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Total, Direct and Indirect Effects of Paan on Oral Cancer

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Abstract

Purpose—Paan (betel leaf and betel nut quid) used with or without tobacco has been positively associated with oral cancer. Oral submucous fibrosis (OSMF), a pre-cancerous condition caused by paan, lies on the causal pathway between paan use and oral cancer. The purpose of this analysis was to estimate the effect of paan consumption on oral cancer risk when it is mediated by OSMF.

Methods—We used mediation methods proposed by Vanderweele, which are based on causal inference principles, to characterize the total, direct and indirect effects of paan, consumed with and without tobacco, on oral cancer mediated by OSMF. We reanalyzed case-control data collected from three hospitals in Karachi, Pakistan between July 1996 and March 1998.

Results—For paan without tobacco the total effect on oral cancer was OR=7.39, 95% CI, 1.01, 38.11, natural indirect effect (due to OSMF among paan users) was OR=2.48, 95% CI, 0.99, 10.44, and the natural direct effect (due to paan with OSMF absent) was OR=3.32, 95% CI, 0.68, 10.07. For paan with tobacco the total direct effect was OR=15.68, 95% CI, 3.00, 54.90, natural indirect effect was OR=2.18, 95% CI, 0.82, 5.52, and the natural direct effect was OR=7.27, 95% CI, 2.15, 20.43.

Conclusions—Paan, whether or not it contained tobacco, raised oral cancer risk irrespective of OSMF. Oral cancer risk was higher among those who used paan with tobacco.

Keywords

oral cancer; betel (areca) nut; chewing tobacco; oral submucous fibrosis

Introduction

We showed in an earlier report that paan use is independently associated with oral cancer whether or not it contains tobacco [1]. Paan contains areca (betel) nut which is a strong risk factor for oral submucous fibrosis (OSMF) [2], an oral condition that restricts mouth opening and increases oral cancer risk [3]. OSMF is thus on the causal pathway between

paan consumption and oral cancer and mediates that relation. Obtaining unbiased estimates of exposure-outcome relation in the presence of a mediator is problematic [4]. VanderWeele recently proposed a new analytical approach to characterize the unbiased relation between exposure and outcome in the presence of a mediator [5,6]. We therefore reanalyzed our data to characterize the direct and indirect effects of paan, with and without tobacco, on oral cancer mediated by OSMF.

Materials and Methods

Population

These analyses were conducted on case-control data collected from three hospitals in Karachi, Pakistan between July 1996 and March 1998. Participants provided informed consent, and the ethical review board of Aga Khan University approved the study [1].

Outcome

Cases of oral cancer were consenting individuals admitted to the participating hospitals with biopsy confirmed primary oral squamous cell carcinoma. All eligible cases in the recruiting period were approached. Controls were recruited contemporaneously with the cases from individuals admitted to the orthopedic and general surgical wards of the hospital where the case was recruited. Controls were matched for age (within 5 years of case) and sex. All individuals with a prior history of cancer were excluded. Details of the study population and data collection are described elsewhere [1].

Mediator (OSMF)

A physician or dentist trained to identify OSMF examined the mouths of all participants and checked for the presence of OSMF by palpating blanched areas in the mouth with a finger for the presence of fibrous bands. Based on the clinical examination OSMF was determined to be present or absent.

Exposure (paan use)

Paan use was determined by questionnaire. Paan use with and without tobacco were evaluated separately. Individuals consuming areca nut only were combined with those who consumed paan without tobacco. A user was defined as an individual who had ever indulged in the habit daily for at least a month.

Covariates

Age, sex, education level, smoking habits, and consumption of alcohol were determined from a questionnaire. Smokers were defined as individuals who had ever indulged in the habit daily for at least a month.

Statistical methods

We evaluated the total, direct and indirect effects of paan use with and without tobacco on oral cancer, with OSMF as the mediator, using the counterfactual framework proposed by VanderWeele [5,6]. With these methods it is possible to decompose the total effect of paan

use on oral cancer into a direct effect (not mediated by OSMF) and indirect effect (mediated by OSMF) [6]. A diagrammatic representation of these effects is described in the causal directed acyclic graph (DAG) in Figure 1.

