RESEARCH ARTICLE

Impact of Chronic Hepatitis B and Hepatitis C on Adverse Hepatic Fibrosis in Hepatocellular Carcinoma Related to Betel Quid Chewing

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Abstract

The pathogenesis of hepatocellular carcinoma (HCC) related to habitual betel quid (BQ) chewing is unclear. Risk of HCCis increased with adverse hepatic fibrosis. This study aimed to assess the impact of chronic viral hepatitis on adverse hepatic fibrosis in HCC related to BQ chewing. This hospital-based case-control study enrolled 200 pairs of age- and gender-matched patients with HCC and unrelated healthy controls. Serologic hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (anti-HCV), α-fetoprotein (AFP), and surrogate markers for significant hepatic fibrosis were measured. Information on substance-use habits was obtained with a questionnaire. By analysis of surrogate markers for hepatic fibrosis, the prevalence of significant hepatic fibrosis in patients chewing BQ was between 45.8% and 91.7%, whereas that for patients without BQ chewing was between 18.4% and 57.9%. The difference was significant (*P* <0.05 for each surrogate marker). Multivariate analysis indicated that cirrhosis with Child-Pugh C (odds ratio (OR) = 3.28; 95% confidence interval (CI), 1.29-8.37), thrombocytopenia (OR = 3.92, 95% CI, 1.77-8.68), AFP >400 mg/L (OR = 2.21, 95% CI, 1.05-4.66) and male gender (OR = 4.06, 95% CI, 1.29-12.77) were independent factors associated with habitual BQ chewing. In conclusion, adverse hepatic fibrosis and severe liver damage play important roles in the pathogenesis of BQ-related HCC, which could be aggravated by chronic hepatitis B and hepatitis C. BQ-cessation programs and prevention of chronic HBV/HCV infection are needed to prevent HCC related to BQ chewing.

Keywords: HCC - chronic hepatitis B and C - betel quid chewing - risk factor - surrogate markers for hepatic fibrosis

Asian Pac J Cancer Prev, **15 (2)**, 637-642

Introduction

Hepatocellular carcinoma (HCC) ranks the fifth in frequency among men and the seventh among women worldwide (Jemal et al., 2011). The well-known environmental risk factors for HCC include chronic infection with the hepatitis B virus (HBV) and hepatitis C virus (HCV) (Bouchard and Navas-Martin, 2011; El-Serag, 2012) and habitual betel quid (BQ) chewing (Tsai et al., 2001, 2004; IARC, 2004; Secretan et al., 2009; Hamed and Ali, 2013). However, the pathogenic mechanisms about HCC related to BQ chewing remain to be determined.

Betel quid chewing is common in 10%-20% of the human population (IARC, 2004). It is estimated that about 600 million people chew various types of BQ worldwide, predominantly in the countries of South and Southeast Asia (Lee et al., 2011). In Taiwan, the estimated number of habitual BQ chewers is about one-tenth of the 23 million inhabitants of Taiwan (Ko et al., 1992). The BQ prepared in Taiwan is quite different from that in other parts of the world. It consists of 2 halves of a fresh areca nut, sandwiched with a piece of the betel leaf, and red slaked lime paste. There is no tobacco added (Ko et al., 1992). BQ with or without added tobacco was classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC, 2004; Secretan et al., 2009). There is sufficient evidence that betel quid without added tobacco causes HCC (Tsai et al., 2001, 2004; IARC, 2004).

Taiwan is an area hyper-endemic for chronic HBV/HCV infection and HCC (Tsai et al., 2001, 2004; IARC, 2004; Chen et al., 2007; Kee and Lu, 2011). Persistent hepatic inflammation is a hallmark of habitual BQ chewing (Sarma et al., 1992; Lin et al., 2008; Angadi and Rao, 2011) and/or chronic HBV/HCV infection (Bouchard and Navas-Martin, 2011; El-Serag, 2012). Hepatic fibrosis, or cirrhosis, is a common end-stage condition of many chronic liver diseases (Bouchard and Navas-Martin,

