International Journal of Epidemiology, 2014, 1174–1182
doi: 10.1093/ije/dyu009
Advance Access Publication Date: 17 February 2014
Original article

NON-COMMUNICABLE DISEASE RISK FACTORS

Association between betel quid chewing and carotid intima-media thickness in rural Bangladesh

Tyler R McClintock,[1] Faruque Parvez,[2] Fen Wu,[1] Weijia Wang,[1] Tariqul Islam,[3] Alauddin Ahmed,[3] Ishrat Shaheen,[3] Golam Sarwar,[3] Ryan T Demmer,[4] Moise Desvarieux,[4,5,6] Habibul Ahsan[7] and Yu Chen[1]*

[1]Departments of Population Health and Environmental Medicine, New York University School of Medicine, New York, NY, USA, [2]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY, USA, [3]U-Chicago Research Bangladesh, Dhaka, Bangladesh, [4]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA, [5]INSERM, UMR-S 707, Université Pierre et Marie Curie-Paris 6, Paris, France, [6]École des Hautes Études en Santé Publique, Paris and Rennes, France and [7]Departments of Health Studies, Medicine and Human Genetics and Comprehensive Cancer Center, University of Chicago, Chicago, IL, USA

*Corresponding author. Department of Population Health, New York University School of Medicine; 650 First Avenue, New York, NY 10016, USA. E-mail: yu.chen@nyumc.org.

Accepted 9 January 2014

Abstract

Background: Areca nut, more commonly known as betel nut, is the fourth most commonly used addictive substance in the world. Though recent evidence suggests it may play a role in the development of cardiovascular disease, no studies have investigated whether betel nut use is related to subclinical atherosclerosis.

Methods: We evaluated the association between betel nut use and subclinical atherosclerosis in 1206 participants randomly sampled from the Health Effects of Arsenic Longitudinal Study (HEALS). Frequency and duration of betel nut use were assessed at baseline, and carotid IMT was measured on average 6.65 years after baseline.

Results: A positive association was observed between duration and cumulative exposure (function of duration and frequency) of betel nut use and IMT, with above-median use for duration (7 or more years) and cumulative exposure (30 or more quid-years) corresponding to a 19.1 μm [95% confidence interval (CI): 5.3-32.8; $P \leq 0.01$] and 16.8 μm (95% CI: 2.9-30.8; $P < 0.05$) higher IMT in an adjusted model, respectively. This association was more pronounced in men [32.8 μm (95% CI: 10.0-55.7) and 30.9 μm (95% CI: 7.4-54.2)]. There was a synergy between cigarette smoking and above-median betel use such that the joint exposure was associated with a 42.4 μm (95% CI: 21.6-63.2; $P \leq 0.01$) difference in IMT.

Conclusion: Betel nut use at long duration or high cumulative exposure levels is associated with subclinical atherosclerosis as manifested through carotid IMT. This effect is especially pronounced among men and cigarette smokers.

Key words: Areca nut, Bangladesh, betel nut, cardiovascular disease, carotid intima-media thickness

Key Messages

- Betel nut is an addictive substance used by more than 10% of the world's population on a daily basis.
- We evaluated the association between betel nut use and carotid intima-media thickness in 1206 participants randomly sampled from the Health Effects of Arsenic Longitudinal Study (HEALS).
- Betel nut use at long duration or high cumulative exposure levels is associated with subclinical atherosclerosis as manifested through carotid IMT. This effect is especially pronounced among men and cigarette smokers.

Introduction

Areca nut, more commonly known as betel nut, is the fourth most commonly used addictive substance in the world, following caffeine, nicotine and alcohol.[1] A seed of the tropical palm *Areca catechu*, betel nut is the major constituent in a mix of slaked lime and flavourings known as betel quid. Wrapped in a Piper-betel leaf, this combination is chewed widely throughout Central, Southeast, and Southern Asia, as well as in some South Pacific Islands. In total, it is believed that roughly 600 million individuals—or more than 10% of the world's population—chew betel nut on a daily basis.[2,3]

Betel nut has been labelled by the International Agency for Research on Cancer as a human carcinogen, with its use having been significantly linked to precancerous oral fibrosis as well as cancer of the oral cavity, pharynx and oesophagus.[2] Similar effects have been observed in the liver, as an increased risk of both cirrhosis and hepatocellular carcinoma has been demonstrated among users.[4] Beyond carcinogenesis, recent evidence suggests that use of betel nut may play a role in the development of such systemic diseases as metabolic syndrome,[5] hypertension,[6,7] diabetes mellitus,[8,9] obesity,[10] and cardiovascular disease.[11–14]

With cardiovascular disease (CVD) standing as the leading cause of death worldwide,[15,16] the possible role of betel nut in its development is a significant and pressing concern. Modifiable lifestyle factors are thought to cause the majority of CVD cases, which makes identification of these risks a crucial first step in the development of targeted prevention strategies and early interventions. Several studies have implicated long-term betel nut chewing as an independent risk factor for CVD; however, little is known about related pathological mechanisms. In addition, all existing studies on this topic have included men only and, as a result, the effect of betel nut use on women is largely unknown. Further limiting the interpretation of prior studies, as there is high concomitant use of cigarettes along with betel nut, is a lack of data on whether the effect of betel nut is independent of or synergistic with the effect of cigarette smoking.

