45	3.542 (2.772, 4.525)	<0.001*	2.399 (1.728, 3.331)	<.001*	3.331 (2.531, 4.383)	<.001*	3.181 (2.457, 4.119)	<.001*
Age × gender								
<45 given males vs <45 given females	–		0.517 (0.311, 0.859)	.011*	–		–	
45–55 vs <45 given females			1.810 (1.066, 3.073)	.028*	–		–	
Age × HCV								
45–55 vs <45 given HCV	–		–		0.558 (0.218, 1.431)	.225	–	
>55 vs <45 given HCV	–		–		0.576 (0.260, 1.275)	.173	–	
Gender × HCV								
females vs males given HCV	–		–		–		0.753 (0.473, 1.199)	.232

Results are presented as odds ratios (OR) with corresponding 95% confidence intervals (95% CI) and *P* values.

HCV = hepatitis C virus.

* $P < .05$, indicates significantly associated.

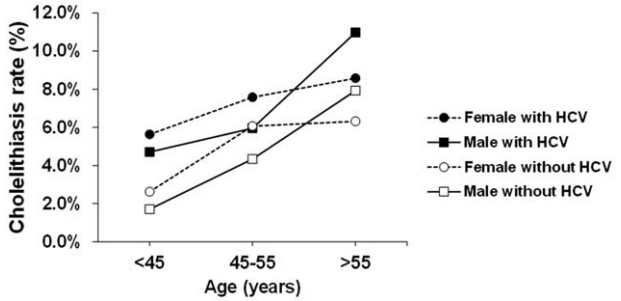

Figure 2. Prevalence of cholelithiasis stratified by age for given gender and HCV status.

7.9%, respectively, in non-HCV males ($P < .001$). The prevalence rate ranged from 5.6% to 8.6% in HCV females and from 4.7% to 11.0% in HCV males (Fig. 2).

Table 3 presents associations between cholelithiasis with 3 noninvasive assessments of liver fibrosis: ALT/AST ratio, APRI, and FIB-4. Univariate analysis showed that elevated APRI or FIB-4 are associated with the occurrence of cholelithiasis (OR = 1.35 for APRI and 1.90 for FIB-4; both $P < .05$). When adjusted for covariates, including HCV status, sex, age, and interaction terms between age and sex, the adjusted OR only showed a significant association between FIB-4 and cholelithiasis (OR = 1.28, $P = .019$) (Table 3).

Table 4 presents multivariate logistic regression analysis of associations between cholelithiasis and all variables by HCV status and gender. Results of Model I showed subjects with HCV, females, age >45 years, female aged >55, elevated FIB-4 level, fat or heavy BMI category, and diabetes were associated with the occurrence of cholelithiasis (all $P < .05$). Model II, including factors with significant associations in Model I plus laboratory examinations, also showed an association between abnormal total bilirubin level and cholelithiasis (OR = 1.494, $P = .009$). Model III, with Model II significant values plus lifestyle measures, showed that abnormal total bilirubin level and smoking were associated with the development of cholelithiasis (bilirubin: OR = 1.52, $P = .006$; smoking: OR = 1.62, $P = .003$) (Table 4).

4. Discussion

Results of our investigation of the effects of age and gender on the prevalence of cholelithiasis in subjects with chronic HCV infection vs non-HCV infected subjects revealed that gallstone disease was more prevalent among HCV-infected individuals than in non-HCV infected. HCV status and age were associated with the development of gallstones, and rates increased with age. Notably, after age of 55, cholelithiasis was more prevalent among HCV-positive males than females. The highest rates in non-HCV females and males were also in individuals over age 55 and were higher in males (7.8%) than in females (6.1%). Significant associations were found between cholelithiasis and age/sex interaction terms. All noninvasive tests for liver fibrosis were associated with the prevalence of cholelithiasis but only the FIB-4 was significantly associated. These findings of the present study have reconfirmed current knowledge of the association between HCV infection and cholelithiasis and highlighted the importance of certain crucial factors associated with gallstones, especially HCV status. Besides showing that age, gender, and HCV infection are associated with increased risk and prevalence of cholelithiasis, we examined the interaction between age and gender, which has seldom been studied previously, finding that after age of 55, gall bladder disease is more prevalent among HCV-positive males than among peri-menopausal females, and that hormonal changes in females over age of 55 may protect against cholelithiasis.

