available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.ejconline.com

Association between different alcoholic beverages and leukoplakia among non- to moderate-drinking adults: A matched case–control study

Stefano Petti[a,*], *Crispian Scully*[b]

[a]G. Sanarelli Department of Public Health Sciences, La Sapienza University, P.le Aldo Moro 5, 00185 Rome, Italy
[b]Eastman Dental Institute, University College, London, UK

ARTICLE INFO

Article history:
Received 23 June 2005
Received in revised form 11 July 2005
Accepted 18 July 2005
Available online 20 January 2006

Keywords:
Leukoplakia
Alcohol
Wine
Beer
Spirits

ABSTRACT

While heavy drinking is a risk factor for oral leukoplakia, the effects of moderate drinking and of different alcoholic beverages are unclear and were investigated in this case–control study. 187 leukoplakia patients, aged 40–65 years, who were not heavy drinkers and did not change their drinking/smoking habits over the last decade, were age/gender matched to 187 controls. The effect of regular, moderate, overall alcohol, beer, wine, spirit drinking, adjusted for the effects of heavy smoking and other important co-variables, were assessed using conditional logistic regression analysis. Overall alcohol (OR, 0.22), wine (OR, 0.20), spirits (OR, 2.93) were significantly associated to leukoplakia. A statistically significant interaction wine/heavy smoking was also found. Such different effects could be explained by the ethanol antagonising potential of other drink components, such as polyphenols, abundant in red wine and scarce in spirits. Among heavy smokers, the preventive effect of wine was more than multiplicative.

© 2005 Elsevier Ltd. All rights reserved.

1. Introduction

With an estimated global prevalence of 1.5–2.6% and a malignant transformation rate of 0.7–2.0%,[1] leukoplakia is the most frequent form of potentially malignant oral lesion. In the development of this condition, tobacco smoking/chewing is a major risk factor,[2–15] but the role played by heavy alcohol drinking also seems very important.[2,6,9,10] The mechanisms implicated in dysplastic transformation of the squamous cells of the oral mucosa caused by heavy alcohol drinking include the toxic activity of ethanol metabolites, the interference with DNA repair mechanisms, and the increased cell membrane permeability, which might promote the entrance of other carcinogens, such as those produced by tobacco smoking/chewing,[16] suggesting that heavy smoking and alcohol drinking may have a synergistic effect.[17,18]

There are few doubts on the role of heavy alcohol drinking in increasing the risk for oral leukoplakia. However, it is not clear whether regular, moderate alcohol drinking is also associated with an increased risk for this condition,[11,15,19–21] and if a real difference exists between specific types of alcoholic beverages in determining the risk associated to a moderate quantity of ethanol intake.[6,13]

The present study sought to investigate the association existing between leukoplakia and a moderate intake of different alcoholic beverages in a sample of adults whose smoking and drinking habits did not vary during the last decade.

2. Patients and methods

A 1:1 matched case–control study, authorized by the local research ethics committee (the ethical committee of the

* *Corresponding author:* Tel.: +3906 4991 4667; fax: +3906 4454 845.
E-mail address: stefano.petti@uniroma1.it (S. Petti).
0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.07.038

University of La Sapienza, Rome, Italy), was designed. In order to control for most potential confounders, pairs were matched for age and gender and odds ratios were adjusted for some important co-variables associated with leukoplakia. Moreover, in order to reduce the information bias regarding the exposure level to tobacco and ethanol, possibly arising from unclear or recently changed smoking and/or alcohol drinking habits, only patients with consolidated habits were recruited.

A consecutive sample of 187 subjects, aged 40–65 years, with a diagnosis of oral leukoplakia, referred to the Oral Surgery Unit of the Eastman Dental Hospital of Rome (Italy), who gave their informed consent to participate in the study, was considered.