Carotid artery intima media thickness (IMT) is a validated surrogate marker of preclinical atherosclerosis that has been shown to predict cardiovascular morbidity and mortality.[17,18] Whereas many studies have sought to examine CVD risk factors in relation to carotid IMT as a mechanistic intermediate, none have investigated whether the theorized cardiovascular effects of betel nut chewing might be manifested as subclinical atherosclerosis. Here, we utilize carotid IMT to assess atherosclerotic change in association with betel nut use within a subgroup of participants in the prospective, population-based Health Effects of Arsenic Longitudinal Study (HEALS).

Materials and Methods

Study population

The parent study, Health Effects of Arsenic Longitudinal Study (HEALS), is an ongoing population-based prospective cohort study in Araihazar, Bangladesh.[19] Briefly, between October 2000 and May 2002, 11 746 men and women ('original cohort') were recruited from a well-defined 25-km^2 geographical area, under the criteria that all were married (to reduce loss to follow-up), between 18 and 75 years old and had resided in the study area for at least 5 years. From 2006 to 2008, HEALS was expanded

to include an additional 8287 participants ('expansion cohort') following the same methodologies. The overall response rate was 97%. Study participants underwent baseline clinical assessment and structured interviews.

A total of 800 participants were randomly selected from the 11 224 original cohort members who provided urine samples at baseline, as part of a previous study on urinary arsenic and IMT.[20] A total of 700 participants were randomly sampled from the 5136 participants older than 30 years of age in the expansion cohort, as the expansion cohort was, on average, younger. In total, carotid IMT was measured for 1206 individuals, consisting of 600 from the original cohort and 606 from the expansion cohort, and 294 participants did not complete IMT measurements due to death, move, serious illness or time constraints. The distributions of demographic and lifestyle variables in the study population and in the overall cohort were very similar (data not shown). Informed consent was obtained from study participants; study procedures were approved bythe Ethical Committee of the Bangladesh Medical Research Council and the Institutional Review Boards of Columbia University and the University of Chicago.

Assessment of betel usage

Baseline in-person interview was conducted by a trained interviewer and included detailed enquiries on lifestyle characteristics. Participants were asked about their present and past use of betel quid, the number of times per day betel was chewed, the duration of betel use and, in the expansion cohort only, whether betel was chewed with jorda (tobacco leaves). They were queried as to their use of cigarettes or bidis (hand-rolled cigarettes), and also answered questions on demographics, religion and socio-economic measures (television and land ownership, occupation, years of education). Clinical evaluations were conducted by local trained physicians. The present study used baseline data from both the original and the expansion cohorts.

Measurement of carotid IMT

Carotid IMT was measured between April 2010 and September 2011, an average of 6.65 years after baseline (collection of data regarding betel use). Detailed methods for measurements of carotid IMT have been described previously.[20] Briefly, the measurements were conducted using a SonoSite MicroMaxx ultrasound machine (SonoSite, Bothell, WA) equipped with a L38e/10-5 MHz transducer. All carotid imaging and IMT measurements were performed by a single physician who was trained and certified to perform carotid ultrasound measurements according to the specific ultrasound imaging and reading protocols developed, implemented and validated in the Oral Infections and Vascular Disease Epidemiology Study (INVEST).[21] The use of this strict INVEST protocol has minimized the variability of scanning due to technical settings and led to a 50% reduction in the standard deviations of IMT values, with both intra- and inter-reader variability >0.94. IMT measurements were analyzed offline with Matlab (Mathworks, Natick, MA), which automatically calculated the distances between boundaries and expressed the results as the mean and maximal values. In accordance with the Mannheim consensus, we used the mean of the near and far walls of the maximum common carotid artery IMT from both sides of the neck (mean of four measurements) as the main outcome variable, similar to previous studies.[22–24] Although IMT values are typically presented in units of tenths of a millimetre, we present our data here in thousandths of a millimetre to better illustrate effect sizes of smaller magnitudes, as well as to remain consistent with our previous studies.[20]

