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Risk of Betel Quid Chewing on the Development of Liver Cirrhosis: A Community-Based Case-Control Study

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PURPOSE: The role of betel quid on the development of liver cirrhosis is unclear; we thus designed a community-based case-control study to evaluate the association between betel quid chewing and liver cirrhosis.
METHODS: A total of 42 cases of liver cirrhosis and 165 matched controls were included for analysis. Questionnaires were administered to obtain histories of betel quid chewing, alcohol consumption, smoking, and family history of liver disease. Hepatitis B surface antigen and anti-hepatitis C antibody were also determined by immunoassay.
RESULTS: Individuals with more betel quid chewing (more than 55 quid-years vs. less than 55 quid-years and never-chewers, matched odds ratio [OR_m] = 2.2; 95% confidence interval [CI]: 1.0–5.0) had higher risks for liver cirrhosis. The combined effects on liver cirrhosis by betel quid chewing and the number of other risk factors, including hepatitis B virus (HBV) infection, smoking, and alcohol drinking, were also observed. When individuals with less betel quid chewing (less than 55 quid-years and never-chewers) and with no other risk factors used as a reference, betel quid chewers expressing greater betel quid chewing (more than 55 quid-years) and more risk factors of HBV infection, cigarette smoking, and habitual alcohol drinking expressed a greater risk of liver cirrhosis (OR_m = 70.8; 95% CI: 4.0–1260.1).
CONCLUSIONS: Our results suggest that betel quid chewing may play an important role in the development of hepatic cirrhosis. Larger study and cohort studies would be necessary to provide further evidence regarding this finding.
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KEY WORDS: Alcohol Drinking, Betel Quid Chewing, Hepatitis B Virus, Liver Cirrhosis, Smoking.

INTRODUCTION

In Southeast Asia, especially in Taiwan and India, betel quid is a natural masticatory and stimulant composed of fresh green areca fruit, *Piper betle* (betel leaf), and slaked lime paste (1). The prevalence of betel chewing in the Taiwanese population is greater than 10% (2). Although the chewing of betel quid is practiced in several different ways in various areas, the major components are relatively consistent. Areca fruit contains some alkaloids, of which arecoline is the major one. *P. betle* contains high concentrations of safrole (1,2-methylenedioxy-4-allylbenzene) (3), which is thought to induce the formation of oxidative stress in liver (4). An association between betel quid with oral submucous fibrosis, oral cancer, and esophageal cancer has been demonstrated in previous studies (5–7). A case report and a case-control study also indicated that betel quid chewing may be a risk factor for hepatocellular carcinoma (8, 9). However, the role of betel quid on the development of chronic liver disease like hepatic cirrhosis remains unknown.

Liver cirrhosis is a consequence of fibrotic changes that occur in a chronically damaged liver. Especially, liver cirrhosis is a highly notable health problem in Taiwan and is the sixth leading cause of death by disease (with chronic liver disease), killing about 5000 people each year (10). Numerous clinical studies have demonstrated that the patients who have chronic hepatitis B virus (HBV) (11) and hepatitis C virus (HCV) (12, 13) infection may progress to liver cirrhosis. However, some liver cirrhosis occurs in patients without evidence of hepatotropic viral infection. In addition to hepatitis virus infection, it has long been known that tobacco smoking (14, 15) or alcohol consumption (14, 16) plays a role in the etiology of liver cirrhosis. Of Taiwanese betel-quid chewers, 86% were smokers and 75% were alcohol drinkers (2). However, there is less understanding about interactions between these environmental risk factors and betel-quid chewing on the development of liver cirrhosis.

Importantly, higher prevalence of substance use for betel quid, cigarette smoke, and alcohol in Taiwanese aborigines has been observed in several surveys (17–19). Furthermore, aboriginal health status is worse than that of the general Taiwanese population. Thus we designed a case-control

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Selected Abbreviations and Acronyms

anti–HCV = anti–hepatitis C virus antibody
CI = confidence interval
HBsAg = hepatitis B surface antigen
HBV = hepatitis B virus
HCV = hepatitis C virus
OR_m = matched odds ratio

study in Taiwanese aboriginal communities to elucidate whether betel-quid chewing is associated with the development of liver cirrhosis and to evaluate the interaction of hepatitis virus infection, cigarette smoking, and alcohol drinking on the development of liver cirrhosis with betel-quid chewing conducted.