Leukoplakia was diagnosed at C3 level of certainty, using the certainty factor scale ranging between C1 and C4,[22] by means of two clinical examinations and a biopsy carried out by the same two trained and calibrated clinicians with more than thirty-years of clinical experience. Inter- and intra-examiner diagnostic consistency was not statistically analysed. At the first examination, the suspected lesion was predominantly white and not classifiable as any other oral mucosal lesion. Mechanical irritations of the oral mucosa, such as sharpened or broken teeth and prostheses, were removed. As far as possible, chemical irritations, such as smoking and heavy alcohol drinking, were also removed. After a four-to-five week interval, if the lesion showed no reduction in size, a biopsy was made, and the lesion had to be histopathologically characterized by presence of dysplasia involving the lower one third of the epithelial layer.

With regard to smoking and alcohol drinking habits, leukoplakia patients who were classified as heavy drinkers, or reported being former smokers and/or drinkers, having drastically changed their smoking and/or drinking habits during the last ten years, chewing tobacco, or smoking cigars were not considered eligible and were excluded. Subjects reporting a mean daily intake frequency of five or more alcohol units (AU) were classified as heavy drinkers[23] and were not considered in the present study. The AU, approximately corresponding to 12 g of ethanol, is usually contained in a can of light beer (i.e. 330 ml), a glass of wine (i.e. 125 ml), or a small glass of spirits (i.e. 40 ml).

The control group consisted of 187 gender and age (±3 years) matched patients unaffected by leukoplakia and consecutively selected among those referred in the same period to the same Oral Surgery Unit for surgical extractions of tooth roots or third molars, and who gave their informed consent for participation. Subjects not affected by either leukoplakia at C1–C4 level of diagnosis, or any other oral mucosal lesions were considered eligible. In relation to the patients' smoking and drinking habits, the same exclusion and classification criteria as for the cases were adopted.

A face-to-face interview based on a structured questionnaire was administered twice to cases and controls at two different occasions with an interval of three to five weeks, to obtain consistent information regarding the following variables:

(1) *Occupation*. The occupational categories were: (i) manual, (ii) technical/artisan/industrial worker, (iii) teacher/office worker, (iv) professional/business, (v) unemployed, (vi) housewife, (vii) retired.[24] For category (vii), the past occupation was considered. For categories (iii), (iv), (v) and (vi), the educational level was also inquired.
(2) *Presence of relatives with cancer*. In order to avoid participants' misunderstandings, cancer was defined using WHO definition as "an uncontrolled growth and spread of cells that may affect almost any tissue of the body";
(3) *Smoking habits*. The number of years subjects reported smoking and the average daily number of smoked cigarettes were investigated.
(4) *Alcohol drinking habits*. The number of years subjects reported drinking and average daily AU intake from overall alcohol, beer, wine and spirits were investigated.

For each subject and variable the value used for the statistical analysis resulted from the average of values obtained from the two interviews.

The effect of habitual, moderate alcohol drinking and of other co-variates on leukoplakia was initially explored by means of a bivariate analysis on matched pairs. The number of discordant pairs, crude odds ratios (OR) with 95% confidence intervals (95% CI), were calculated for each predictor variable, dichotomised as in Table 1. To statistically analyse the homogeneity between cases and controls in the marginal distributions of predictors variables, the McNemar's test was used, and an α level of 0.05 was considered statistically significant, yet not discriminatory for their use in the multivariable analysis.

The OR for leukoplakia of regular, moderate alcohol drinking, adjusted for the effect of covariates, was investigated by means of multiple conditional logistic regression analysis with backward elimination procedure. Variables were removed from the model until the remaining coefficient estimates showed $P \leqslant 0.05$. The predictor variables were alcohol drinking, heavy smoking, occupation, familial predisposition and mechanical irritations of oral mucosa, which were preliminarily tested for collinearity and multicollinearity by means of pairwise Pearson's correlation coefficient (r) and variance inflation factor (VIF). The highest acceptable values for r and VIF were set at 0.6 and 10, respectively.

