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Dietary exposures and oral precancerous lesions in Srikakulam District, Andhra Pradesh, India

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Abstract

Objective: To test the effect of dietary nutrients on oral precancerous lesions in a reverse-smoking (i.e. smoking with the glowing end inside the mouth) population in South India.

Design: Case–control. Cases with precancerous lesions were matched to an equal number of lesion-free controls matched on age (±5 years), sex and village. All subjects used tobacco in some form. Dietary data were obtained using an interviewer-administered food-frequency questionnaire, designed for use in this population. All interviews were conducted blinded to the disease status of the subject. Data were analysed using logistic regression.

Setting: Nineteen rural villages in Srikakulam District, Andhra Pradesh.

Subjects: From a survey of 6007 tobacco users, 485 (79% women) were found to have precancerous, mostly palatal, lesions (cases), and 487 lesion-free subjects were selected as controls.

Results: All eligible subjects consented to participate and nearly all (>99%) had complete data for analyses. Reverse smoking was the most common form of tobacco use among cases (81.9%) and controls (73.5%), and reverse smokers were 5.19 times more likely than chewers to have these lesions (95% confidence interval = 1.35, 19.9). After controlling for relevant covariates, including the type of tobacco use, protective linear effects were observed for zinc (70% reduction across the interquartile range, $P < 0.002$), calcium (34% reduction, $P < 0.002$), fibre (30% reduction, $P < 0.009$), riboflavin (22% reduction, $P < 0.03$) and iron (17% reduction, $P < 0.05$).

Conclusions: Several dietary nutrients appear to protect against oral precancerous lesions that are strongly associated with reverse smoking. The results of this study indicate scope for targeting dietary factors in preventing oral cancer, which should be coupled with aggressive anti-tobacco use efforts.

Keywords
India
Oral neoplasms
Precancerous conditions
Dietary nutrients

Oral cancer is the sixth commonest cancer in the world[1]. Its incidence is particularly high in India, some other countries in Asia, and in certain places in the Western hemisphere, e.g. parts of France and Brazil, where smoking and alcohol drinking are major risk factors. In India, chewing and smoking of tobacco products in various forms is primarily responsible for the high incidence. The World Health Organization (WHO) has estimated that 90% of oral cancers in India among men were attributable to chewing and smoking habits[2]. In previous work, it has been shown that reverse smoking (i.e. with the glowing end inside the mouth), a practice common among women in a coastal region of Andhra Pradesh in east–central India, is strongly associated with oral, particularly palatal, precancerous lesions that may progress to carcinoma and may exhibit epithelial atypia of the palate[3–5].

Nutritional risk factors also have been implicated in cancers of the oral cavity. A number of studies have indicated that the consumption of various vegetables and fruits reduces risk. These relationships may be independent of other risk factors and show a dose–response effect[6–10]. However, any cancer of the alimentary tract can

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affect dietary intake, which in turn may affect the accuracy of assessment of usual dietary habits among cases[11].

Within the oral cavity, cancer generally develops on the tongue, buccal mucosa, gingiva, lips, floor of the mouth, but less often on the palate, except in reverse smokers among whom it is the most common location. In the region of this study, Srikakulam District, Andhra Pradesh, reverse smoking is practised using *chutta*, a coarsely made cheroot (cigar with both ends open) about 5–12 cm long[5]. The prevalence and incidence of these precancerous palatal and other mucosal changes are very high among such reverse smokers[12].

Oral, including palatal, cancer often is preceded by precancerous lesions[13]. The relative risk of developing oral cancer among individuals with oral precancerous lesions has been found to be very high (i.e. >200), demonstrating the fact that such lesions lie on the causal pathway to cancer[14]. The association of oral precancerous lesions with tobacco habits follows a pattern similar to that of oral cancer[12]. Because the prevalence of oral precancerous lesions is much higher than that of oral cancer, these conditions provide a useful clinical marker for oral cancer. For this reason, they have been used as such in large-scale intervention trials[15]. In addition to improving the outcome yield of such studies, using precancerous lesions provides an opportunity to avoid some of the biases associated with measuring dietary intake in individuals with oral cancer. Clearly, the high rate of oral cancer underlines this as a matter of great public concern and the presence of a precancerous marker lesion makes careful epidemiological study more feasible. A probable wide range of variability in nutrient exposures[16–18] that could overcome a common problem with limitations in the distribution of nutrient intake[19] provided an additional rationale for evaluating dietary factors for oral cancer precursors in India.

The primary goal of this research was to test the relationship between the presence of precancerous changes in the mouth and the dietary intake of: the antioxidants, β-carotene and ascorbic acid; the B vitamins, thiamine and riboflavin; and the trace elements, iron, copper, calcium and zinc. These micronutrients were chosen on the basis of a variety of laboratory studies[20,21], human experimental studies[22–26], observational studies[27–30] and the availability of data in the nutrient database[31].