We used the SAS macro developed by Valeri and Vanderweele [7] to evaluate the natural direct effect, natural indirect effect, and total effect of paan use on oral cancer with OSMF as a mediator from case-control data [8]. We assumed that there was first, no unmeasured confounding of paan use and oral cancer; second, no unmeasured confounding of OSMF and oral cancer; third, no unmeasured confounding of the paan-OSMF relation; fourth, that no OSMF-oral cancer confounder was affected by paan use; and finally that the outcome (oral cancer) was rare [7]. Vanderweele showed that under these assumptions the odds ratio (OR) for total effects evaluating exposure and outcome in the presence of a mediator, can be decomposed into ORs for direct and indirect effects as shown below. Moreover, in this situation the OR approximates the relative risk [8].

$$OR_{1,0|c}^{TE} = \frac{P(Y_{1M1}=1|c)/\{1 - P(Y_{1M1}=1|c)\}}{P(Y_{1M0}=1|c)/\{1 - P(Y_{1M0}=1|c)\}} \times \frac{P(Y_{1M0}=1|c)/\{1 - P(Y_{1M0}=1|c)\}}{P(Y_{0M0}=1|c)/\{1 - P(Y_{0M0}=1|c)\}} = OR_{1,0|c}^{NIE} \times OR_{1,0|c}^{NDE}$$

Where $Y_{1M1}=1$, indicates outcome is present, exposure (paan use) and mediator (OSMF) are present

$Y_{1M0}=1$, indicates outcome is present, exposure (paan use) is present, mediator (OSMF) is absent

$Y_{0M0}=1$, indicates outcome is present, exposure (paan use) is absent, mediator (OSMF) is absent

c represents vector of confounders

TE indicates Total Effects

NIE indicates Natural Indirect Effects

NDE indicates Natural Direct Effects

The OR estimates from these analyses would approximate causal estimates when the assumptions listed above were satisfied. All analyses were conducted in SAS 9.3 (SAS Institute, Cary, NC).

Results

In this study there were 79 cases of oral cancer and 143 controls; 56 persons had OSMF and 166 did not; 41 persons consumed paan without tobacco and 181 did not; and 55 persons used paan with tobacco and 167 did not. Individuals with OSMF were more likely to have never attended school, report smoking, use alcohol, and consume paan with and without tobacco. OSMF was more prevalent among cases (58.2%) than controls (7.0%) (Table 1).

Paan without tobacco increased oral cancer risk 7 fold (OR=7.39, 95% CI, 1.01, 38.11). The natural indirect effect (oral cancer risk due to OSMF among users of paan without tobacco) was (OR=2.48, 95% CI, 0.99, 10.44), and the natural direct effect (oral cancer risk due to

paan without tobacco in the absence of OSMF) was (OR=3.32, 95% CI, 0.68, 10.07) (Table 2).

Paan with tobacco raised the risk of oral cancer 16 fold (OR=15.68, 95% CI, 3.00, 54.90). The natural indirect effect (oral cancer risk due to OSMF among users of paan with tobacco) was (OR=2.18, 95% CI, 0.82, 5.52), and the natural direct effect (oral cancer risk due to paan with tobacco in the absence of OSMF) was (OR=7.27, 95% CI, 2.15, 20.43) (Table 2).

The models evaluating interaction between paan use and OSMF on oral cancer risk converged but the standard errors of the estimates were highly inflated. These results are therefore not presented.

Discussion

This is the first report, to the best of our knowledge, which separated the direct and indirect effects of paan use on oral cancer risk. Paan consumed with or without tobacco increased oral cancer risk. The risk of oral cancer was higher among those who used paan with tobacco than those who used it without tobacco.