Different regression models were developed, testing moderate alcohol drinking both globally and split into beer, wine and spirits; both including and excluding two sets of important interaction terms. Namely, among patients with consolidated smoking and drinking behaviour, like those recruited in this study, older subjects were judged more likely to develop the condition than younger subjects, because of the longer tobacco and/or ethanol exposure duration, the first set of interaction terms included those between heavy smoking and age (dichotomised into a reference value of <55 years and a risk value of $\geqslant$55 years) and between alcohol drinking and age. The second set included those between heavy smoking and alcohol drinking variables, based on the supposed synergistic activity of smoking and drinking in oral cancer development.[16,17] When interaction terms were tested, the presence of the corresponding main effects was forced in the regression model until interaction terms remained.

The whole models' goodness of fit was investigated by means of likelihood ratio χ^2 test and R^2. In order to increase

Table 1 – List of the investigated predictor variables and their coding

Variable	Reference value	Risk value
Overall alcohol	None/occasional drinker (<1 daily AUs of overall alcohol intake)	Habitual, moderate drinker (1–4 daily AUs of overall alcohol intake)
Beer	None/occasional drinker (<1 daily AUs of beer intake)	Habitual, moderate drinker (1–4 daily AUs of beer intake)
Wine	None/occasional drinker (<1 daily AUs of wine intake)	Habitual, moderate drinker (1–4 daily AUs of wine intake)
Spirits	None/occasional drinker (<1 daily AUs of spirit intake)	Habitual, moderate drinker (1–4 daily AUs of spirit intake)
Smoking	None/light/moderate smoker (<20 daily cigarettes)	Heavy smoker (⩾20 daily cigarettes)
Occupation	Non manual or intellectual (professional, business. Teacher, office worker, unemployed and housewife with high school/academic degree)	Manual (truly manual. Technical, artisan, industrial worker. Teacher, office worker, unemployed and housewife without high school/academic degree)
Familial predisposition	Non predisposed (no first-grade relative with cancer)	Predisposed (one or more first-grade relatives with cancer)
Mechanical irritation of oral mucosa	No irritation (0–1 signs and/or symptoms of inflammation associable with mechanical irritation at the first clinical examination)	Irritation (⩾2 signs and/or symptoms of inflammation associable with mechanical irritation at the first clinical examination)

the model accuracy and to avoid the phenomenon of overfit, by whom the inclusion of too many variables in a model influences the coefficient estimates and produces an apparently high level of goodness of fit, the number of matched pairs to the number of predictor variables ratio had to be ⩾10. Finally, in order to explore the robustness of the results with respect to spurious associations, the final models were validated splitting the data in half and re-estimating the predictor variable coefficients using one half of the data and recalculating the whole model's goodness of fit using the other half. The validation criteria of the models were that the newly point estimates of the coefficients fall within the 95% CI of the coefficients estimated using the whole sample and that the model goodness of fit be statistically significant at 95% level.

3. Results

Case and control groups included 61.5% ($n = 115$) males and 38.5% ($n = 72$) females, with mean ages of 54.3 ± 5.3 and 54.5 ± 5.5 years, respectively.

40.1% of cases and 59.9% of controls reported habitually drinking alcoholic beverages. Wine was the most frequently consumed alcoholic beverage in both groups, followed by spirits among cases, and beer among controls. Red wine was preferred by 81.2% of all wine drinkers ($n/N = 125/154$), the remainder reported drinking both red and white wine, while heavier liquorish wines, such as sherry were not regularly consumed. Light beer was preferred by 67.5% of beer drinkers ($n/N = 52/77$), the remaining 32.5% had no preference between light and heavy beer. None of the patients had an exclusive preference for white wine or heavy beer (data not shown).

In order to formulate a tentative judgment on the smoking and alcohol drinking behaviour of cases and controls, the two groups' profiles were compared to those of the Italian adult population as reported by the National Institute of Statistics (ISTAT) (Table 2). Heavy smoking proportion and daily number of smoked cigarettes were considerably higher among cases and controls, than among the Italian population aged 15 years or more, while the overall alcohol drinking habits of controls were similar to those of the Italian adult population aged 45–64 years. In contrast, cases drank generally less overall alcohol, wine and beer, but significantly more spirits.

