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Cotinine Levels Among Betel Quid Users and Cigarette Smokers in Cambodia

Pramil N. Singh, DrPH¹, Zuhair Natto, BDS, MPH, DrPH^{1,2}, Rituraj Saxena, MPH¹, Hiya Bannerjee, PhD³, Daravuth Yel, MD, MA⁴, Sothy Khieng, MA^{5,6}, and Jayakaran S. Job, MD, DrPH¹

¹Loma Linda University, Loma Linda, CA, USA ²King Abdulaziz University, Jeddah, Saudi Arabia

³Covance, Inc, Monona, WI ⁴World Health Organization (Tobacco Free Initiative), Phnom Penh, Cambodia ⁵Royal University of Phnom Penh, Phnom Penh, Cambodia ⁶VU University, Amsterdam, The Netherlands

Abstract

Smokeless tobacco use in the form of the betel quid is common in the Western Pacific Region, and yet few studies have determined the nicotine delivery of this habit. During a validation substudy, we randomly sampled 201 adults from a rural province of Cambodia and determined nonparametric (bootstrapped) confidence intervals (CIs) for salivary cotinine levels in tobacco users. We found that cotinine levels for daily betel quid use among women (95% CI = 218.6–350.0 ng/mL) were (1) similar to the levels for daily cigarette smoking in men (95% CI = 240.2–317.1 ng/mL) and (2) significantly higher than the levels for daily cigarette smoking in women (95% CI = 71.8–202.7 ng/mL). The 95% confidence range for these habits exceeded the threshold for addiction. Our findings from rural Cambodia indicate that the typical betel quid habit among women supports the same level of nicotine addiction as the typical cigarette habit in men.

Keywords

cotinine; betel quid; cigarette smoking; Cambodia

Introduction

Smokeless tobacco in the form of a betel quid (tobacco leaves, areca nut, betel leaf, and slaked lime [calcium hydroxide]) is consumed by 600 million persons in Asia, South Asia, and Southeast Asia and also among migrants from those regions to Western nations.^{1–3} In the Western Pacific Region (WPR), betel quid use is a common and socially acceptable form of tobacco use among women, particularly older women.³

Corresponding Author: Pramil N. Singh, Center for Health Research, School of Public Health, Loma Linda University, Nichol Hall 2010, Loma Linda, CA 92350, USA. psingh@llu.edu.

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In some of the WPR nations (ie, Indonesia, Malaysia, Palau, and Papua New Guinea), the rate of use among the oldest women exceeds 50%.^{3–6} In 2 large national prevalence surveys of Cambodia (2006, 2010), we found that of the 1.9 million tobacco users, more than 500 000 were women who used betel quid that contained smokeless tobacco.^{7,8}

Despite the high prevalence of this form of betel quid use in Asia, there is very little data that measures and characterizes the nicotine delivery profile of this addictive tobacco habit. In South Asia, betel quid is often sold as a manufactured product (ie, *paan*) with a tobacco additive. In contrast, the amount of tobacco added to homemade betel quids common in the WPR nations is variable, and the nicotine released from chewing the quid could also vary based on other ingredients in the quid.

In WPR nations it is noteworthy that betel quid chewing appears to be a highly addictive habit; the rate of cessation tends to be very low and typically occur after the onset of disease in older adults.^{9–11} In the 2011 National Adult Tobacco Survey of Cambodia, survey estimates indicated that of the 537 000 adults who had ever been daily betel quid chewers, only 9000 had successfully quit the habit.¹¹ The addictive nature of this form of tobacco use is not only attributable to nicotine but to other psychoactive alkaloids derived from the areca nut³ and betel leaf.¹² As policy-driven efforts to curtail cigarette smoking emerge in the WPR (ie, price per pack increases and health labels), we note that the availability of highly addictive noncigarette forms of tobacco provide an alternative to tobacco cessation.

In the present study, we determined the salivary cotinine levels of betel quid users and cigarette smokers in a rural sample of adults from Cambodia. Gender norms in this nation identify men as primarily cigarette smokers and women as primarily betel quid users.⁷ Our main objective was to characterize the nicotine delivered by daily and less-than-daily tobacco habits in men and women. Implications for regional tobacco control measures are discussed.