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2011; Luedde and Schwabe, 2011; El-Serag, 2012). During fibrosis progression, hepatocellular damage and inflammation trigger complex cellular events that result in collagen deposition (Angadi and Rao, 2011; Bouchard and Navas-Martin, 2011; Luedde and Schwabe, 2011; El-Serag, 2012; Kong et al., 2012). Serial liver biopsies are the current gold standard to evaluate the progression of fibrosis. However, the procedure is limited by its invasive nature, expense, morbidity, intra- and inter-observer variability, and sampling errors (Lackner et al., 2005; Gumusay et al., 2013; Yan et al., 2013). To date, several routine laboratory tests, single or in combination, have been used as surrogate markers for predicting significant hepatic fibrosis (Lackner et al., 2005; Gumusay et al., 2013; Yan et al., 2013). These surrogate markers were confirmed to predict the presence of significant fibrosis, or cirrhosis with considerable diagnostic accuracy, which can be useful in predicting the progression of chronic liver disease (Lackner et al., 2005; Gumusay et al., 2013; Yan et al., 2013).

BQ chewers generally swallow the saliva completely, thus bathing the epithelial lining of the upper digestive tract with the toxins released during chewing. This increases the possibility of severe toxic effects of betel quid at sites other than the oral cavity (Gupta and Warnakulasuriya, 2002). Habitual BQ chewing independently contributes to the risk of oral submucous fibrosis (IARC, 2004; Angadi and Rao, 2011). It is a precancerous lesion of oral squamous cell carcinoma (IARC, 2004; Angadi and Rao, 2011). On the other hand, animals feeding BQ in the long term developed both chronic hepatocyte necroinflammation (Sarma et al., 1992) and liver cancer (Bhide et al, 1979; Nishikawa et al., 1992). Epidemiological data indicated that habitual BQ chewing is an independent risk factor for liver cirrhosis (Tsai et al., 2003; Hsiao et al., 2007). Cirrhosis itself is a preneoplastic lesion of HCC. The hallmark of these two premalignant diseases is fibrosis (IARC, 2004; Angadi and Rao, 2011; Bouchard and Navas-Martin, 2011; El-Serag, 2012) The risk for HCC increased with severity of liver injury and adverse fibrosis or cirrhosis (Bouchard and Navas-Martin, 2011; Luedde and Schwabe, 2011; El-Serag, 2012; Kong et al., 2012). Habitual BQ chewing has been documented as an independent risk factor for HCC (Tsai et al., 2001, 2004; IARC, 2004; Hamed and Ali, 2013). In addition, there is an interaction between habitual BQ chewing and chronic HBV/HCV infection on risk for HCC (Tsai et al., 2004). However, no data is available concerning the pathogenic mechanisms and prevention of HCC related to BQ chewing. We speculated that habitual BQ chewing may predispose to HCC via adverse hepatic fibrosis, which may be aggravated by chronic HBV/HCV infection. This case-control study was performed to prove this hypothesis.

Materials and Methods

Study population

A hospital-based case-control study was undertaken in the southern Taiwan. Two hundred consecutive newly diagnosed patients with HCC were enrolled as the case group. During the same study period, 200 unrelated healthy community residents who entered the hospital for health check-up were enrolled as the control group. Each healthy control was pair-matched by gender and age (±3 year) to a patient with HCC. These subjects were hospitalized or had visited outpatient clinics at Kaohsiung Medical University Hospital from January 2004 to December 2005. All study subjects were Han Chinese. Signed informed consent forms were obtained from all study subjects. The study was approved and the procedures followed were in accordance with the ethical standards of the Investigation and Ethics Committee of the Kaohsiung Medical University Hospital. Patients with HCC were eligible for the study if they were newly diagnosed by aspiration cytology or biopsy. HCC was staged according to the tumor-node-metastasis (TNM) system (Greene et al., 2002). Cirrhosis was diagnosed by liver biopsy, abdominal sonography, biochemical evidence of parenchymal damage plus endoscopic esophageal or gastric varices (Tsai et al., 1993). Patients with cirrhosis were classified into the 3 Child-Pugh grades based on their clinical status. There was no space-occupying lesion in the liver in any healthy control, as confirmed by normal abdominal sonography. None of the controls had symptoms, signs, or biochemical evidence of liver disease. All studied subjects were proved not to have other cancer in an initial screening examination.