4.1. Prevalence and risk factors

Regarding the association between HCV infection and the prevalence of gallstones, results of the present study agree with previous findings. In another study conducted in Taiwan, the prevalence rate of cholelithiasis was 6.8% in an HCV hyperendemic area and was associated with a higher mean age as in our study.[19] Conte et al[25] found an overall prevalence of gallstones of 29.5%, which increased significantly with age without regard to gender or the cause of cirrhosis. Shah et al[26] reported that risk of gallstone disease was increased in HCV-related chronic liver disease and that the association was especially pronounced in HCV-positive males; this was an important finding because those investigators had excluded subjects with other risk factors for gallstones. In fact, the association between chronic HCV infection and cholelithiasis is even more likely than with the other 2 common etiologies for liver cirrhosis, alcohol, and chronic HBV infection.[27] Given that HCV is a common infection among older adults in certain areas of China[11] it is particularly noteworthy that the present study was able to confirm the greater prevalence of gallstones in HCV-infected individuals compared to non-HCV-infected individuals in a large Taiwan population, also showing the association with older age.

In terms of risk factors, the present study found that HCV infection in females was significantly associated with age, BMI,

Table 3

Associations between cholelithiasis and noninvasive assessments of liver fibrosis.

	Univariate		Multivariate	
Variables	**OR (95% CI)**	***P* value**	**Adj. OR† (95% CI)**	***P* value**
ALT/AST ratio	0.992 (0.781, 1.260)	.948	1.174 (0.896, 1.538)	.244
APRI	1.355 (1.130, 1.625)	.001*	1.077 (0.853, 1.358)	.534
FIB-4	1.909 (1.571, 2.320)	<.001*	1.287 (1.043, 1.588)	.019*

Results are presented as odds ratios (OR) with corresponding 95% confidence intervals (95% CI) and *P* values.

ALT = alanine aminotransferase, APRI = AST to platelet ratio index, AST = aspartate aminotransferase, FIB-4 = Fibrosis-4 index.

† Adj. OR, adjusted OR derived after adjustments for HCV, gender, age, and interaction between gender and age for each assessment of liver fibrosis.

* $P < .05$, indicates significantly associated.

Table 4

Multivariate logistic regression analyses of cholelithiasis and subjects' demographics and clinical characteristics, noninvasive assessments of liver fibrosis, laboratory examinations, and lifestyle measures.