Statistical analyses

Descriptive analyses were conducted first to characterize the study population in terms of demographics, smoking status and betel use. A series of linear regression models were constructed to analyse the association of betel use with carotid intima-media thickness. These compared differences in IMT between ever and never betel users; never, low (one to three times/day) and high (four or more times/day) frequency users; never, low (1–6 years), and high (7 or more years) duration users; and never, low (1–30 quid-years), and high (over 30 quid-years) total exposure users. The total exposure variable (quid-years) signified the product of years and frequency of use, similar to pack-years for cigarette smoking. The delineation of high and low users within each category was based upon the median value of each respective metric among betel nut users. Two models were constructed in the assessment of each measure of betel use, with the model 1 adjusting for age, body mass index (BMI) and sex; model 2 additionally adjusted for education duration, baseline systolic blood pressure and any past or current cigarette smoking. For each model, results were computed for the overall cohort, as well as for men and women separately. Additional models adjusting for diabetes did not yield results significantly different from the results presented here; hence, those data are not shown.

We also evaluated whether the effects of betel nut on IMT differ by sex or cigarette smoking, and whether the joint effect of betel nut use and cigarette smoking on IMT are synergistic (positive interaction on the additive scale).

Here, cigarette smoking was defined as ever or never, whereas betel consumption intensity was defined as never, low, and high. Again, low and high betel consumption was based on the median value of 30 quid-years in users. Interaction was tested using two cross-product terms; one between the dummy variable for low betel nut consumption and sex (or cigarette smoking), and the other between the dummy variable for high betel nut consumption and sex (or cigarette smoking). *P*-values associated with the cross-product terms were used to judge significance of the interaction. We also assessed differences in IMT by joint status of cigarette smoking and levels of betel nut consumption overall and by sex, with non-users of betel nut and never smokers as the reference group. In addition, in the expansion cohort ($n = 606$), we assessed whether IMT levels differ among never users of betel nut, users of betel nut chewed with jorda (tobacco leaves) and users of betel nut alone. All analyses were conducted using SPSS version 20 (SPSS , Chicago, IL).

Results

Characteristics of the study population are shown in Table 1. Higher IMT was associated with the male sex ($P < 0.01$), increasing age ($P < 0.01$), higher BMI ($P = 0.02$) and elevated blood pressure ($P < 0.01$). An unadjusted analysis showed cigarette smoking to be associated with higher IMT as ever smokers increased from 17.6% prevalence in the first IMT quartile to 54.7% in the fourth ($P < 0.01$). Betel nut chewing displayed a similar trend, as current use was reported among 27%, 28.3%, 39.5% and 49.3% of those in the first through fourth quartiles, respectively ($P < 0.01$).

Table 2 shows associations between a variety of betel use metrics and IMT. Duration of use was related to IMT, with a difference of 18.20 μm (95% CI: 4.36, 32.05; $P \leq 0.01$) comparing those using >6 years (median use time) with non-users, adjusting for age, BMI and sex. This association was strengthened when further adjusting for education, baseline systolic blood pressure and ever smoking [19.06 μm (95% CI: 5.30, 32.82; $P \leq 0.01$)]. Sex-specific analysis showed the relationship with duration of use to persist among men [32.81 μm (95% CI: 9.95, 55.67; $P \leq 0.01$)], though not in women (*P* for interaction < 0.01). Intensity of use was similarly related to increased IMT in the overall population [16.83 μm (95% CI: 2.89, 30.78; $P < 0.05$)] and within men [30.92 μm (95% CI: 7.41, 54.21; $P \leq 0.01$)], though not for women [11.51μm (95% CI: -5.91, 28.93; $P = 0.20$)]. Neither frequency of betel use nor ever use exhibited a relationship with IMT.

Cigarette smoking in never betel nut users was associated with a non-significant increase of 6.19 μm (95% CI: -11.11, 23.49) in IMT, and high betel nut consumption alone was related to a non-significant increase of 3.91 μm (95% CI: -13.31, 21.12). There was evidence for a synergy between betel nut use and smoking on IMT levels such that smoking and high betel nut consumption (>30 quid-years) were associated with an IMT difference of 42.39 μm (95% CI: 21.59, 63.20) relative to individuals who reported never smoking or betel nut use. The test for interaction

Table 1. Distribution of population characteristics and betel nut use by carotid IMT quartile