Betel (areca) nut, a component of paan with or without tobacco, was positively associated with oral cancer (OR 3.5, 95% CI 2.2, 4.3) [9] and leukoplakia (OR 7.9, 95% CI 4.3, 14.6) [10] in meta-analyses of observational studies that adjusted for smoking and did not include individuals who chewed tobacco. Betel nut contains alkaloids and other substances that are hypothesized to contribute to carcinogenesis by generation of reactive oxygen species, formation DNA adducts, and causing DNA strand breaks [11]. Betel nut is hypothesized to be a strong risk factor for OSMF which is a precancerous lesion [3]. A very strong association between betel nut and OSMF was reported in case-control study conducted in Karachi, Pakistan [12]. In addition to being carcinogenic [13] betel nut is addictive [14]. It is consumed in South Asia, China, Taiwan, countries of Southeast Asia and the Pacific Rim, and in western countries among immigrants from these areas [15]. The annual incidence of OSMF among betel nut users is estimated to be 21.3 per 100,000 for men and 45.7 per 100,000 in women [16]. The cumulative incidence of oral cancer among individuals with OSMF was 7.6% over 17 years [17]. Murti et al reported lower point estimates of incidence of OSMF among participants of an intervention group aimed at reducing betel nut use [16]. Even though this study was likely underpowered, because there were just 11 cases of OSMF in the intervention group, the results were consistent with evidence from observational and mechanistic studies. We found that paan without tobacco raised the risk of oral cancer overall irrespective of OSMF after adjusting for other confounders. Even though the estimates of the natural direct and natural indirect effects of paan without tobacco failed to reach statistical significance in this analysis (likely because of small sample size) they were consistent with the hypothesis that paan without tobacco causes oral cancer whether or not it is mediated by OSMF. This finding concurs with current knowledge, and further clarifies potential pathways through which paan without tobacco can impact oral cancer risk.

The general consensus is that chewing tobacco raises oral cancer risk, particularly based on evidence from US and Asian studies [18], even though this is not a consistent finding in

European reports [18,19]. The discrepant findings depending upon where the study is conducted is attributed to the type of chewing tobacco used in those communities [19]. However, there is wide agreement that the form in which chewing tobacco that is used in South Asia is potentially carcinogenic [11]. An intervention aimed at reducing tobacco use in India resulted in lower incidence of oral precancerous lesions in the intervention group [20]. In South Asia chewing tobacco is largely used in paan [11,21]. We found that while paan with tobacco raised the risk of oral cancer overall it had a stronger direct than indirect effect. This is consistent with current evidence which suggests that chewing tobacco acts directly on oral tissues [18], while betel nut has potential to cause OSMF [3] in addition to a direct effect on tissues of the mouth [9]. As paan with tobacco has both betel nut and chewing tobacco it is plausible that it would be more harmful.

Our study had limitations. First, we were unable to evaluate possible interaction between paan use and OSMF. Even though the models evaluating these interactions converged the standard errors associated with the parameter estimates were large rendering them unreliable. We therefore did not report them. Evaluation of these interactions would provide additional insights and we recommend that they be evaluated in studies with larger sample size. Second, some of the confidence intervals of our estimates were wide and included the null value. This was probably due to the small sample size as was observed in the example provided by Valeri and Vanderweele [7]. However, the product of the point estimates of the natural direct effect and natural indirect effect were approximately equal to the estimated total effects for paan without ($3.32 \times 2.48 = 8.23$ versus 7.39) and with tobacco ($7.27 \times 2.18 = 15.84$ versus 15.68) suggesting that the direct and indirect effect estimates approached their true values. Third, the study population was recruited from a hospital and may not be representative of the general population. Among the controls the prevalence of key risk factors were as follows [1]: smoking (32%), paan without tobacco (10.7%), and paan with tobacco (10.1%). Estimates of prevalence in the community during the time period in which the data were collected were similar to those observed among the controls for smoking [22,23], and paan use [21]. Finally, the case control study design is prone to differential assessment of exposures and participant selection in cases and controls. To minimize interviewer bias we used a standardized questionnaire and concealed the main study hypothesis from the interviewers. To minimize potential for selection bias we used clear and consistent criteria to include and exclude persons into the study and approached all potential cases in the data collection phase.