	Model I		Model II		Model III	
Variables	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**	**OR (95% CI)**	***P* value**
HCV (yes vs no)	1.395 (1.088, 1.787)	.009*	1.370 (1.062, 1.766)	.015*	1.351 (1.048, 1.743)	.020*
Gender (female vs male)	1.782 (1.155, 2.751)	.009*	1.924 (1.213, 3.052)	.005*	2.093 (1.302, 3.366)	.002*
Age						
45–55 vs <45 y	2.043 (1.233, 3.384)	.006*	2.091 (1.261, 3.470)	.004*	2.075 (1.248, 3.450)	.005*
>55 vs <45 y	3.154 (2.051, 4.851)	<.001*	3.137 (2.035, 4.835)	<.001*	3.266 (2.114, 5.046)	<.001*
Age × gender						
Aged 45–55 vs <45 y given female	0.897 (0.480, 1.676)	.733	0.839 (0.447, 1.575)	.586	0.862 (0.458, 1.620)	.644
Aged >55 vs <45 y given female	0.460 (0.275, 0.769)	.003*	0.458 (0.272, 0.772)	.003*	0.458 (0.271, 0.773)	.003*
FIB-4	1.407 (1.137, 1.741)	.002*	1.344 (1.081, 1.670)	.008*	1.345 (1.082, 1.673)	.008*
BMI						
Fat vs normal	2.117 (1.626, 2.756)	<.001*	2.132 (1.625, 2.796)	<.001*	2.174 (1.657, 2.853)	<.001*
Heavy vs normal	1.554 (1.180, 2.048)	.002*	1.553 (1.173, 2.058)	.002*	1.575 (1.189, 2.086)	.002*
Hypertension (yes vs no)	1.159 (0.900, 1.492)	.252	1.156 (0.896, 1.492)	.265	1.167 (0.904, 1.507)	.236
DM (yes vs no)	1.667 (1.242, 2.238)	.001*	1.660 (1.228, 2.242)	.001*	1.643 (1.216, 2.221)	.001*
TGs (abnormal vs normal)	–		1.000 (0.763, 1.310)	1.000	0.991 (0.755, 1.301)	.947
Cholesterol (abnormal vs normal)	–		1.032 (0.750, 1.421)	.845	1.047 (0.761, 1.441)	.778
HDL (abnormal vs normal)	–		1.027 (0.769, 1.372)	.856	0.974 (0.726, 1.305)	.858
LDL (abnormal vs normal)	–		1.028 (0.748, 1.413)	.866	1.005 (0.731, 1.382)	.973
Bilirubin (abnormal vs normal)	–		1.089 (0.589, 2.014)	.787	1.071 (0.578, 1.986)	.828
Total bilirubin (abnormal vs normal)	–		1.494 (1.107, 2.018)	.009*	1.529 (1.131, 2.066)	.006*
HGB (abnormal vs normal)	–		1.281 (0.942, 1.743)	.114	1.280 (0.941, 1.743)	.116
Chewing (yes vs no)	–		–		0.995 (0.678, 1.461)	.980
Smoking (yes vs no)	–		–		1.624 (1.180, 2.234)	.003*
Drinking (yes vs no)	–		–		0.703 (0.488, 1.013)	.058

Results are presented as odds ratios (OR) with corresponding 95% confidence intervals (95% CI) and *P* values.

BMI = body mass index, DM = diabetes mellitus, FIB-4 = fibrosis-4 index, HDL = high-density lipoprotein, HCV = hepatitis C virus, HGB = hemoglobin, LDL = low-density lipoprotein, TG = triglyceride.

* $P < .05$, indicates significantly associated.

hypertension, DM, hyperlipidemia, total bilirubin, betel nuts chewing, and smoking, while HCV in males was significantly associated with age, hypertension, DM, TG, hyperlipidemia, direct bilirubin, total bilirubin, HGB level, and betel nuts chewing. Other authors have shown or suggested that lifestyle factors had more influence on the development of gallstones than HCV status.[8]

4.2. Gender differences

Our findings relative to gender suggest that females older than 55 years are protected from cholelithiasis. This concept was true despite adjusting for all the possible known risk factors. Although the exact etiology was unknown, our results suggest the influence of female hormonal changes in conjunction with aging. The results of other studies bear this out. Results of another study among a Taiwanese population showed that anti-HCV was associated with gallstones formation in males but not in females.[19] Cirillo et al[28] investigated the effect of estrogen therapy on gallbladder disease, finding that postmenopausal women who had received estrogen therapy had an increased risk of developing biliary tract disease; results suggested a causal association between estrogen and gallstone disease. Another study indicated that the risk of cholecystectomy was increased among women receiving oral estrogen therapy during menopause.[29] In the present study, more females were diagnosed with cholelithiasis during child-bearing years, which is consistent with other studies. A study of pregnancy and cholelithiasis concluded that pregnancy was an important pathogenetic factor favoring gallstone formation in young Chilean women.[30] A higher prevalence of gallstones has been observed in women of all age groups and is particularly evident among younger adults. Among large cohorts included in the noted GREPCO study, Attili et al[31] found a female-to-male ratio of 2.9 between the ages of 30 and 39 years; the ratio narrowed to 1.6 between the ages of 40 and 49 years and 1.2 between the ages of 50 and 59 years. The higher rates in young women were believed to be a result of pregnancy and sex steroids, as subsequent studies have also reported.[28,30] Although aging is a risk factor for cholelithiasis, it appears to be inversely associated among females. Nevertheless, given the abundant evidence, female gender is obviously a risk factor for gallstones formation, as compared to the risk in males, which is more associated with liver disease. In the present study, although this phenomenon was observed in female subjects, it did not reach statistical significance. In subjects older than 55 years, more males had cholelithiasis than females, which could be associated with HCV-infected liver disease in males and hormonal influences in females. The aforementioned evidence regarding increased risk of cholelithiasis as a result of pregnancy and in women receiving estrogen therapy suggests that females at postmenopausal age have a level of protection from gallstones formation.