Characteristics	Overall [$n = 1206$]	Quartile 1 (557.5–717.5 μm) [$n = 307$]	Quartile 2 (717.5–770.0 μm) [$n = 300$]	Quartile 3 (770.0–840.0 μm) [$n = 299$]	Quartile 4 (840.0–1242.5 μm) [$n = 300$]	*P**
Carotid IMT (μm)	790.2	682.9	745.2	803.0	932.1	<0.01
Male (%)	39.0	23.5	37.0	38.8	57.0	<0.01
Age at IMT measurement (years)	45.5	39.6	43.6	46.7	52.1	<0.01
Body mass index (kg/m^2)	20.0	19.5	20.1	20.2	20.1	0.02
Cigarette use (%)						<0.01
Past	6.1	2.0	3.3	8.0	11.0	
Current	28.5	15.6	27.3	27.8	43.7	
Betel nut use (%)						<0.01
Past	3.6	2.3	2.7	3.3	6.0	
Current	36.0	27.0	28.3	39.5	49.3	
Betel frequency among ever users (times/day)	5.2	4.3	5.2	5.4	5.6	0.04
Betel duration among ever users (years)	11.0	6.1	10.4	11.1	13.8	<0.01
Education (years)	3.0	3.3	2.9	3.1	2.8	0.46
Prior diagnosis of diabetes mellitus (%)	1.9	1.0	0.7	1.7	4.3	<0.01
Systolic blood pressure (mmHg)	117.4	112.1	115.1	118.4	124.2	<0.01
Diastolic blood pressure (mmHg)	75.5	73.5	74.0	76.3	78.3	<0.01

*Chi-square test was used for categorical variables and ANOVA for continuous variables.

Table 2. Associations between betel use and intima-media thickness

	Effect estimate (95% CI)									
	Overall ($n = 1206$)			Men ($n = 470$)			Women ($n = 736$)			P for interaction by sex
	N[a]	Model 1[b]	Model 2[c]	n	Model 1	Model 2	n	Model 1	Model 2	
Betel nut use										
Never	729	Reference	Reference	287	Reference	Reference	442	Reference	Reference	
Ever	477	3.85 (−7.01, 14.72)	4.68 (−6.20, 15.56)	183	11.76 (−6.23, 29.74)	10.50 (−7.61, 28.60)	294	−0.35 (−14.00, 13.30)	2.53 (−11.10, 16.16)	0.06
Frequency of use										
None	729	Reference	Reference	287	Reference	Reference	442	Reference	Reference	
Low (1–3 times/day)	179	7.96 (−6.41, 22.34)	7.44 (−6.93, 21.81)	90	11.81 (−10.65, 34.27)	8.91 (−13.61, 31.44)	89	4.19 (−14.54, 22.93)	6.19 (−12.47, 24.85)	0.52
High (>3 times/day)	296	0.86 (−11.92, 13.64)	2.57 (−10.12, 15.26)	93	11.70 (−10.87, 34.27)	12.06 (−10.36, 34.48)	203	−2.83 (−18.38, 12.71)	0.53 (−14.87, 15.93)	0.04
Duration of use										
None	729	Reference	Reference	287	Reference	Reference	442	Reference	Reference	
Low (1–6 years)	229	−7.94 (−20.87, 4.99)	−7.30 (−20.18, 5.58)	93	−6.82 (−28.61, 14.97)	−9.03 (−30.84, 12.77)	136	−8.41 (−24.29, 7.48)	−5.53 (−21.31, 10.24)	0.85
High (>6 years)	246	18.20 (4.36, 32.05)**	19.06 (5.30, 32.82)**	90	33.32 (10.32, 56.33)**	32.81 (9.95, 55.67)**	156	10.69 (−6.77, 28.15)	13.47 (−3.83, 30.78)	<0.01
Intensity of use										
None	729	Reference	Reference	287	Reference	Reference	442	Reference	Reference	
Low (1–30 quid-years)	235	−4.82 (−17.65, 8.01)	−4.84 (−17.61, 7.94)	99	−3.54 (−24.91, 17.82)	−5.22 (−26.65, 16.21)	136	−5.62 (−21.50, 10.25)	−3.90 (−19.64, 11.84)	0.69
High (>30 quid-years)	240	15.06 (1.03, 29.09)*	16.83 (2.89, 30.78)*	84	31.95 (8.33, 55.58)**	30.92 (7.41, 54.21)**	156	7.05 (−10.50, 24.60)	11.51 (−5.91, 28.93)	<0.01

[a]Incomplete data on betel frequency and duration for 2 participants.
[b]Adjusted for age, BMI, and sex.
[c]Adjusted for age, BMI, sex, education duration, baseline systolic blood pressure and ever smoking.
*$P < 0.05$
**$P \leq 0.01$

yielded a *P*-value for interaction equal to 0.01 (Table 3), suggesting that the joint effect of cigarette smoking and high level of betel nut consumption in IMT was greater than the sum of their individual effects. This synergy was driven by findings among men (Table 3). It should be noted that confounding due to differences in cigarette consumption were not likely to have played a role in this effect as cigarettes per day ($P = 0.18$), years of smoking ($P = 0.82$) and pack-years of cigarette smoking ($P = 0.82$) did not differ by the levels of intensity of betel use.