MATERIAL AND METHODS

Study Population and Epidemiological Data

During the period from August 1997 to June 1999, a community-based liver disease screening project was performed in Fuhsing, located in the northern mountain areas of Taiwan. The study protocol conformed to the Declaration of Helsinki and had been approved by the relevant ethics committees of the participating institutions. The population of Fuhsing was approximately 11,000 persons as determined over recent years. Over 7000 residents were indigenous people, which is the largest indigenous group apart from the Atayal tribe. The traditional life style for residents in Fuhsing is farming.

Initially, research assistants and local nurses abstracted name, personal identification number, gender, and date of birth from the records of local housing offices. There were 4597 male and 3205 female residents over 20 years of age who were invited to participate. A total of 1379 men and 1469 women were enrolled in the final health examination. The response rate from 8102 residents over 20 years of age was 36.5%. To improve the response rate, we used a variety of strategies, including sending letters when phones were disconnected, sending research staff to the last known address, and using contacts (friends and neighbors) to get updated information on the participants or to pass a message along. The reasons given by subjects who rejected inclusion in or could not participate in the study were that they were too busy to be interviewed, were out of town, and could not be located. Because of the lack of job opportunities, most men or young people have to earn their livings from areas outside their hometown, and they keep their children and parents at home to save expenses. Hence, it is typical in this society that each household has some family members moving out of town. During our study, most of the time we found that the elderly, women, and children are more likely to stay in their hometown. Residents with a shorter distance between their residence and local research station had a higher rate of response.

Information pertaining to personal characteristics was collected from study subjects using interviewer-administered questionnaires during the medical surveillance. Informed consent was obtained from all participants. The structured questionnaire contained questions that covered demographic characteristics, and life style, including habits of cigarette smoking, alcohol drinking, and betel-quid chewing as well as personal and family history of chronic liver diseases. If the subjects were found to have once used a substance, then the interviewer obtained a detailed history of their smoking, alcohol drinking, and betel-quid chewing habits, including when they began and stopped using the substance, how much of the substance they consumed daily, and how long they had used the substance. If they were found to be former users, then they were asked how long it had been since they gave up the use of the substance. The question asked to ascertain betel-quid use was "how often do you chew betel quid (per day or per week)?" The frequency of betel-quid use was asked again in a different part of the interview to ensure accuracy. A parameter termed "pack-years" was coined as an indicator of cumulative smoking dose and was defined as the number of packs of cigarettes smoked daily multiplied by the number of years of active smoking. Habitual alcohol drinking was defined as alcohol consumption on at least one occasion weekly and consuming more than 80 g of alcohol weekly, as in our previous work (20). Habitual betel-quid chewing was defined as a person chewing 1 quid or more daily for at least 1 year. Similarly, a parameter termed "quid-years" was coined as an indicator of cumulative betel-quid chewing level and was defined as the number of betel quids chewed daily multiplied by the number of years of betel-quid chewing as reported by Wu et al. (7). Family history of chronic liver disease was defined as chronic liver disease within the first-degree relatives of the test subjects.

Health Examination and Laboratory Analyses

For those subjects who participated in the medical surveillance process, abdominal ultrasonography was conducted by a qualified gastroenterologist, using a high-resolution real-time ultrasound machine (Toshiba, model SAL-38B, Toshiba Co. Ltd., Tokyo, Japan) equipped with a linear-type 3.75 MHz convex-type transducer. In addition, 10 mL of venous blood was collected into a heparinized Vacutainer. Samples were centrifuged and blood components separated and stored at –70°C. Serum samples were further assayed for hepatitis B surface antigen (HBsAg) status by enzyme immunoassay (EIA; Austrial-II, Abbott Laboratories, Chicago, IL) with commercial kits and for anti-hepatitis C virus (HCV) by EIA with second-generation commercial

kits (Abbott Laboratories). Conventional liver function tests were conducted by an autoanalyzer.