From the bivariate analysis, depicted in Table 3, overall alcohol, beer and wine drinking were inversely associated with the condition, while a weak, non-significant, direct association was found for spirits.

No important levels of collinearity and multicollinearity among predictor variables were found. In fact, the highest r values resulted between beer and spirit ($r = 0.50$) and between

Table 2 – General smoking and alcohol drinking profiles of case and control groups and of the Italian adult population aged 15 years or more (smoking) and 45–64 years (alcohol)

	Cases	Controls	Italian adults
Proportion of heavy smokers (⩾20 daily cigarettes)	29.4%	26.2%	13.6%
Mean number of daily smoked cigarettes	10.2	7.3	4.7
Mean overall alcohol intake (litres per year)	5.7	9.3	9.2
Intake-litres per year (%) – from beer	0.5 (8.2%)	1.3 (14.4%)	1.5 (16.0%)
Intake-litres per year (%) – from wine	2.9 (51.0%)	7.0 (75.7%)	7.1 (77.6%)
Intake-litres per year (%) – from spirits	2.3 (40.8%)	0.9 (9.9%)	0.6 (6.3%)

Table 3 – Crude odds ratios (OR), and 95% confidence intervals (95% CI), for leukoplakia among the 187 matched pairs

Variable	Exposed cases / Non-exposed controls	Exposed controls / Non-exposed cases	OR (standard error)	95% CI
Overall alcohol	23	60	0.38 (0.09)[a]	0.20–0.56
Beer	15	65	0.23 (0.07)[a]	0.09–0.58
Wine	27	68	0.40 (0.09)[a]	0.22–0.58
Spirits	35	27	1.30 (0.33)	0.65–1.95
Heavy smoking	25	22	1.14 (0.33)	0.49–1.79
Occupation	60	35	1.71 (0.36)[a]	1.00–2.42
Familial predisposition	72	20	3.60 (0.91)[a]	1.81–5.38
Mucosal irritations	32	38	0.84 (0.20)	0.45–1.23

a $P < 0.05$ with McNemar's test.

Table 4 – Adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for leukoplakia among the 187 matched pairs, from the best fitting and validated conditional logistic regression models

Variable	Coefficient (standard error)	OR	95% CI
1st model (the results were the same in all cases, including or excluding the interaction terms alcohol*age, heavy smoking*age, heavy smoking*alcohol)			
Whole model goodness of fit, likelihood ratio $\chi^2_{4df} = 71.98$ ($P < 0.0001$); $R^2 = 0.14$			
Overall alcohol	−1.54 (0.28)	0.22	0.12–0.37
Heavy smoking	1.14 (0.30)	3.13	1.74–5.60
Occupation	1.44 (0.28)	4.21	2.42–7.32
Familial predisposition	1.08 (0.24)	2.93	1.83–4.70
2nd model (beer, wine, spirits, heavy smoking forced in the model until interaction terms between alcoholic drinks and heavy smoking, alcoholic drinks and age, heavy smoking and age, remained)			
Whole model goodness of fit, likelihood ratio $\chi^2_{6df} = 120.21$ ($P < 0.0001$); $R^2 = 0.23$			
Wine	−1.62 (0.36)	0.20	0.10–0.40
Spirits	1.07 (0.41)	2.93	1.31–6.55
Heavy smoking	1.57 (0.40)	4.82	2.18–10.66
Occupation	1.86 (0.32)	6.41	3.45–11.90
Familial predisposition	1.72 (0.28)	5.59	3.23–9.69
Wine*smoking	−2.28 (0.64)	0.10	0.03–0.36

Only variables with statistically significant ($P < .05$) coefficient estimates (assessed by the coefficient to its standard error ratio) are listed.

presence/absence of oral mucosa irritations and occupation ($r = 0.41$), with the remainder being lower than 0.4. The highest VIF value resulted for occupation (VIF = 6.90), using overall alcohol not split into the three beverages as predictor variable (data not shown).