The study also had strengths. First the definition of oral cancer was based on biopsy which is the gold standard. Second, OSMF was identified by clinical examination. Third, we had data on important confounders and adjusted for those in the analyses (Figure 1). For example, even though social factors influence paan use [24], they do not influence the estimates in these analyses after adjustment for the measured confounders in this study as show in the DAG in Figure 1. Fourth, these analyses provided an unbiased relation between paan use and oral cancer. For instance the association between paan use with tobacco and oral cancer was 8.4 using conventional analyses [1] and 15.68 using the newer method. Finally, the assumptions described by Vanderweele to correctly identify direct and indirect effects from case-control data using the counterfactual model were met [8].

The 5-year survival of oral cancer is 62.7% based on US data where OSMF is very rare [25], but varies from approximately 38–60% in Asian countries where OSMF is prevalent [26]. However, these data need to be interpreted with the caveat that survival may vary between regions by factors affecting it, such as age at disease incidence, and access to health care and its quality. Few studies have compared outcomes among oral cancer cases occurring with and without OSMF. Chaturvedi and colleagues compared oral cancer cases with (n=112) and without OSMF (n=259) in India [27]. They found that individuals with both oral cancer and OSMF were more likely to be male, younger, and have better prognostic factors (grade, nodal metastasis, and extracapsular spread) as compared to those with oral cancer without OSMF [27]. They hypothesized that mucosal constriction characterizing OSMF may prevent cancerous cells from migrating to regional lymph nodes. Alternatively, oral cancer that is associated with OSMF may be detected earlier than that occurring without OSMF. The younger age of individuals with OSMF and oral cancer may reflect patterns of paan and betel nut use in the region [28]. Paan use has been identified as a major public health problem, particularly among the South Asia diaspora eliciting calls for urgent action [21,29]. Our study clarifies the relation between paan use and oral cancer which will give clinicians more specific information about oral cancer prevention to convey to their patients; basic science researchers directions to explore disease mechanisms; public health professionals information to more accurately estimate disease burden related to paan use and influence policy makers; and individuals conducting clinical trials to frame their research questions more accurately.

In conclusion, paan use raised oral cancer risk about seven fold, directly and mediated by OSMF, even when it was consumed without tobacco. There was a fifteen fold increase in oral cancer risk when paan was consumed with tobacco. Reducing paan and chewing tobacco use may decrease the burden of oral cancer in communities where this habit is common.

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Figure 1.

Causal directed acyclic graph (DAG) describing relations between paan use and oral cancer mediated by oral submucous fibrosis (OSMF)

C1: Age, sex, education, smoking, alcohol use

A: Paan without tobacco

M: OSMF

C2: Paan with tobacco

Y: Oral cancer

U: Unmeasured factor impacting paan use (with or without tobacco)

Table 1

Characteristics of study population by oral submucous fibrosis (OSMF)

	OSMF (n=56)	No OSMF (n=166)
Age in years, mean $\pm$ SD	46.4 $\pm$ 12.2	47.9 $\pm$ 13.1
Male, n (%)	36 (64.3)	108 (65.1)
Never attended school, n (%)	32 (59.3)	77 (51.3)
Smoker, n (%)	26 (46.4)	57 (34.3)
Alcohol use, n (%)	13 (23.2)	25 (15.7)
Paan without tobacco, n (%)	20 (35.7)	21 (12.7)
Paan with tobacco, n (%)	31 (55.4)	24 (14.5)
Oral cancer, n (%)	46 (82.1)	33 (19.9)

Table 2

Total, direct, indirect, and total effects of paan and oral cancer mediated by oral submucous fibrosis

	Odds Ratio (OR)	95% Confidence Interval (CI)
Paan Without Tobacco¹		
Natural Direct Effect	3.32	0.68, 10.07
Natural Indirect Effect	2.48	0.99, 10.44
Total Effect	7.39	1.01, 38.11
Paan with Tobacco²		
Natural Direct Effect	7.27	2.15, 20.43
Natural Indirect Effect	2.18	0.82, 5.52
Total Effect	15.68	3.00, 54.90

¹ Adjusted for age, sex, education, smoking and alcohol use, and use of paan with tobacco² Adjusted for age, sex, education, smoking and alcohol use, and use of paan without tobacco