4.3. Liver disease severity and gallstones

For the present study, we evaluated the extent of liver fibrosis to determine whether the degree of liver disease was associated with gallstone development. Although liver biopsy is still the debated gold standard for assessing the degree of liver fibrosis, available noninvasive methods of assessing liver fibrosis were reviewed by

Papastergiou et al[24] who suggested that several tests had merit. After performing 3 noninvasive tests of liver fibrosis, we found that only the FIB-4 was significantly associated with the prevalence of cholelithiasis. Among all noninvasive tests of liver fibrosis, FIB-4 provides good discrimination between severe fibrosis (AUROC 0.85) and cirrhosis (AUROC 0.91).[24] Therefore, we were able to understand the status of liver cirrhosis of each participant better than relying on subjective recall of cirrhosis diagnosis in the questionnaires. Results showed that in females older than 45 years, an elevated FIB-4 level indicating increased fibrosis was associated with the occurrence of cholelithiasis.

Cirrhosis is a major risk factor for gallstones.[25,27,32] The increased risk was demonstrated in a longitudinal, cross-sectional study of 1010 patients with cirrhosis[25] in which both prevalence and incidence of gallstones were far higher in patients with cirrhosis than among the general population of the study area; the authors reported this as a "close relation between cirrhosis and gallstones" and "a major risk factor." Acalovschi et al[32] documented liver cirrhosis as a risk factor for gallstones and were among the first investigators to show other significant risk factors for gallstone disease, including HCV infection, in a large patient population with chronic HCV hepatitis, excluding cirrhosis.[17] In addition, Zhang et al[13] reported that the prevalence of gallstone disease was significantly associated with cirrhosis in HCV-infected individuals of both genders. In an attempt to explain these associations, several authors investigated gallbladder motility in patients with chronic HCV hepatitis and cirrhosis, finding that a decrease in gallbladder motility was present in HCV-related cirrhosis and chronic HCV hepatitis.[33,34] Buzas et al[35] attributed this phenomenon partly to an increase in gallbladder wall thickness, which might be a risk factor for gallstone development.

4.4. Limitations

This study has some limitations. First, it was a cross-sectional analysis, which limits making inferences regarding causality. Future longitudinal studies are required, especially to verify possible causal relationships between postmenopausal females and cholelithiasis. Interview (questionnaire) data are based on self-reports and are therefore subject to recall problems and misunderstanding of questions by participants, and various other factors. To overcome this possible bias, we chose objective laboratory parameters or anthropometric measures rather than variables that may be highly sensitive to subjective alterations. Family history and genetics were not included in the community health screening examinations even though family history studies suggest that genetics has a significant role in the development of gallstones.[35] Also, the precise timeframe of gallstones development and HCV infection was not available in the database so we could not consider this in our analysis. The cohort for this study was not large enough to generate state or local prevalence estimates, and study conclusions may not apply to all populations. Further prospective study of the prevalence of cholelithiasis among HCV-infected individuals is needed to confirm results of the present study, especially gender differences and the influence of female hormones on gallstone formation.

5. Conclusion

Gallstone disease is more prevalent among HCV-infected individuals than among non-HCV infected individuals. HCV status, gender, and age are associated with the development of gallstones, and rates of gallstone development increase with age in males but not in peri- or menopausal females. Females older than 55 years appear to be protected from cholelithiasis, possibly associated with hormone levels although further study is needed to confirm this. These findings may increase clinicians' awareness of contributing factors to gallstone development and help to identify possible candidates for cholelithiasis based on age, gender, and HCV status. Our results may benefit clinical practice by helping to develop better screening plans and prevention measures.

Author contributions

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