Selection of Cases and Control Subjects

Detection for liver cirrhosis was based on criteria developed by Yang et al. (21). Liver cirrhosis was diagnosed in the presence of coarse echo patterns with and without irregular surface outlines. Furthermore, the ultrasonographic criteria which were used to diagnose a fatty liver included liver and kidney echo discrepancy, presence of an increased liver echogenicity (bright), echo penetration into the deep portion of the liver, and clarity of the liver blood vessel structures. Because subjects with fatty liver and no cirrhosis might have coarse echo patterns on ultrasound, fatty liver was also recognized by increased liver echogenicity and then excluded. The control subjects were matched individually to the cases on age ($\pm$ 5 years), gender, and race. A 1:4 ratio of cases to control subjects was used in this study. Healthy control subjects were selected randomly from a pool of eligible participants who underwent detailed questionnaires, whose HBsAg and anti-HCV status were tested, and who were free from any evidence of chronic liver disease. All controls had normal serum aminotransferase levels and with no space-occupying lesions in the liver, as evidenced by normal abdominal sonography. However, one patient ended up with incomplete matching. Overall, 42 case examples and 165 control subjects were included in the analysis conducted herein.

Statistical Analysis

Comparisons between the case group and control group for gender, ethnicity, age, proportion of HBV infection and HCV infection, betel-quid chewing, smoking behavior, alcohol consumption status, and family history of chronic liver disease were conducted using the Student *t* test for continuous variables and χ^2 test or Fisher's exact test for discrete variables. Subsequently, a conditional logistic model was employed to obtain the matched odds ratio (OR_m) and 95% confidence interval (CI) for each variable. Kappa test was used to evaluate the agreement of habits of betel quid chewing, cigarette smoking, and alcohol drinking of our subjects. Likelihood ratio χ^2 tests were also used to test the interaction between hepatitis viral infection with habits of betel-quid chewing, cigarette smoking, and alcohol drinking. Additionally, the number of risk factors together was also taken into multiple logistic regression model, and the trend of the association between the number of risk factors and liver cirrhosis development by betel-quid chewing was also assessed. We used SAS statistical software, version 9.1 (SAS Institute Inc., Cary, NC) for all analyses. All *p* values were calculated using two-tailed statistical tests. A probability of 0.05 or less was considered as significant.

RESULTS

The baseline characteristics of 42 cases of liver cirrhosis and 165 matched controls are presented in Table 1. The mean age of the subjects was 50.2 $\pm$ 14.0 (standard deviation [SD]) years. The Atayal tribe accounted for 84.5% of the study subjects. Cases of liver cirrhosis revealed a significantly greater prevalence of HBsAg than controls (45.2% vs. 24.2%; $p < 0.01$, χ^2 test). Comparing case subjects and controls revealed no real difference between these groups for anti-HCV status (2.4% vs. 2.4%). In addition, case subjects were more likely than controls to have histories of cigarette smoking (78.6% vs. 52.1%; $p < 0.01$), or alcohol drinking (78.6% vs. 46.7%; $p < 0.01$). Case subjects were also more likely than controls to have habits of betel-quid chewing (31.0% vs. 20.6%; $p = 0.15$), or any family history of chronic liver disease (7.1% vs. 3.0%; $p = 0.22$), however, the difference didn't reach statistical significance.

The potential risk factors for and associated ORs of liver cirrhosis are summarized in Table 2. All of the following categories were significantly associated with liver cirrhosis: HBsAg carrier status ($OR_m = 2.7$; 95% CI, 1.3–5.4), cigarette smoking (>5 pack-years vs. <5 pack-years and never-smokers, $OR_m = 3.3$; 95% CI, 1.5–7.0), and habitual alcohol drinking ($OR_m = 4.2$; 95% CI, 1.9–9.2). The mean value of total amount of betel quid consumed in our subjects