In expansion cohort members (for whom we have information on whether betel nut was chewed with jorda), we evaluated the association between betel nut consumption and IMT by the use of jorda. Among 606 members of the expansion cohort, 203 (33.5%) used betel nut together with jorda, whereas 50 (8.3%) used betel without jorda. In linear regression, levels of IMT were non-significantly higher in individuals who used betel nut with or without jorda, with an estimate of 3.28 μm (95% CI: −13.87, 20.43) and 6.53 μm (95% CI: −20.71, 33.77), respectively, compared with never users. We were not able to stratify the analyses on the intensity of betel nut use and IMT by the use of jorda due to limited sample size.

Discussion

To the best of our knowledge, we believe the present analysis to be the first describing a positive association of betel nut with a surrogate marker of subclinical atherosclerosis or CVD. In particular, we have shown that betel nut use at long duration or high cumulative exposure levels is associated with higher levels of preclinical atherosclerosis. Long-term betel nut consumption was related to a difference of 19.06 μm (95% CI: 5.30, 32.82) and 32.81 μm (95% CI: 9.95, 55.67) in IMT overall and among men, respectively. Within our population, this effect was especially pronounced among men and cigarette smokers. Prior literature has shown clinically relevant differences in morbidity and mortality corresponding to IMT increases of 8.2 to 40 μm (albeit in studies of varying design types)[25–28] and, more specifically, that a 100-μm increase in IMT could relate to a 50% increased risk of coronary heart disease (CHD).[29] Hence, the IMT difference (increase of 19.1 μm) associated with above-median betel use (7 or more years) in our population could translate to a roughly 10% increased risk of CHD, although more research is needed for confirmation. Given the high prevalence of CHD, a small increase in this subclinical marker could translate to a substantial number of additional cases. This is especially significant as betel use is widespread in Bangladesh, and the country is now entering a period where prevalence of chronic disease is on the rise, coincident with economic development.

Our findings are consistent with prior studies suggesting a link between betel nut use and CVD. To date, all such investigations have taken place among Taiwanese men. The first, by Yen *et al.*, was a prospective cohort study of nearly

Table 3. Associations between joint intensity of baseline betel and tobacco consumption with intima-media thickness

Cigarette smoking status	Betel consumption intensity	Overall		Men		Women	
		n[b]	Effect estimate (95% CI)[a]	*n*	Effect estimate (95% CI)	*n*	Effect estimate (95% CI)
Never	No	530	Reference	101	Reference	429	Reference
Never	Low[c]	121	−7.54 (−24.38, 9.30)	6	−40.35 (−114.21, 33.51)	115	−2.63 (−19.22, 13.96)
Never	High[d]	136	3.91 (−13.31, 21.12)	12	−16.74 (−70.59, 37.12)	124	13.18 (−5.10, 31.45)
Ever	No	199	6.19 (−11.11, 23.49)	186	5.98 (−16.59, 28.55)	13	29.76 (−13.05, 72.56)
Ever	Low	114	7.90 (−11.68, 27.48)	93	6.39 (−20.06, 32.85)	21	6.74 (−28.18, 41.66)
Ever	High	104	42.39 (21.59, 63.20)*	72	48.09 (17.98, 78.20)*	32	22.12 (−8.45, 52.70)
P for interaction between smoking and low level consumption			0.47		0.30		0.47
P for interaction between smoking and high level consumption			0.01		0.05		0.43

[a]Adjusted for age, BMI, sex, education duration and baseline systolic blood pressure.
[b]Incomplete data on betel consumption intensity for 2 participants.
[c]Defined as median intensity or below (1–30 quid-years).
[d]Defined as above median intensity (>30 quid-years).
*$P < 0.01$.