The best fitting logistic regression models, among those addressing the validation criteria are displayed in Table 4. When overall alcohol was used as predictor (first model), such variable was significantly associated to a decreased risk for leukoplakia occurrence (adjusted OR, 0.22; 95% CI 0.12–0.37) and the different regression models, both including and excluding the interaction terms, provided the same results. Among covariates, heavy smoking was directly associated with the condition at a statistically significant level (adjusted OR, 3.13; 95% CI, 1.74–5.60), as well as occupation and presence of close relatives with cancer.

The best fitting model, among those analysing the effect of beer, wine and spirit drinking separately, was obtained including the interaction terms (second model displayed). Significant effects for wine (adjusted OR, 0.20; 95% CI, 0.10–0.40), spirits (adjusted OR, 2.93; 95% CI, 1.31–6.55) and heavy smoking (adjusted OR, 4.82; 95% CI, 2.18–10.66) were reported. Moreover, the interaction between wine and heavy smoking was statistically significant, implying that among heavy smoking subjects, the inverse association between wine drinking and leukoplakia was magnified (coefficient, −2.28; standard error 0.64). Among the other co-variates, occupation and relatives with cancer were once again directly and statistically significantly associated with leukoplakia.

4. Discussion

In the present study, regular moderate alcohol consumption was associated with a decreased probability of leukoplakia occurrence, with respect to occasional or no alcohol consumption. Such leukoplakia preventing activity was attributable to wine. Conversely, spirit drinking was associated to an increased risk for leukoplakia, while the effect of moderate beer drinking was not significant, which does not necessarily mean that it had no effect on the condition. Moreover, among

regular wine users, moderate drinking was also able to neutralise the reported leukoplakia promoting activity of smoking more than twenty cigarettes daily.

Once again it is important to emphasise that patients who were classified as heavy drinkers, on the basis of the mean declared daily AU intake were not included in the study, because heavy drinking is unanimously recognised as a leukoplakia risk factor.[6,9,10] The patient selection procedure was likely to be responsible for the reported statistically significant leukoplakia preventing activity of overall alcohol and wine drinking. Moreover, information on alcohol intake was subjected to information bias, more specifically, interviewer and respondent bias.[25,26] This could lead to overestimate alcohol intake among none/light drinkers and, more frequently, to underestimate it among heavy drinkers, with a potential misclassification of some of them in the group of moderate drinkers.[26]

The effect of such misclassification on the risk estimate is not clear. Assuming that under-reporting is systematically larger among high consumers than among other drinking categories,[26] there are two possible scenarios. The first is that the leukoplakia risk curve of alcohol consumption would be J- or U-shaped, as in the case of cardiovascular diseases and of overall mortality, with moderate drinkers at lower risk than high and to none/occasional drinkers.[27] In this case, the potential inclusion of some heavy drinkers in the group of moderate drinkers would cause an artificially higher estimate of the risk for leukoplakia among moderate drinkers. The second is that the leukoplakia risk would progressively increase with parallel increase of alcohol consumption, as in the case of oral cancer.[28] In this instance, the estimated leukoplakia risk among moderate drinkers would be artificially lower.

The method to report alcohol consumption that was adopted in this study (face-to-face interview), was considered the most effective, as self-administered questionnaires are generally less sensitive in detecting heavy drinkers, and at present, there is no perfect biochemical marker available. Particularly, there is no reliable indicator of alcohol consumption in the low to moderate range,[26] which was the alcohol consumption level investigated in the present survey. In order to increase data reliability, particularly in classifying heavy drinkers, subjects were interviewed twice with an interval of three to five weeks. According to Greenfield, this procedure detects a high proportion of under-reporting subjects at the second interview.[29] Finally, the consideration that the other surveys cited in the present study are subjected to the same information bias renders the present data, if not completely reliable, at least comparable.