Methods

Study Population

We collaborated with the National Institute of Statistics (Ministry of Planning, Cambodia) and used census base maps to randomly sample 201 adults (ages 21 to 84 years) from a rural province (Kampong Thom) of Cambodia. Specifically, we used a circular systematic sampling method to ensure geographic coverage of all villages and enumerated households on the census base map of the province.⁷ Of these 201 adults, 196 (98% response rate) provided interviewer-administered survey data on tobacco use and saliva samples. Informed consent was obtained in the participant's language and in written form at the beginning of the data collection. Institutional review board approval of the survey, salivary cotinine measurements, and sampling protocols of this study was obtained from the Ethics Committee of the Ministry of Health (Phnom Penh, Cambodia) and from the US-based ethics review committee at Loma Linda University (Loma Linda, California).

Questionnaire

The survey items used were part of the national tobacco survey items that have been previously described.⁷ Briefly, the survey items were designed during focus groups^{13,14} involving survey research training of Ministry of Health (Cambodia) personnel,⁹ in consultation with local nongovernmental organizations (ie, ADRA) who had conducted tobacco surveys. The final survey was conducted in the local language (Khmer) after being translated using methods described by Flaherty.¹⁵

The survey included items on demographics and tobacco use (commercial cigarettes, hand-rolled cigarettes, chewing tobacco and betel quid, and tobacco pipe). Pictograms of the local tobacco products were also shown when surveying on the specific forms of tobacco.¹⁴

Salivary Cotinine Measure

Salivary cotinine was measured for each participant using the NicAlert test (NyMox Corporation)—a semiquantitative immunoassay technology that uses rapid test strips to assess salivary cotinine levels from exposures to cigarettes, chewing tobacco, or tobacco pipes that occurred during the past 48 to 72 hours.¹⁶ The resulting data are an ordinal score from 0 to 6 that represent, respectively, 0 to 10 ng/mL, 10 to 30 ng/mL, median of 30 to 100 ng/mL, 100 to 200 ng/mL, 200 to 500 ng/mL, 500 to 1000 ng/mL, and >1000 ng/mL. Cooke et al¹⁶ reported that the NicAlert test has a sensitivity of 93%, specificity of 95%, and positive predictive value of 95% when compared with salivary cotinine measured by gas chromatography nitrogen phosphorus detection.

Statistical Analysis

For current (daily, less than daily) and daily betel quid users and cigarette smokers means and 95% confidence intervals (CIs) were computed. For the final analytic salivary cotinine variable, the ordinal scores were recoded to the midpoint of the range of cotinine values represented by each score value (given in the last section). To estimate the salivary cotinine level for current and daily users of tobacco use, we then computed a nonparametric 95% CI by bootstrapping the variance from 2000 samples using the BC_a (bias corrected and accelerated method).

Results

The demographics and tobacco use data of the population, given in Table 1, indicate similar trends to the national sample^{7,11} as evidenced by a mean age of about 43 years, 60% being married, and a tendency for men to smoke cigarettes (39.7% smoked commercial brands and 24% smoked hand-rolled cigarettes) and women to chew tobacco with the betel quid (24.4%).

Because national prevalence data from Cambodia indicate that the 2 largest groups of tobacco users are men who smoke cigarettes (about 1.4 million) and women who chew betel quid (500 000), we examined the cotinine levels of these groups and found the following. We found that for women who were current users of smokeless tobacco, the mean cotinine level (273.3 ng/mL, *n* = 27) was quite similar to that of men who were current smokers of

cigarettes (262.2 ng/mL, n = 44); CIs for these estimates were overlapping and with precision that supports comparability of the ranges. There was evidence that for women who smoked cigarettes (a small proportion [$<3\%$] of the national sample), the cotinine level (132.8 ng/mL, n = 15) was significantly lower than that for (1) women who used smokeless tobacco (273.3 ng/mL, n = 27) or (2) men who smoked cigarettes (262.1 ng/mL, n = 44).