75 000 men taking part in a community-based screening programme with 3 years of follow-up. In a final model adjusted for age, education, smoking status, dietary intakes and a number of additional confounders, ever chewers were at a 24% higher risk of CVD ($P < 0.01$). Furthermore, a significant dose-response relationship was observed ($P < 0.05$).[12] A study by Lin *et al.* set mortality rather than disease incidence as the primary endpoint, utilizing over 56 000 men pooled from four nationwide health screen centres. The hazard ratio for CVD mortality was 1.56 (95% CI: 1.02, 2.38) and 2.02 (95% CI: 1.31, 3.13) comparing former betel nut chewers and current chewers with never chewers, respectively.[11] Lastly, a hospital-based case-control study by Tsai *et al.* in 2012 demonstrated a 3.5-fold increased risk of obstructive coronary artery disease among men who had ever been betel nut chewers.[14]

We found that the effects of high betel nut consumption on IMT may be greater in men and smokers. For both duration of betel use and cumulative exposure, there was a highly significant association with IMT in men, though not women. All existing studies on CVD risk are restricted to men, likely reflecting the fact that betel nut was mostly used by men and rarely by women among Taiwanese.[30] This stands in contrast to our study population, as the prevalence of betel nut use was similar by sex (38.4% of men and 32.5% women). The observation of a synergistic effect between cigarette smoking and betel nut use in IMT may explain the sex difference, as 64.0% of men and only 14.4% of women were ever smokers in our study population. This finding, therefore, may also have important public health implications in that cessation of smoking or betel nut use may lead to a greater than expected reduction in IMT and possibly subsequent CVD morbidity and mortality. As smokers were also more likely to use betel nut in other populations,[11,12] future interventions may consider targeting men or smokers if findings from the present study are confirmed.

Many of the detrimental effects of betel nut chewing could explain its association with CVD and the atherosclerotic changes observed here. Betel nut use activates the sympathetic nervous system, triggering release of adrenal catecholamines that cause vasoconstriction along with elevated heart rate and blood pressure.[1,2,31] Second, betel nut contains four arecal alkaloids (primarily arecoline, along with arecaidine, guvacine and guvacoline), which have been associated with both short-term hypoglycaemia and eventual development of diabetes mellitus. This effect is thought to be due to adrenal alkaloids inhibiting at the γ-aminobutyric acid (GABA) receptor, thereby blocking GABA's inhibitory effects on glucagon and somatotropin. A resultant elevation in glucagon secretion increases appetite and glucose intolerance, leaving the individual more susceptible to developing diabetes mellitus.[1,5,8,11,32] Third, betel nut chewing is associated with periodontal infection, which might increase the risk of CVD through a variety of mechanisms such as the development of a chronic low-grade inflammation in response to infection.[21,33–35] Fourth, betel nut use increases oxidative stress, a potent inductor of endothelial dysfunction and significant influence in the development of atherosclerotic plaque.[36,37]

Strengths of our study include its large, population-based sample size as well as use of standardized IMT protocols. Additionally, only 3% of the study population reported use of prescription drugs at baseline, removing a potential cofounder. Limitations include the time gap between questionnaire administration to assess betel habits (during baseline enrolment periods: 2000–02 or 2006–08) and IMT measurement (2010–11). Because duration and intensity of betel use were not assessed during biennial follow-up, we could not evaluate effect of betel nut use closer to the time when carotid IMT was measured. However, data on ever and never status available biennially indicated that cigarette and betel habits were stable between baseline enrolment and IMT measurement (incidence of smoking = 0.3 per 100 person-years and incidence of betel use = 2.7 per 100 person-years during the study period). Given atherosclerosis is a lifelong process that develops over a long period of time due to both past and persistent factors, our findings that betel nut use at baseline was associated with IMT measured an average of 6.65 years later indicate that high intensity of betel nut use in the past is positively related to subsequent IMT levels. Such a relationship between distant exposure to cardiovascular risk factors and subsequent atherosclerotic processes is also evident in several prior studies showing childhood CVD risk factors were related to increased IMT later in life.[38,39] Nevertheless, future studies are needed to fully elucidate a cause-effect relationship between betel use and IMT, as well as to assess whether the effect of betel nut on cIMT differs at different stages of life. It should also be noted that it is unlikely that subjects with high levels of betel nut consumption and IMT were preferentially included in this study (a necessary condition for a bias) as the distributions of demographic, lifestyle and diet patterns in the study population and in the overall cohort were very similar (data not shown).

Though we did not possess comprehensive data on concurrent use of chewing or smokeless tobacco as a component of betel quid, we do not believe this would have altered the interpretation of our findings given that existing studies on the association between chewing tobacco and CVD have been inconclusive, and none have shown a significant effect on carotid IMT.[40–42] Metrics such as cholesterol and waist-hip ratio measurements also could not be

included in this analysis as those data were not collected. However, because there is no conclusive evidence of an association between betel use and low-density lipoprotein (LDL) or high-density lipoprotein (HDL),[43] we do not believe that inclusion of these variables would have significantly impacted on our results. Certain characteristics of this rural study population limit generalizability, as the population possessed a mean age of 45.5 years at time of IMT measurement and a mean BMI of 20.0 kg/m^2. This relatively young age of our participants may obscure the effects of cumulative betel exposure as overall average duration of use was, necessarily, shorter; it may also explain the lack of significance between ever and never users in our population as well as between high- and low-frequency users. Lastly, though all participants were married, we believe this would not significantly bias our findings, as marriage is nearly universal in rural Bangladesh.