The findings reported in the present study could be helpful to reconcile the only apparently contrasting results from the literature concerning the effect of alcohol drinking on leukoplakia, since most studies investigating the effect of overall alcohol consumption, without any distinction between heavy and moderate drinkers, generally report not definitive results. Specifically, in only one case was alcohol drinking found associated with an increased risk for leukoplakia at a statistically significant level,[2] with the remainder reporting non-significant results.[11,12,19–21] When heavy drinkers were investigated independently, a clear, direct, statistically significant association of such condition with alcohol intake was reported,[6,9,10] whereas the effect of moderate drinking alone was not significant.[14] Therefore, on the basis of the present and the cited data, it could be speculated that heavy drinking can be recognised as an important risk factor for leukoplakia, whatever type of beverage is consumed. On the other hand, moderate but regular drinking seems not to affect, if not decreasing, the risk for this condition.

As for the role of various alcoholic beverages on leukoplakia, some authors have reported beer drinking, unlike wine or spirit, increasing the risk for leukoplakia among alcohol misusing subjects.[13] In a sample of adult Kenyans, commercial beer, wine and spirits were found to significantly, yet weakly, increase the risk for the condition, irrespectively of the exposure level to ethanol.[7] However, according to WHO, in surveys from developing countries, it is difficult to account for unrecorded consumption of smuggled or home-or informally-produced alcohol, and so alcohol drinking may generally be underestimated.[23] Such findings and those from the present study lead us to hypothesize that for moderate drinkers, wine could decrease the risk for leukoplakia occurrence, while the role of other drinks is still under debate.

Finally, an important issue is if alcohol drinking has a synergistic, multiplicative leukoplakia promoting activity with heavy smoking, like in oral cancer.[17,18] Data from heavy drinkers suggest no interaction,[10,13] whereas among moderate wine drinkers of the present study, the activity of such drink was useful in decreasing the risk for the condition.

The differences between different alcohol consumption levels and between beer, wine and spirit drinking, in affecting leukoplakia development could be explained by the different composition of the various alcoholic drinks, which contain substances synergistically or antagonistically interacting with ethanol.

A group of metabolically active, carcinogenic components of alcoholic drinks are nitrosamines. Ethanol blocks nitrosamine metabolism in the liver, thus allowing them to circulate to other organs, such as kidneys and oesophagus,where they can be activated into carcinogens. Beer usually contains high nitrosamine levels, although concentrations have declined recently, spirits also contain nitrosamines, while wine does not.[16,30]

Another important group of substances contained in beer, wine and spirits are polyphenols. These are plant secondary metabolites, which play an important role in many biological processes, such as plant protection against ultraviolet light and their defence against pathogenic micro-organisms. The great interest in polyphenols for human health is associated with their potent antioxidant activity. Generally, it is established that an oxidation process is involved in the initial steps of human cell dysplasia development. Indeed, highly reactive oxygen species, such as singlet oxygen, superoxide anion and hydrogen peroxide, naturally formed during normal human metabolism, can react with DNA, proteins and lipids, thus disrupting important cellular structures and functions. Human metabolism counts on an efficient antioxidant defensive system involving enzymes and proteins to prevent these detrimental effects. However, under certain circumstances the defences can be overwhelmed so that cancer and dysplasia might occur. Polyphenols appear to exert their effect by

inhibiting the metabolic activation of carcinogenic free radicals[31] and also by inducing apoptosis.[32,33]