These trends were also evident for daily users of tobacco and are depicted in Table 2 and Figure 1. All the CIs in Table 2 and Figure 1 exceed the threshold for addiction of 50 to 70 ng/mL, which is based on intake of 4 to 6 mg of nicotine.¹⁷

Discussion

In Cambodia, national estimates of adult tobacco use indicate that, of the approximately 2 million tobacco users, the 2 largest groups (by tobacco type and gender) are men who smoke cigarettes (estimated at 1.4 million) and women who use smokeless tobacco in the form of a betel quid (estimated at 500 000).^{7,11} Our findings from rural Cambodia indicate that the typical, daily smokeless tobacco use of women results in the same salivary cotinine levels (279.2 ng/mL [95% CI = 218.6–350.0]) as the typical daily cigarette smoking habit in men (277.9 ng/mL [95% CI = 240.2–317.1]). The range for both habits is well above the threshold for addiction.¹⁷ These data complement recent findings from the 2011 National Adult Tobacco Survey of Cambodia, which indicated that among the 1.9 million tobacco users in the nation, 90% needed to use smoked or smokeless tobacco within an hour of waking up.¹¹

Betel Quid Use Among Women of Cambodia

We have found evidence that the daily betel quid habit in Cambodian women delivers about the same nicotine as the daily cigarette habit in Cambodian men—the latter smoking about 1 pack of cigarettes per day.¹¹ In Cambodia, the betel quid that women use is combined into a “leaf packet” (ie, the betel leaf, *Areca catechu*) of about 10 to 30 g of tobacco leaves, areca nuts, a mineral slaked limestone paste of aqueous calcium hydroxide, and catechu bark. This leaf packet is then sucked in the lateral gingival pocket for periods ranging from 20 minutes to as long as overnight.^{12,18} During mastication, the alkaline effect of the calcium hydroxide in the betel quid releases alkaloids and phenols¹⁹ from the tobacco leaves (nicotine), the areca nut (ie, arecoline, arecaidine, guvacine, and guvacoline), and the betel leaf (cadinene)¹²; these have psychoactive and addictive effects. Thus, measurement of the addictive properties of the homemade betel quid is complex when considering that the amount of nicotine and other additives released will vary based on the relative composition of the quid.

In Cambodia, the median age at initiation into the habit is 33 years, and by percentile, the middle 80% of the age at initiation range occurs between ages 20 and 50 years and, thus, primarily in women of reproductive age.¹² In previous reports^{7,8} and in an article by Singh et al²⁰ in this issue of the journal, we have reported that pregnancy is a time when many Cambodian women first try the betel quid as part of a traditional medicine remedy for morning sickness. The pathway by which this sporadic use for symptomatic relief leads to habitual use has not been well studied but is a familiar scenario with other addictive

substances (ie, opiates). The possible role of older female relatives and traditional midwives in promoting a “first use” of this drug among pregnant women and/or women of reproductive age has been suggested but also needs further investigation.⁷

Nicotine and Nicotine Metabolite Levels in Betel Quid and Smokeless Tobacco

Our findings indicating comparable salivary cotinine levels between betel quid chewers and smokers in Cambodia show some agreement with work done in small samples of South Asian adults. Among Bangladeshi women (n = 229) living in the United Kingdom, Croucher et al,²¹ found very similar mean salivary cotinine levels among those who chewed packaged betel quid or *paan* (316.48 ng/mL, 95% CI = 266.47–370.79) and those who smoked tobacco (315.77 ng/mL, 95% CI = 179.02–491.19). In an early study of healthy male volunteers (n = 47) working at a research institute in Mumbai,²² India, salivary cotinine levels of betel quid chewers were higher (202 ng/mL, 95% CI [103–388]) but within the range of that for cigarette smokers (51 ng/mL, 95% CI [0–517]), with the small sample size preventing further comparison. In a larger sample of 130 men and women volunteers in Chandigarh, India, urinary cotinine values for the cigarette smokers (mean $\pm$ SD = 2736 $\pm$ 983 ng/mL) and betel quid chewers (2178 $\pm$ 334.29 ng/mL) were within a similar range.²³ Overall, our findings from Cambodia add to the emerging data from small samples of Asian adults indicating that betel quid products appear to be supporting a similar level of nicotine addiction as the cigarettes smoked in the same communities.

When considering the variability in nicotine delivery from samples of betel quid, some factors need mention. With smokeless tobacco use, systemic absorption will of course be dependent on how much is absorbed through buccal membranes and also, if the quid is swallowed, how much escapes first pass metabolism by the liver. Practices relating to swallowing the quid after a session vary as a matter of cultural practice within the WPR. Other factors include (1) the amount of alkalizing agent (ie, lime paste) and catechu bark added to the quid, both of which enhance the release of nicotine when mixed with tobacco leaves in the quid; (2) the strain of tobacco leaf used because there is some variability by strain in capacity to absorb additives; and (3) the pattern of use (ie, repeated and frequent dipping of small amounts versus sustained retention of a single large bolus).