TABLE 1. Characteristics of study cases of liver cirrhosis and matched controls

Characteristic	Case subjects ($N = 42$)	Control subjects ($N = 165$)	Total ($N = 207$)
Age, yr	50.9 $\pm$ 14.4*	50.0 $\pm$ 13.9	50.2 $\pm$ 14.0
Gender, male	37† (88.1%)	145 (87.9%)	182 (87.9%)
Ethnicity			
Ataya	35 (83.3%)	140 (84.8%)	175 (84.5%)
Han	7 (16.7%)	25 (15.2%)	32 (15.5%)
HBsAg positive status	19 (45.2%)‡	40 (24.2%)	59 (28.5%)
Anti-HCV positive status	1 (2.4%)	4 (2.4%)	5 (2.4%)
Betel quid chewers§	13 (31.0%)	34 (20.6%)	47 (22.7%)
Quid-years	59.8 $\pm$ 135.2	49.3 $\pm$ 201.4	51.4 $\pm$ 189.6
Smokers‖	33 (78.6%)‡	86 (52.1%)	119 (57.5%)
Pack-years	17.4 $\pm$ 21.3	12.5 $\pm$ 20.2	13.5 $\pm$ 20.4
Habitual alcohol drinkers	33 (78.6%)‡	77 (46.7%)	110 (53.1%)
Alcohol consumption (g/wk)	246.1 $\pm$ 236.0‡	106.2 $\pm$ 137.5	134.6 $\pm$ 171.2
Family history of chronic liver disease	3 (7.1%)	5 (3.0%)	8 (3.9%)

*Mean $\pm$ standard deviation.
†Number of subjects.
‡$p < 0.01$; χ^2 test or *t* test.
§Included current and ex-chewers.
‖Included current and ex-smokers.

TABLE 2. HBsAg and anti-HCV status, habits of betel quid chewing, cigarette smoking, and alcohol drinking, and family history of chronic liver disease of study cases of liver cirrhosis and matched controls

Characteristic	Cases (*N*)	Controls (*N*)	OR_m* (95% CI)	HBsAg-adjusted OR_m* (95% CI)
HBsAg status				
Positive	19	40	2.7 (1.3–5.4)	
Negative	23	125	1.0	
Anti-HCV status				
Positive	1	4	1.0 (0.1–9.1)	1.3 (0.1–11.9)
Negative	41	161	1.0	1.0
Betel quid chewing				
>55 quid-years	11	24	2.1 (0.9–4.7)†	2.2 (1.0–5.0)‡
≤55 quid-years§	31	141	1.0	1.0
Smoking				
>5 pack-years	31	76	3.3 (1.5–7.0)	4.3 (1.8–10.1)
≤5 pack-years‖	11	89	1.0	1.0
Alcohol drinking (> 80 g/wk)				
Yes	33	77	4.2 (1.9–9.2)	6.0 (2.4–14.7)
No	9	88	1.0	1.0
Family history of chronic liver disease				
Yes	3	5	2.4 (0.6–10.6)	2.4 (0.5–10.6)
No	39	160	1.0	1.0

*Matched for age, gender and race.
†$p = 0.08$.
‡$p = 0.06$.
§Included never-chewers.
‖Included never-smokers.

was 51.4 quid-years (see Table 1). Thus betel-quid chewers with less than 55 quid-years (closest available value to the average of 51.4) and never-chewers were defined as less betel quid chewing. Subjects with habits of betel-quid chewing also had a higher risk of liver cirrhosis (>55 quid-years vs. <55 quid-years and never-chewers, $OR_m = 2.1$; 95% CI, 0.9–4.7), although it did not reach statistical significance. Following adjustment for the status of HBsAg carrier, the association of liver cirrhosis with either cigarette smoking of more than 5 pack-years ($OR_m = 4.3$; 95% CI, 1.8–10.1) or habitual alcohol drinking ($OR_m = 6.0$; 95% CI, 2.4–14.7) remained clearly apparent. The adjusted OR of liver cirrhosis for betel-quid chewing was of borderline significance ($OR_m = 2.2$; 95% CI, 1.0–5.0, $p = 0.06$).