All plant derivative foods and beverages, such as the investigated alcoholic drinks, contain different types of polyphenols to different extent. Namely, beer contains high levels of anthocyanins and their polymers. Wine, particularly red wine, has a high content of different polyphenols, such as quercetin, resveratrol, anthocyanins and tannins. On the other hand, polyphenol concentration in spirits is generally low, with the partial exception of spirits aged in wooden barrels, such as brandy and whisky, which contain tannins extracted from the barrel walls by the ethanol–water solution during aging. The decrease in polyphenol concentration is artificially made by the distilled alcoholic drink producers, in order to increase the concentration of volatile aromatic compounds, which are generally bound to polyphenols.[31] Among the investigated drinks, the highest antioxidant potential has been found for red wine, which has a reducing power 10–20-fold higher than the normal reducing power of human plasma[34] and the ingestion of two glasses of red wine causes an increase of the total antioxidant capacity of serum by 18% after 1 h and 11% after 2 h.[35]

On the basis of such considerations, it could be speculated that the effect of moderate alcohol drinking on leukoplakia may depend on the activity of the carcinogenic drink components. If they have greater activity than the carcinogen neutralising components, then regular intake of a moderate quantity of wine could reduce the risk for oral leukoplakia, while moderate spirit intake would increase such risk.

Conflict of interest statement

All the authors of the present paper disclose, under their responsibility, any financial and personal relationship with other people or organisations that could inappropriately influence the work.

Acknowledgements

The authors thank Dr. Francesco Riva and Dr. Maurizio Baraglia for their skilful oral examinations, Dr. Stefania Iannazzo and Ms. Chiara Riva for the collection of the anamnasestic information. This study has been partly supported by grants from the Italian Ministry of Agricultural Politics and Forests.

REFERENCES

1. Petti S. Pooled estimate of World leukoplakia prevalence: a systematic review. *Oral Oncol* 2003;**39**:770–80.
2. Zheng TZ, Boyle P, Hu HF, et al. Dentition, oral hygiene, and risk of oral cancer: a case–control study in Beijing, People's Republic of China. *Cancer Causes Control* 1990;**1**:235–41.
3. Banoczy J, Rigo O. Prevalence study of oral precancerous lesions within a complex screening system in Hungary. *Community Dent Oral Epidemiol* 1991;**19**:265–7.
4. Hashibe M, Sankaranarayanan R, Thomas G, et al. Alcohol drinking, body mass index and the risk of oral leukoplakia in an Indian population. *Int J Cancer* 2000;**88**:129–34.
5. Datta K, Saha RK, Chakrabarti RN. A simple risk estimates study for oral cavity cancer: practical approach in Indian context. *J Indian Med Assoc* 1997;**95**:70–1.
6. Nagao T, Warnakulasuriya S, Gelbier S, et al. Oral pre-cancer and associated risk factors among industrial workers in Japan's overseas enterprises in the UK. *J Oral Pathol Med* 2002;**32**:257–64.
7. Macigo FG, Mwaniki DL, Guthua SW. The association between oral leukoplakia and use of tobacco, alcohol and khat based on relative risks assessment in Kenya. *Eur J Oral Sci* 1995;**103**:268–73.
8. Macigo FG, Mwaniki DL, Guthua SW, et al. Influence of cigarette filters on the risk of developing oral leukoplakia in a Kenyan population. *Oral Dis* 2001;**7**:101–5.
9. Garcia-Pola Vallejo MJ, Martinez Diaz-Canel AI, Garcia Martin JM, et al. Risk factors for oral soft tissue lesions in an adult Spanish population. *Community Dent Oral Epidemiol* 2002;**30**:277–85.
10. Lee CH, Ko YC, Huang HL, et al. The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in southern Taiwan. *Br J Cancer* 2003;**88**:366–72.
11. Shiu MN, Chen TH, Chang SH, et al. Risk factors for leukoplakia and malignant transformation to oral carcinoma: a leukoplakia cohort in Taiwan. *Br J Cancer* 2000;**82**: 1871–4.
12. Shiu MN, Chen TH. Impact of betel quid, tobacco and alcohol on three-stage disease natural history of oral leukoplakia and cancer: implication for prevention of oral cancer. *Eur J Cancer Prev* 2004;**13**:39–45.
13. Harris CK, Warnakulasuriya KAAS, Cooper DJ, et al. Prevalence of oral mucosal lesions in alcohol misusers in south London. *J Oral Pathol Med* 2004;**33**:253–9.
14. Dietrich T, Reichart PA, Scheifele C. Clinical risk factors of oral leukoplakia in a representative sample of the US population. *Oral Oncol* 2004;**40**:158–63.
15. Evstifeeva TV, Zaridze DG. Nass use, cigarette smoking, alcohol consumption and risk of oral and oesophageal precancer. *Eur J Cancer B: Oral Oncol* 1992;**28B**:29–35.
16. Ogden GR, Wight AJ. Aetiology of oral cancer: alcohol. *Br J Oral Maxillofac Surg* 1998;**36**:247–51.
17. La Vecchia C, Tavani A, Franceschi S, et al. Epidemiology and prevention of oral cancer. *Oral Oncol* 1997;**33**:302–12.
18. Ke L, Ping Y, Ge H. Protective action of fresh fruit and vegetable consumption on the synergic effect of alcohol and tobacco use on esophageal cancer in south China. *Nutrition* 2003;**19**:821.
19. Thomas G, Hashibe M, Jacob BJ, et al. Risk factors for multiple oral premalignant lesions. *Int J Cancer* 2003;**107**:285–91.
20. Campisi G, Margiotta V. Oral mucosal lesions and risk habits among men in an Italian study population. *J Oral Pathol Med* 2001;**30**:22–8.
21. Kaugars GE, Brandt RB, Chan W, et al. Evaluation of risk factors in smokeless tobacco-associated oral lesions. *Oral Surg Oral Med Oral Pathol* 1991;**72**:326–31.
22. van der Waal I, Axell T. Oral leukoplakia: a proposal for uniform reporting. *Oral Oncol* 2002;**38**:521–6.
23. World Health Organisation. *Global status report on alcohol*. Geneva: World Health Organisation; 1999.. p. 9–31.
24. Hashibe M, Jacob BJ, Thomas J, et al. Socioeconomic status, lifestyle factors and oral premalignant lesions. *Oral Oncol* 2003;**39**:664–71.
25. Edwards SL, Slattery ML, Ma K-N. Measurement errors stemming from nonrespondents present at in-person interviews. *Ann Epidemiol* 1998;**8**:272–7.
26. Carlsson S, Hammar N, Hakala P, et al. Assessment of alcohol consumption by mailed questionnaire in epidemiological studies: Evaluation of misclassification using a dietary history