The study of nicotine delivery by commonly used smokeless tobacco products is receiving more global attention. Of particular interest is the trend where women use or switch to smokeless tobacco products during pregnancy—often as part of a perceived harm reduction. In Sweden, pregnant mothers who smoke are known to opt for smokeless tobacco in the form of *snus* during their pregnancy.²⁴ *Iqmik*, a form of smokeless tobacco and ash, is formed into an alkali quid in the oral cavity and preferentially used by Alaskan Native women during pregnancy as a substitute for other forms of tobacco.²⁵ It is interesting to note that Hurt et al²⁵ found that *Iqmik* users had significantly higher serum cotinine levels (167 $\pm$ 116 vs 81 $\pm$ 100 ng/mL) than other tobacco users (commercial smokeless tobacco or cigarettes). Taken together with the findings from Asia indicating use of betel quid during pregnancy, the current global data raise the important question of whether prenatal nicotine exposure from a smokeless source is harmful and, if so, at what levels. Epidemiological

studies, including one in this issue of this journal, have linked prenatal use of snuff,²⁴ betel quid,²⁶ and/or *Iqmi*²⁵ to adverse infant outcomes.

Implications for Tobacco Control in the WPR

In the WPR, several nations have ratified the WHO Framework Convention on Tobacco Control Treaty and are in the process of implementing initiatives to control manufactured-cigarette smoking by increasing the price per pack (ie, by taxation), affixing health warning labels to the packs, and enforcing smoke-free areas. We note that these measures will not affect smokeless tobacco use in the rural areas of the WPR, which occurs through the sale of loose tobacco in traditional markets. In Cambodia, our findings in this report add to evidence that several markers of tobacco dependence (salivary cotinine, time to use on waking, and cessation/quit ratio)¹¹ indicate that the smokeless tobacco habits of rural women of the region are at least as addictive as the daily cigarette habit and highly prevalent—factors that will certainly keep the demand for loose tobacco at current levels. Such loose tobacco alternatives also provide alternatives to manufactured-cigarette smokers.

Limitations

One of the limitations of the design of our study involving rural Cambodian adults was the small sample size. Also, to measure salivary cotinine, we used a semiquantitative test strip with 6 categories of salivary cotinine. Despite the validity of the test, it is noteworthy that a continuous measure of salivary cotinine would have enhanced our statistical power to detect a difference between tobacco user groups. Further studies with larger sample sizes and a more sensitive test of nicotine exposure are needed.

Conclusion

Our findings from Cambodia indicate that the typical betel quid habit among women supports the same level of nicotine addiction as the typical cigarette habit in men. As policy-driven efforts to decrease daily cigarette smoking (ie, taxation to increase the price of a pack) in the region take effect, the loose tobacco for betel quid chewing provides a readily available and cheaper alternative for a similar daily dose of nicotine.

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Figure 1.
Mean and 95% confidence intervals for daily tobacco users in rural Cambodia.

Table 1

Demographic Characteristics and Tobacco Use in a Sample of 196 Adults in Cambodia.

Variable	All (n = 196)	Men (n = 78)	Women (n = 118)
Age (mean $\pm$ SD)	42.8 ($\pm$ 17.2)	40.6 ($\pm$ 17.9)	44.2 ($\pm$ 16.7)
Marital status (%)			
Never married	12.2	19.2	7.6
Currently married	60.0	66.7	55.1
Live together	13.7	14.1	13.6
Widowed	9.6	0	16.1
Divorced/Separated	4.5	0	7.6
Current tobacco use (%)	43.9	59.0	34.0
Cigarette (commercial brand)	20.4	39.7	7.6
Cigarette (hand-rolled)	13.8	24.4	6.8
Chewing tobacco	14.8	2.6	23.0

Abbreviation: SD, standard deviation.

Table 2

Mean Salivary Cotinine [95% CI] Levels for Different Forms of Tobacco Use Among Cambodian Men and Women.

	Men	Women
Current cigarette smokers		
All	262.1 ng/mL [95% CI = 224.3–305.7]	132.8 ng/mL [95% CI = 76.3–200.0]
Daily	277.9 ng/mL [95% CI = 240.2–317.1]	135.5 ng/mL [95% CI = 71.8–202.7]
Current users of chewed tobacco		
All	—	273.3 ng/mL [95% CI = 205.0–346.7]
Daily	—	279.2 ng/mL [95% CI = 218.6–350.0]

Abbreviation: CI, confidence interval.