Furthermore, matched ORs of liver cirrhosis were calculated to investigate the joint effect of betel-quid chewing, cigarette smoking, and habitual alcohol drinking with HBV infection, respectively. Since betel-quid chewing with cigarette smoking (agreement, 0.60, κ, 0.23; 95% CI, 0.13–0.32, $p < 0.01$), betel-quid chewing with alcohol drinking (agreement, 0.54, κ, 0.12; 95% CI, 0.02–0.21, $p = 0.02$), and cigarette smoking and alcohol drinking (agreement, 0.66, κ, 0.31; 95% CI, 0.18–0.44, $p < 0.01$) have higher collinearity; thus the effects of these potential confounders were not adjusted in our conditional logistical regression model. When the HBsAg-negative subjects with less betel quid chewing (<55 quid-years and never-chewers) were selected as a reference ($OR_m = 1.0$; Table 3), a prominent risk of liver cirrhosis was observed for HBsAg carriers having experienced greater betel-quid chewing ($OR_m = 4.8$; 95% CI, 1.2–19.3). Subjects with less betel-quid chewing and HBV infection also had a 2.9-fold risk of liver cirrhosis (95% CI, 1.3–6.6), and those with more betel-quid chewing and without the history of HBV infection also had a 2.6-fold risk of liver cirrhosis (95% CI, 0.9–7.2). The interaction between HBsAg status and betel-quid chewing on the risk of liver cirrhosis was significant ($p = 0.02$). Similarly, subjects with less betel-quid chewing and less cigarette smoking (<5 pack-years and never-smokers) were used as a reference ($OR_m = 1.0$); an obvious risk of liver cirrhosis was observed for subjects having experienced more cigarette smoking and greater betel-quid chewing ($OR_m = 5.2$; 95% CI, 1.8–14.8). Subjects with more cigarette smoking and less betel-quid chewing also revealed a significant risk to develop liver cirrhosis ($OR_m = 3.2$; 95% CI, 1.3–7.8). Moreover, subjects with less betel-quid chewing and without habitual alcohol drinking were also selected as a reference ($OR_m = 1.0$); an elevated risk of liver cirrhosis was observed for those individuals having experienced alcohol drinking and greater betel-quid chewing ($OR_m = 7.7$;

TABLE 3. Combined effects of habits of HBsAg status, cigarette smoking, and alcohol drinking, with betel-quid chewing on the development of liver cirrhosis

Characteristic	Betel quid chewing (quid-yr)	Cases (*N*)	Controls (*N*)	OR_m* (95% CI)
HBsAg status				
Positive	>55	4	6	4.8 (1.2–19.3)
Positive	≤55†	15	34	2.9 (1.3–6.6)
Negative	>55	7	18	2.6 (0.9–7.2)
Negative	≤55	16	107	1.0
		Test for interaction: $\chi^2 = 6.79$ (1 *df*); $p = 0.02$		
Smoking				
>5 pack-years‡	>55	11	19	5.2 (1.8–14.8)
>5 pack-years	≤55	20	57	3.2 (1.3–7.8)
≤ 5pack-years	>55	0	5	—
≤ 5 pack-years	≤55	11	84	1.0
		Test for interaction: $\chi^2 = 6.61$ (1 *df*); $p = 0.01$		
Alcohol drinking (>80 g/wk)				
Yes	>55	9	16	7.7 (2.3–25.8)
Yes	≤55	24	61	5.8 (2.1–16.2)
No	>55	2	8	3.9 (0.6–23.9)
No	≤55	7	80	1.0
		Test for interaction: $\chi^2 = 11.21$ (1 *df*); $p < 0.01$		

*Matched for age, gender, and race.
†Included never-chewers.
‡Included never-smokers.

95% CI, 2.3–25.8), followed by those with habitual alcohol drinking and less betel-quid chewing (OR_m = 5.8; 95% CI, 2.1–16.2), and those without habitual alcohol drinking and with more betel-quid chewing (OR_m = 3.9; 95% CI, 0.6–23.9).

Subsequently, we also evaluated the combined effects on liver cirrhosis by betel-quid chewing and the number of other risk factors, including HBV infection, smoking, and alcohol drinking (Fig. 1). When individuals with less betel-quid chewing (<55 quid-years and never-chewers), and with none of the other risk factors, were used as a reference, those expressing more risk factors of HBV infection, cigarette smoking, and habitual alcohol drinking expressed a greater risk of liver cirrhosis. The trend was obvious both for subjects in more than 55 quid-years groups of betel chewing (test for trend, $p = 0.01$) and for those in less than 55 quid-years groups ($p < 0.01$).

DISCUSSION

In the current study, the results showed that individuals with more betel-quid chewing had higher risks for liver cirrhosis. The combined effects on liver cirrhosis by betel-quid chewing and the number of other risk factors, including HBV infection, smoking, and alcohol drinking, were also observed.