Structured questionnaire and standardized interview

We designed a structured questionnaire, as described previously (Tsai et al., 2003), to obtain information on age, sex, habits of smoking (the quantity of cigarettes smoked per day and the duration of smoking), alcohol drinking (the quantity and duration of drinking, types of alcoholic beverage), and BQ chewing practice (the duration of habit, daily amount consumed, type of BQ consumed). A habitual BQ chewer was defined as chewing one quid or more daily for at least one year. A habitual cigarette smoker was defined as smoking one cigarette or more per day for at least one year. A habitual alcohol drinker was defined as drinking an alcohol beverage for more than 4 days a week for a total of at least one year. All cases and matched controls were interviewed by interviewers trained in study details and questionnaire contents. All interviews were conducted in person using the structured questionnaire.

Surrogate markers for hepatic fibrosis

From routine laboratory data, surrogate markers associated with hepatic fibrosis were calculated exactly as previously described (Lackner et al., 2005). These markers included peripheral blood platelet count, aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR), AST/platelet ratio index (APRI), Pohl score, and cirrhosis discriminant score (CDS). The selected cutoff values for significant fibrosis (platelet count ≤ 130 x10^9/L), APRI≥ 1.5, Pohl score ≥0, CDS ≥8, and AAR≥1.0) were adapted from Lackner et al. (2005).

Serologic examination

Hepatitis B surface antigen (HBsAg), antibodies to HCV (anti-HCV), and α-fetoprotein (AFP) were detected

Table 1. Basic Characteristics of the Subjects Studied

Parameters	Cases (n=200)	Controls (n=200)	P^a
Gender (M:F)	154:46	154: 46	NS
Age (median (ranges)) (yrs)	52 (41-72)	51 (41-70)	NS
HBsAg/anti-HCV			0.0001
Negative/negative	24	152	
Negative/positive	46	10	
Positive/negative	110	38	
Positive/positive	20	0	
Cirrhosis	174	-	
Child-Pugh grade			
A	70	-	
B	69	-	
C	35	-	
Tumor stage (I/II/III/IV)	22/86/84/8	-	

anti-HCV, antibodies to hepatitis C virus; HBsAg, hepatitis B surface antigen; NS, nonsignificant; [a]Continuous variables and category variables were analyzed by Man-Whitney U test and χ^2 test with Yates' correction, respectively

Table 2. Risk for HCC by Univariate and Multivariate Analyses

Risk factors	cases (n=200)	controls (n=200)	OR (95% CI)	adjusted OR[a] (95% CI)
HBsAg				
Positive	130	38	7.92 (5.01-12.51)	17.31 (9.77-30.66)
Negative	70	162	1.0	1.0
Anti-HCV				
Positive	66	10	9.36 (4.64-18.86)	28.57 (12.73-64.10)
Negative	134	190	1.0	1.0
Betel quid chewing[b]				
Yes	48	12	4.95 (2.54- 9.65)	3.73 (1.63-8.53)
No	152	188	1.0	1.0
Alcohol drinking[b]				
Yes	50	24	2.44 (1.43- 4.17)	-
No	150	176	1.0	
Cigarette smoking[b]				
Yes	102	80	1.56 (1.06-2.32)	-
No	98	120	1.0	

anti-HCV, antibodies to hepatitis C virus; CI, confidence interval; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; OR, odds ratio; [a]Adjusted for sex, age >50 years, HBsAg, anti-HCV, habitual betel quid chewing, alcohol drinking, and cigarette smoking by logistic regression analysis; [b]Multivariate analysis indicated that alcohol drinking (OR, 6.23; 95% CI, 2.85-13.65) and cigarette smoking (OR, 4.26; 95% CI, 1.80-10.1) were independent risk factors for habitual betel quid chewing

by Ausria-II, second generation Abbott HCV EIA, and α-feto RIABEAD (Abbott Laboratories, North Chicago, IL), respectively. For anti-HCV, reactive specimens were retested. Only repeatedly reactive specimens were interpreted as anti-HCV positive. Conventional liver function tests were measured by an autoanalyzer (Hitachi, Model 736, Tokyo, Japan).