In conclusion, we found that betel nut use at long duration or high cumulative exposure levels is associated with higher levels of preclinical atherosclerosis as manifested through carotid IMT. This positive association is especially pronounced among men and cigarette smokers. Development of CVD is a multifactorial process often influenced by decades of lifestyle factors and environmental exposures. These findings may enhance our understanding of the pathophysiology underlying the association between betel and CVD, and direct future prevention or intervention strategies to curtail this practice that has longstanding roots throughout Southeast Asia. Our findings are of particular relevance in Bangladesh and other Asian populations, where betel nut use is prevalent and incidence of CVD is rising.

Funding

This work was supported by the National Institutes of Health (grants R01 ES017541, P42 ES010349 and P30 ES000260), New York University grant 14-A0-00-002282 and in part by grant UL1 TR000038 from the National Center for the Advancement of Translational Science (NCATS), National Institutes of Health.

Acknowledgements

The authors thank the dedicated project staff and fieldworkers in Bangladesh, without whom this work would not have been possible.

Conflict of interest: None declared.

References

1. Boucher BJ, Mannan N. Metabolic effects of the consumption of Areca catechu. *Addict Biol* 2002;7:103–10.
2. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans / World Health Organization IAfRoC. Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans / World Health Organization, International Agency for Research on Cancer* 2004;85:1–334.
3. Gupta PC, Warnakulasuriya S. Global epidemiology of areca nut usage. *Addict Biol* 2002;7:77–83.
4. Wu GH, Boucher BJ, Chiu YH, Liao CS, Chen TH. Impact of chewing betel-nut (Areca catechu) on liver cirrhosis and hepatocellular carcinoma: a population-based study from an area with a high prevalence of hepatitis B and C infections. *Public Health Nutr* 2009;12:129–35.
5. Yen AM, Chiu YH, Chen LS *et al*. A population-based study of the association between betel-quid chewing and the metabolic syndrome in men. *Am J Clin Nutr* 2006;83:1153–60.
6. Tseng CH. Betel nut chewing is associated with hypertension in Taiwanese type 2 diabetic patients. *Hypertens Res* 2008;31:417–23.
7. Heck JE, Marcotte EL, Argos M *et al*. Betel quid chewing in rural Bangladesh: prevalence, predictors and relationship to blood pressure. *Int J Epidemiol* 2012;41:462–71.
8. Tung TH, Chiu YH, Chen LS, Wu HM, Boucher BJ, Chen TH. A population-based study of the association between areca nut chewing and type 2 diabetes mellitus in men (Keelung Community-based Integrated Screening programme No. 2). *Diabetologia* 2004;47:1776–81.
9. Benjamin AL. Community screening for diabetes in the National Capital District, Papua New Guinea: is betelnut chewing a risk factor for diabetes? *Papua N Guinea Med J* 2001;44:101–07.
10. Javed F, Bello Correra FO, Chotai M, Tappuni AR, Almas K. Systemic conditions associated with areca nut usage: a literature review. *Scand J Public Health* 2010;38:838–44.
11. Lin WY, Chiu TY, Lee LT, Lin CC, Huang CY, Huang KC. Betel nut chewing is associated with increased risk of cardiovascular disease and all-cause mortality in Taiwanese men. *Am J Clin Nutr* 2008;87:1204–11.
12. Yen AM, Chen LS, Chiu YH, Boucher BJ, Chen TH. A prospective community-population-registry based cohort study of the association between betel-quid chewing and cardiovascular disease in men in Taiwan (KCIS no. 19). *Am J Clin Nutr* 2008;87:70–78.
13. Chu NS. Cardiovascular responses to betel chewing. *J Formos Med Assoc* 1993;92:835–37.
14. Tsai WC, Wu MT, Wang GJ *et al*. Chewing areca nut increases the risk of coronary artery disease in Taiwanese men: a case-control study. *BMC Public Health* 2012;12:162.
15. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases – Part I: General considerations, the epidemiologic transition, risk factors, and impact of urbanization. *Circulation* 2001;104:2746–53.
16. Lopez AD, Mathers CD. Measuring the global burden of disease and epidemiological transitions: 2002-2030. *Ann Trop Med Parasitol* 2006;100:481–99.
17. de Groot E, Hovingh GK, Wiegman A *et al*. Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. *Circulation* 2004;109:III33–38.
18. Stein JH, Korcarz CE, Hurst RT *et al*. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness

Task Force. Endorsed by the Society for Vascular Medicine. *Je Am Soc Echocardiogr* 2008;21:93–111; quiz 89–90.
19. Ahsan H, Chen Y, Parvez F *et al*. Health Effects of Arsenic Longitudinal Study (HEALS): description of a multidisciplinary epidemiologic investigation. *J Expo Sci Environ Epidemiol* 2006;16:191–205.
20. Chen Y, Wu F, Graziano J *et al*. Arsenic exposure from drinking water, arsenic methylation capacity, and carotid intima-media thickness in Bangladesh. *Am J Epidemiol* 2013;178:372–81.
21. Desvarieux M, Demmer RT, Rundek T *et al*. Periodontal microbiota and carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology Study (INVEST). *Circulation* 2005;111:576–82.
22. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 1999;340:14–22.
23. Kitamura A, Iso H, Imano H *et al*. Carotid intima-media thickness and plaque characteristics as a risk factor for stroke in Japanese elderly men. *Stroke* 2004;35:2788–94.
24. Touboul PJ, Hennerici MG, Meairs S *et al*. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th Watching the Risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006 and Hamburg, Germany, 2011. *Cerebrovasc Dis* 2012;34:290–96.
25. Zureik M, Touboul PJ, Bonithon-Kopp C *et al*. Cross-sectional and 4-year longitudinal associations between brachial pulse pressure and common carotid intima-media thickness in a general population. The EVA study. *Stroke* 1999;30:550–55.
26. Ando F, Takekuma K, Niino N, Shimokata H. Ultrasonic evaluation of common carotid intima-media thickness (IMT) – influence of local plaque on the relationship between IMT and age. *J Epidemiol* 2000;10:S10–17.
27. Hodis HN, Mack WJ, LaBree L *et al*. The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med* 1998;128:262–69.
28. Crouse JR 3rd, Byington RP, Bond MG *et al*. Pravastatin, lipids, and atherosclerosis in the carotid arteries (PLAC-II). *Am J Cardiol* 1995;75:455–59.
29. Chambless LE, Heiss G, Folsom AR *et al*. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. *Am J Epidemiol* 1997;146:483–94.
30. Guh JY, Chen HC, Tsai JF, Chuang LY. Betel-quid use is associated with heart disease in women. *Am J Clin Nutr* 2007;85:1229–35.
31. Chu NS. Neurological aspects of areca and betel chewing. *Addict Biol* 2002;7:111–14.
32. Guh JY, Chuang LY, Chen HC. Betel-quid use is associated with the risk of the metabolic syndrome in adults. *Am J Clin Nutr* 2006;83:1313–20.
33. Jeng JH, Wang YJ, Chiang BL *et al*. Roles of keratinocyte inflammation in oral cancer: regulating the prostaglandin E2, interleukin-6 and TNF-alpha production of oral epithelial cells by areca nut extract and arecoline. *Carcinogenesis* 2003;24:1301–15.
34. Janket SJ, Baird AE, Chuang SK, Jones JA. Meta-analysis of periodontal disease and risk of coronary heart disease and stroke. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;95:559–69.
35. Demmer RT, Desvarieux M. Periodontal infections and cardiovascular disease: the heart of the matter. *J Am Dent Assoc* 2006;137 Suppl:14S–20S; quiz 38S.
36. Lai KC, Lee TC. Genetic damage in cultured human keratinocytes stressed by long-term exposure to areca nut extracts. *Mutat Res* 2006;599:66–75.
37. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens* 2000;18:655–73.
38. Raitakari OT, Juonala M, Kahonen M *et al*. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *JAMA* 2003;290:2277–-83.
39. Li S, Chen W, Srinivasan SR *et al*. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. *JAMA* 2003;290:2271–76.
40. Lee PN. Circulatory disease and smokeless tobacco in Western populations: a review of the evidence. *Int J Epidemiol* 2007;36:789–804.
41. Critchley JA, Unal B. Is smokeless tobacco a risk factor for coronary heart disease? A systematic review of epidemiological studies. *Eur J Cardiovasc Prev Rehabil* 2004;11:101–12.
42. Bolinder G, Noren A, de Faire U, Wahren J. Smokeless tobacco use and atherosclerosis: an ultrasonographic investigation of carotid intima media thickness in healthy middle-aged men. *Atherosclerosis* 1997;132:95–103.
43. Iqbal MP, Mehboobali N, Haider G, Pervez S, Azam I. Effects of betel nut on cardiovascular risk factors in a rat model. *BMC Cardiovasc Disord* 2012;12:94.