Early epidemiological studies have demonstrated that HBV infection is the most important risk factor for liver cirrhosis (11). Chen and Sung (22) reported that the prevalence of HBsAg was 85.4% among Taiwanese liver cirrhosis patients. In the present study, individuals suffering from liver cirrhosis also demonstrated a higher prevalence of the HBsAg (45.2%) than those not suffering from liver cirrhosis, and caused a 2.7-fold risk for liver cirrhosis. However, only half of liver cirrhosis patients were HBsAg carriers, suggesting that other etiological factors on the development of chronic liver damage may also be important. Additionally, it was anticipated that the prevalence of liver cirrhosis among hepatitis virus carriers in this community-based study might be lower than those from liver clinics because the latter generally represent patients with clinically significance disease. Moreover, the number of our subjects with HCV infection was relatively small; thus it would seem likely that this was the reason that we observed no significant association between HCV infection and liver cirrhosis risk in this study.

Both hepatitis virus (11–13) and hepatotoxic chemicals (14–16) may be involved in the pathogenesis of cirrhosis. In our study we further observed an elevated risk for liver cirrhosis in betel-quid chewers, with either the presence or absence of hepatitis viral infection. There are a number of theoretical reasons to support our findings in this study. An animal study showed that obvious changes were observed in hepatic levels of biotransformation enzymes and antioxidants in mice fed diets containing areca nut (23). Sarma et al. (24) also indicated that animals with long-term betel-quid feeding can develop chronic hepatocyte necroinflammation. A significant amount of reactive oxygen species production can also be induced by betel-nut extract (25, 26). Evidence of oxidative reactions is associated with fibrogenesis occurring in the liver (27), and many etiological agents of fibrogenesis stimulate free radical reactions either directly or through inflammatory stimuli (28). Fibrotic liver injury results in activation of the hepatic stellate cell which undergoes a phenotypic change to a proliferative myofibroblast-like cell that synthesizes excess interstitial collagens and other matrix components (29, 30). Importantly, exposure of fibroblasts to the extracts of betel nut has been suggested to trigger collagen synthesis and stabilize collagen fibrils (31, 32). Thus our results provide preliminary epidemiological evidence to suggest that betel-quid chewing may play an important role in the development of liver cirrhosis in the Taiwanese population. However, the exact mechanism of betel quid–induced liver damage needs to be further explored.

In addition to HBV infection, smoking (14, 15) and alcohol consumption (14, 16) are known determinants for liver cirrhosis. Our study also revealed that the risk of liver cirrhosis increased among individuals with smoking and alcohol

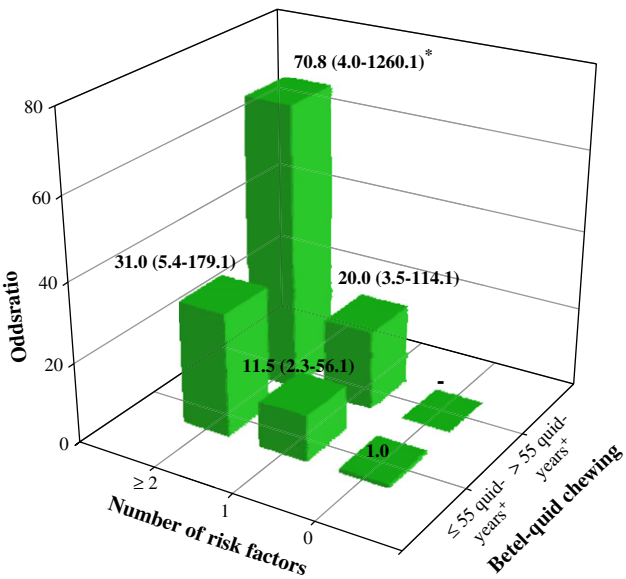

FIGURE 1. Odds ratio (OR) of liver cirrhosis by betel-quid chewing and number of risk factors, including HBsAg positivity, smoking, and alcohol drinking. *Matched OR (95% CI). †The trend in OR of liver cirrhosis with the number of risk factors was at $p = 0.01$ in the more than 55 quid-years group and at $p < 0.01$ in the less than 55 quid-years group (included never-chewers).