Statistical analysis

The following statistical analyses were performed using the IBM SPSS Statistics (IBM Co., Armonk, NY, USA) computer software for Windows (version 19.0).

Mann-Whitney U test was used to compare the difference among medians of continuous variables. Categorical variables were compared using the χ^2 test with Yates' correction or Fisher's exact test where appropriate. Odds ratio (OR) with 95% confidence interval (95% CI) was used to estimate causal relations between risk factors and exposure. Stepwise logistic regression analysis was used for multivariate analysis. Adjusted OR and 95% CI were derived from logistic regression coefficients to provide an estimate of the statistical association between a given variable and the disease (HCC) with the other variables held constant. Two-tailed *P* values and 95% CI were given where appropriate. An α of 0.05 was used as the indicator of statistical significance.

Table 3. Surrogate Markers for Hepatic Fibrosis in Patients with HCC by Status of Habitual Betel Quid Chewing

Parameters	Betel quid chewing		P^a
	Yes (n=48)	No (n=152)	
Platelet ($x10^9$/L)	91 (28-299)[b]	135 (28-299)	0.002
AAR	1.2 (0.4-3.0)	1.0 (0.3-2.8)	0.0001
APRI	4.3 (0.5-20.6)	1.5 (0.3- 5.4)	0.0001
CDS	7 (4-10)	6 (2- 10)	0.001
Platelet <130 $x10^9$/L[c] (%)	75.0	46.1	0.0001
AAR ≥ 1.0[c] (%)	81.3	42.8	0.0001
APRI ≥ 1.5[c] (%)	91.7	48.7	0.0001
CDS ≥ 8[c] (%)	45.8	18.4	0.0001
Pohl score ≥ 1.0[c] (%)	83.3	57.9	0.001

AST, aspartic aminotransferase; ALT, alanine aminotransferase; AAR, AST/ALT ratio; APRI, AST/platelet ratio index; CDS, cirrhosis discriminant score; HCC, hepatocellular carcinoma. [a]Continuous variables and category variables were analyzed by Man-Whitney U test and χ^2 test with Yates' correction, respectively; [b]Continuous variables were expressed as medium (ranges); [c]Cutoff values for significant hepatic fibrosis were adapted from Lackner et al. (2005)

Results

Demographic information of cases and controls

Details of the demographic characteristics of subjects studied are given in Table 1. Cirrhosis was found in 87.0% of patients with HCC. At least one marker of HBsAg or anti-HCV was found in 88% of patients with HCC.

Independent risk factors for HCC by univariate and multivariate analyses

The frequencies of habitual BQ chewing, HBsAg and anti-HCV in patients with HCC (24.0%, 65% and 33.0%, respectively) were higher than those in controls (6.0%, 19% and 5.0%, respectively; each $P = 0.0001$; Table 2). Using healthy controls as a reference group, univariate analysis indicated that HBsAg (OR=7.92; $P = 0.0001$), anti-HCV (OR=9.36; $P = 0.0001$), habitual BQ chewing (OR=4.95; $P = 0.0001$), habitual alcohol drinking (OR=2.44; $P = 0.001$), and habitual cigarette smoking (OR=1.56; $P = 0.027$) were associated with HCC (Table 2). Multivariate analysis indicated that HBsAg (OR= 17.31, $P = 0.0001$), anti-HCV (OR=28.57, $P = 0.0001$), and habitual BQ chewing (OR=3.73, $P = 0.002$) were independent risk factors for HCC (Table 2).

Further analysis indicated that habitual cigarette smoking (OR=4.26, $P = 0.0001$) and habitual alcohol drinking (OR=6.23, $P = 0.0001$) were independent factors for habitual BQ chewing (Table 2).