interview and biochemical markers. *Eur J Epidemiol* 2003;**18**:493–501.
27. Stanley LL, Mazier MJP. Potential explanations for the French paradox. *Nutrition Research* 1999;**19**:3–15.
28. Blagnardi V, Blangiardo M, La Vecchia C, et al. A meta-analysis of alcohol drinking and cancer risk. *Br J Cancer* 2001;**85**:1700–5.
29. Greenfield TK. Ways of measuring drinking patterns and the difference they make: experience with graduated frequencies. *J Subst Abuse* 2000;**12**:33–49.
30. Berger A. Why wine might be less harmful than beer and spirits. *Br Med J* 1998;**317**:848.
31. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr* 2000;**130**(Suppl. 1):2073S–85S.
32. Hsu SD, Singh BB, Lewis JB, et al. Chemoprevention of oral cancer by green tea. *Gen Dent* 2002;**50**:140–6.
33. Hakimuddin F, Paliyath G, Meckling K. Selective cytotoxicity of a red grape wine flavonoid fraction against MCF-7 cells. *Breast Cancer Res Treat* 2004;**85**:65–79.
34. Lugasi A, Hovari J. Antioxidant properties of commercial alcoholic and nonalcoholic beverages. *Nahrung* 2003;**47**:79–86.
35. Whitehead TP, Robinson D, Allaway S, et al. Effect of red wine ingestion on the antioxidant capacity of serum. *Clin Chem* 1995;**41**:32–5.