AEP Vol. 17, No. 6
June 2007: 479–485

drinking. Importantly, betel-quid chewers were usually also habitual smokers and alcohol drinkers in Taiwan (2). A similar finding was also observed from our kappa test. It is reasonable that betel-quid chewing might indeed be merely a marker for greater alcohol or cigarette consumption. The chewing of betel quid is popular in Taiwan (2). The habit of betel-quid chewing appears to be acquired usually at the ages of 12 and 15 years (33). In the current study, our patients usually began chewing betel quid many years before cirrhosis developed. They began chewing betel quid at a median age of 25 years (range, 12–58 years). Moreover, although none of our patients began chewing betel quid after the diagnosis of cirrhosis, it may be that cirrhotic patients are more likely to chew betel quid. Moreover, as shown in Table 3, there was an additive interaction between smoking and alcohol drinking with betel-quid chewing on liver cirrhosis. A similar result was found for betel-quid chewing with HBV infection. Our results further revealed individuals with more risk factors have a higher risk for development of liver cirrhosis. Taken together, these observations suggest an independent effect and an additive interaction in betel-quid chewing with smoking, alcohol drinking, and HBV infection on the development of liver cirrhosis. Prolonged exposure to chemicals may cause hypertrophy and hyperplasia of hepatocytes. Substance use, such as cigarette smoking, alcohol use, combined with betel-quid chewing, may accelerate the progression of hepatitis viral infection and the development of liver cirrhosis (34, 35). One explanation for the proposed interactions between hepatitis virus with betel-quid chewing, alcohol, and cigarette smoke is that liver cells infected with hepatitis viruses exist in a persistently proliferative status; thus they are more readily susceptible to challenges with hepatotoxic chemicals. Nonetheless, such an epidemiological approach may provide general indications on risks associated with putative determinants of liver cirrhosis. Results should then be tested by appropriate experimental models.

Because ultrasonographic machines with high resolution have become portable, scanning was performed in our community-based health examinations for study subjects. However, there is still a risk of misclassification if the diagnosis of liver cirrhosis is based solely on ultrasonography. A previous study showed that the sensitivity and specificity of detecting liver cirrhosis was 62.5% and 86.6%, respectively (36). One qualified gastroenterologist in our study performing the ultrasonographic examinations was blinded as to the subjects' detailed history of their smoking, alcohol drinking, and betel-quid chewing habits. Thus the misclassification was assumed to be nondifferential.

One must be aware of several limitations when interpreting results from this study. Since betel-quid chewing has not heretofore been known to be a risk factor for cirrhosis, it is important to validate that our finding is not due to confounding bias. The prevalence of HBsAg (24.2%) and anti-HCV (2.4%) in our healthy control subjects showed no significant difference from those in volunteer blood donors (37) or community controls in previous Taiwanese studies (38, 39). Therefore our controls might be representative of the general population in this community. On the other hand, previous reports have indicated that male gender and older age were associated with a higher prevalence of betel-quid chewing (2, 33). To control for the possible confounding effect of age and sex, we enrolled study subjects by matching these two variables and adjusted our results by multivariate analysis. In addition, we tried to minimize this possible bias by selecting control subjects originating from the same geographic area and ethnicity as case patients. Selection bias could have resulted when the participation rate was low. We also wanted to ensure that the control subjects were selected on an unbiased basis; comparisons of demographic characteristics between cases and controls are reassuring. In addition, a bias may have been introduced in retrospectively measuring lifetime consumptions of betel quid, cigarette smoke, and alcohol by a questionnaire. However, most of our study subjects (Atayi aborigines) daily consume constant amounts of alcohol throughout their lives, mainly in the form of rice wine. Because subjects did not know the hypothesis of the present study, recall bias, if existent, should have been limited and should not have influenced our conclusion. Decreased levels of cellular oxidative damage have been found in those subjects with increased intake of antioxidant-rich vegetables and fruits (40). However, data on fruit and vegetable intake were not available in our study. There is concern that estimates of fruit and vegetable consumption derived from a questionnaire are never free of errors, and most subjects have difficulty in estimating the frequency of consumption of specific fruits or vegetables. Further study is needed to understand the role of antioxidants such as green vegetable and fruits intake on betel quid–induced health effect. Lastly, there is concern about the small sample size in our study, which limits the statistical power to detect a small increase in risk.

In conclusion, this study suggests that higher consumption of betel quid will act as a possible risk factor for higher risks in the development of liver cirrhosis, and individuals who consume cigarettes and alcohol and who have hepatitis viral infection combined with betel-quid chewing will possess an evident risk for the development of liver cirrhosis. However, these associations should be examined again in a large sample, as the number of liver cirrhosis cases was insufficient in this study.

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