Table 4. Surrogate Markers for Hepatic Fibrosis in Patients with Hepatocellular Carcinoma by Betel Quid Chewing and Status of Viral Hepatitis

Hepatitis	BQ chewing	n	Platelet[a,b] (%)	AAR[a,b] (%)	APRI[a,b] (%)	CDS[a,b] (%)	Pohl score[a,b] (%)
NBNC	nonuser	8	12.5	25.0	50.0	12.5	0.0
NBNC	user	6	83.3[c]	100[c]	100[c]	83.3[c]	83.3[d]
HBV	nonuser	46	26.1	56.5	56.5	8.7	23.9
HBV	user	26	92.3[d]	84.6[c]	84.6[c]	30.8[c]	76.9
HCV	nonuser	20	50.0	15.0	55.0	20.0	15.0
HCV	user	12	100[c]	58.3[c]	100[c]	66.7[c]	58.8[c]
HBV/HCV	nonuser	8	12.5	12.5	12.5	0.0	12.5
HBV/HCV	user	4	100[c]	100[c]	100[c]	75.0[c]	100[c]

AST, aspartic aminotransferase; ALT, alanine aminotransferase; AAR, AST/ALT ratio; APRI, AST/platelet ratio index; BQ, betel quid; CDS, cirrhosis discriminant score; HBV, hepatitis B virus; HCV, hepatitis C virus; hepatitis NBNC, hepatitis non-B, non-C; [a]χ^2 test with Yates' correction; [b]Cutoff values for significant hepatic fibrosis (Platelet <130 x10^9/L; AAR ≥ 1.0; APRI ≥ 1.5; CDS ≥ 8; Pohl score ≥ 1.0) were adapted from Lackner et al (2005); [c]P <0.05 (patients with BQ chewing vs. those without); [d]P <0.01 (patients with BQ chewing vs. those without)

Table 6. Multivariate Analysis of Independent Factors for Betel Quid Chewing in Patients with HCC[†]

Variables	β	SE	P value	OR (95% CI)
Cirrhosis with Child-Pugh C	1.19	0.48	0.013	3.28 (1.29-8.37)
Platelet count <130 x 10^9/L	1.37	0.41	0.001	3.92 (1.77-8.68)
AFP > 400 mg/L	0.79	0.38	0.037	2.21 (1.05-4.66)
Male gender	1.40	0.58	0.016	4.06 (1.29-12.77)

AFP, α-fetoprotein; β, coefficient; CI, confidence interval; HCC, hepatocellular carcinoma; OR, odds ratio; SE, standard error; [†]Stepwise logistic regression analysis: Dependent variable: habitual betel quid chewing. Independent variables: male gender, age >50 years, cirrhosis with Child-Pugh C, AFP > 400 mg/L, and platelet count <130 x 10^9/L

Surrogate markers for hepatic fibrosis in patients with HCC by status of BQ chewing and types of chronic viral hepatitis

As shown in Table 3, HCC patients with habitual BQ chewers tended to have lower peripheral blood platelet count (P = 0.002), and higher levels of AAR (P = 0.0001), APRI (P = 0.0001), and CDS (P = 0.001). Using cutoff values for significant hepatic fibrosis defined by Lackner et al. (2005), the frequencies of thrombocytopenia (platelet < 130 x 109/L, P = 0.0001), AAR ≥1.0 (P = 0.0001), APRI ≥ 1.5 (P = 0.0001), CDS ≥ 8 (P = 0.0001), and Pohl score ≥ 1.0 (P = 0.001) in HCC patients with BQ chewers were higher than those in BQ non-chewers. Putting data together, significant hepatic fibrosis was noted between 45.8% and 91.7% of patients with BQ chewing, and between 18.4% and 57.9% of patients without BQ chewing (Table 3). Furthermore, when patients were divided by types of chronic viral hepatitis (Table 4), surrogate markers for hepatic fibrosis in patients with BQ chewers were still significantly higher than those in BQ non-users regardless of type of hepatitis (Table 4).

Clinical characteristics in patients with HCC by status of habitual BQ chewing

As shown in Table 5, habitual BQ chewers in patients with HCC were male predominant (P = 0.006). All of them were patients with cirrhosis (P = 0.0001; Fisher's exact test). Moreover, the higher the Child-Pugh grade, the higher the frequency of being a habitual BQ chewer (P for trend = 0.0001). Further analysis indicated that the frequency of chewing BQ in patients with Child-Pugh grade C was higher than that in patients with Child-Pugh grade B (P = 0.003) or grade A (P= 0.0001). Compared to patients with Child-Pugh A, those with Child-Pugh B also had higher frequency of being BQ chewers (P = 0.028). HCC patients with BQ chewing had higher frequency of serum AFP > 400 mg/L (P = 0.030). There was no significant difference with regard to sex, older age (>50 years), status of HBsAg or anti-HCV, stage and size of HCC (Table 5). Additionally, there were significantly lower serum albumin level and higher levels of bilirubin, AST, ALT and alkaline phosphatase in HCC patients chewing BQ (data not shown). Multivariate analysis

Table 5. Clinical Parameters in Patients with Hepatocellular Carcinoma by Status of Habitual Betel Quid Chewing

Parameters/ groups	n	With betel quid chewing n (%)	P^a
Gender			0.006
Male	154	44 (28.6)	
Female	46	4 (8.7)	
Age (yr)			NS
≤ 50	84	22 (26.2)	
> 50	116	26 (22.4)	
Cirrhosis			0.0001
Yes	174	48 (27.6)	
No	26	0 (0.0)	
Child-Pugh grade			0.0001
A[b]	70	8 (11.4)[c,d]	
B[b]	69	19 (27.5)[d,e]	
C[b]	35	21 (60.0)[c,e]	
HBsAg/anti-HCV			NS
Negative/Negative	20	4 (20.0)	
Negative/Positive	46	12 (26.1)	
Positive/Negative	110	26 (23.6)	
Positive/Positive	24	6 (25.0)	
AFP (mg/L)			0.030
≤ 400	134	26 (19.4)	
> 400	66	22 (33.3)	
AST (IU/L)			NS
≤ 40 (ULN)	36	10 (27.7)	
> 40	164	38 (23.2)	
ALT (IU/L)			0.001
≤ 40 (ULN)	42	2 (4.8)	
> 40	158	46 (29.1)	
HCC stages			NS
I + II	108	26 (24.1)	
III + IV	92	22 (23.9)	
Tumor size			NS
Diffuse type	44	10 (22.7)	
≥ 5 cm	48	12 (25.0)	
< 5 cm	108	26 (24.0)	

AFP, α-fetoprotein; anti-HCV, antibodies to hepatitis C virus; AST, aspartic aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; NS, not significant; ULN, upper limit of normal; [a]χ^2 test with Yates' correction or Fisher's exact test where appropriate; [b]P for trend = 0.0001 (Mantel-extension test for trend); [c]P = 0.0001; [d]P = 0.028; [e]P = 0.003

indicated that cirrhosis with Child-Pugh C (OR=3.28, P =0.013), thrombocytopenia (OR=3.92, P = 0.001), AFP >400 mg/L (OR=2.21, P = 0.037), and male gender (OR=4.06, P = 0.016) were independent factors for habitual BQ chewing (Table 6).

Discussion

By a formal epidemiological approach and application of non-invasive surrogate markers for hepatic fibrosis, this study indicated that habitual BQ chewing was correlated with adverse hepatic fibrosis (Table 3 and Table 4) and more severe liver damage (Table 5 and Table 6). Further analysis indicated that chronic HBV/HCV infection aggravated the adverse hepatic fibrosis (Table 4) which may contribute to the pathogenesis of BQ-related HCC (Table 4, Table 5, and Table 6).

The principal strengths of the current study are the compelling association identified. It is known that persistent hepatic inflammation is a hallmark of habitual BQ chewing (Sarma et al., 1992; Gupta and Warnakulasuriya, 2002; Lin et al., 2008) and/or chronic HBV/HCV infection (Bouchard and Navas-Martin, 2011; El-Serag, 2012). Our results indicated that habitual BQ chewing do increase risk for HCC (Table 2) and hepatic fibrosis regardless of chronic hepatitis B or hepatitis C (Table 4 and Table 5). Habitual BQ chewing in patients with chronic HBV/HCV infection may cause more adverse hepatic fibrosis and disease severity (Table 5 and Table 6). Hence, we validate our hypothesis that habitual BQ chewing increases the risk for HCC via adverse fibrosis and more severe liver damage.

The pathogenic mechanisms between habitual BQ chewing and HCC risk have not been fully clarified. Our results indicated that the frequency of significant hepatic fibrosis in HCC patients with BQ chewing was significantly higher than those without (Table 3 and Table 4). In addition, among the independent factors associated with habitual BQ chewing (Table 5 and Table 6), cirrhosis with Child-Pugh C implies adverse fibrosis and severe liver damage. Earlier study implies an association between elevated serum AFP level and hepatic fibrosis (Bruce et al., 2008). Moreover, there is a strong correlation between low platelet count and hepatic fibrosis and/or disease severity (Lu et al., 2006; Rodrıguez-Dıaz et al., 2007). Thrombocytopenia itself is also a predictor of HCC development (Lu et al., 2006; Rodrıguez-Dıaz et al., 2007). In this context, adverse hepatic fibrosis (or cirrhosis) and severe liver injury may predispose to HCC development. Our observation was in agreement with previous reports that the risk for HCC increased with severity of liver injury and adverse fibrosis (Lu et al., 2006; Rodrıguez-Dıaz et al., 2007; Angadi and Rao, 2011; Bouchard and Navas-Martin, 2011; Luedde and Schwabe, 2011; El-Serag, 2012). BQ chewing may accelerate progression of chronic liver disease (or cirrhosis) to HCC owing to its accelerated progression of hepatic fibrosis and liver injury and worsens chronic liver disease.

Habitual BQ chewing results in a chronic inflammatory process characterized by activation of inflammatory cells like T cells and macrophages. These cells release and/or stimulate the synthesis of various cytokines (Jeng et al., 2007; Chang et al., 2009; Angadi and Rao, 2011). We had reported previously that there were independent and additive interaction among the tumor necrosis factor 308.2 allele, substance use habits, and chronic HBV/HCV infection on risk for HCC (Jeng et al., 2009). In fact, areca nut may mediate proinflammatory and fibrogenic cytokines such as tumor necrosis factor α and transforming growth factor (TGF) β and reduce antifibrotic cytokine (interferon-γ) (Jeng et al., 2007; Matsuzaki et al., 2007; Angadi and Rao, 2011). Additionally, TGF β favors collagen production and decreases the degradation of collagen. Moreover, chronic inflammation associated with HBV/HCV infection shifts hepatic TGF β signaling from tumor-suppression to fibrogenesis, accelerating liver fibrosis and increasing risk for HCC (Matsuzaki et al., 2007; Murata et al., 2009). This information may interpret the interaction between habitual BQ chewing and chronic HBV/HCV infection on risk of HCC (Tsai et al., 2004). Hence, habitual BQ chewing predisposes to adverse fibrosis progression and aggravates liver damage in the presence of chronic HBV/HCV infection.

It is known that chronic oxidative stress is a potentially important pathogenic mechanism in habitual BQ chewing (IARC, 2004) and chronic HBV/HC0V infection (Bouchard and Navas-Martin, 2011; El-Serag, 2012). . Chronic liver injury produces oxygen-derived free radicals and other reactive oxygen or nitrogen species that may activate NFκB (Luedde and Schwabe, 2011). Additively, both areca nut and safrole in betel leaf can activate NFκB (Ni et al., 2007; Lu et al., 2010; Lin et al., 2012). They have been implicated as important mediators of hepatic fibrogenesis in liver injury (Luedde and Schwabe 2011).

Our study had some limitations. As this is a case-control study, a prospective study to confirm our hypothesis is urgent. Another shortcoming is the rather small sample size and, therefore, the results should be confirmed in a larger series as well as in patients from different ethic origin. In conclusion, habitual BQ chewing, with or without chronic HBV/HCV infection, correlates with adverse fibrosis and more severe liver damage that contributes to a higher risk for HCC. Our study points out that BQ-cessation programs and prevention of chronic HBV/HCV infection should be developed to prevent BQ-related HCC.

Acknowledgements

This study was supported in part by grants from the National Science Council of Taiwan (NSC 93-2314-B-037-079).

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