Methods

Subject recruitment/data collection

This population-based case–control study was conducted in 19 villages not included in earlier studies in Srikakulam District, Andhra Pradesh[12,32]. A preliminary census was conducted for listing households along with the identification information for each member of the household and their tobacco use status.

A team consisting of dentists, field investigators and a social scientist especially trained in conducting diet–nutrition interviews visited each household on the house lists with the aim of examining all tobacco users aged 15 years and over. As the first step in the recruitment/data collection process, a field investigator interviewed the potential study subject and filled out a questionnaire containing basic demographic information and details of tobacco and alcohol habits. An experienced dentist then examined the subject for the presence of oral precancerous lesions. The subject was then classified as a case if she or he had an eligible precancerous lesion (palatal changes consisting of patches and red areas, leukoplakia, erythroplakia, submucous fibrosis, and an ulcer or a growth suspicious of oral cancer). In the initial survey, 6007 tobacco users were examined. Of these, 485 were found to have one or more lesions necessary to qualify them as a case. The potential control pool consisted of all examined persons who were found to be free of lesions. A control ($n = 487$) was identified as the next available examinee found to be free of lesions, and matched on sex, age (±5 years) and village. Because of the design of the study all cases and controls used tobacco in some form. Therefore, type of tobacco habit (yes/no for each category, chewing, smoking, reverse smoking) was recorded and used as a control variable in all statistical analyses. All selected cases and controls consented to participate in the study.

An 80-item food-frequency questionnaire (FFQ) specific to this population was developed with an aim of estimating nutrient intake. This was similar to instruments developed for use in Kerala[33] and Gujarat[34]. The FFQ interview for the case–control study was conducted only if a subject was selected to participate and after obtaining informed consent. To minimise the likelihood of bias, all data were collected in a blinded fashion (i.e. the interviewer was not aware of the status of the subject and the subject was not told of the presence or absence of the lesion until completion of the interview, within 5 days of the exam). Therefore, unlike in most case–control studies, the FFQ was administered without anyone involved in the collection of the dietary data having knowledge of the subject's disease status.

The FFQ took approximately 25 minutes to complete. It consisted of questions on the typical frequency and quantity of consumption of 80 food items representing >95% of exposure to total energy, fat, fibre, iron, copper, zinc, calcium, ascorbic acid, β-carotene and the B vitamins in this population.

FFQ validation

The FFQ specifically developed for use in this population was validated for collecting dietary information and estimating nutrient intake. The nutrient database[31] was the same as used in previous work by our group[33,34]. Some 60 people (30 male/female pairs) living in the broad area

of this study, but not in the villages sampled for the case–control study, were selected for the validation study (i.e. it was an external validation study). On eight randomly selected days over the year, subjects were administered 24-hour diet recall interviews (24HR). The FFQ was administered twice, exactly a year apart. A brief description of the results of the comparison between the FFQ- and 24HR-derived nutrient values is included in this paper.

Oral precancerous lesions

Palatal changes constitute the most important precancerous changes among reverse *chutta* smokers, the most common form of tobacco use in this region. Two components of these palatal changes, namely patches and red areas, were included in this study. Patches were defined as well-demarcated, slightly elevated plaques, which qualify for the clinical term leukoplakia[4]. Red area was defined as palatal mucosa showing well-defined reddening without ulceration[4]. Other non-palatal lesions included in this study were leukoplakia classified into homogeneous, nodular and ulcerated (for a detailed description see Pindborg[13]) and oral submucous fibrosis. In two females, lesions suspicious of being oral cancer were confirmed as such on histopathological examination and referred for care. It is important to note that both heat from reverse smoking and products of tobacco combustion play important roles in carcinogenesis, although it is not feasible to delineate the effect of each[12,15].

Tobacco habits

Reverse *chutta* smoking was the common form of smoking in this region[5], especially among women; 98% of women tobacco users engaged in this practice. In this study, overall, a minority of individuals smoked *bidis* (2.6%), cigarettes (1.7%) and *chutta* in the conventional manner (14.3%), or chewed tobacco (2.2%). *Chutta* is a coarsely prepared cheroot. *Bidi* is a smoking stick prepared by rolling 0.15–0.25 g of sun-dried flake-form of tobacco in a rectangular dried piece of *temburni* leaf (*Diospyrous melanoxylon*). Details of these and other forms of tobacco habits in India are described elsewhere[35].

Statistical methods

For the external validation study, nutrient scores derived from the FFQ were compared with those derived from the eight 24HR administered on randomly selected days over the one-year study period. Pearson product moment and Spearman rank order correlations were used as the criteria for comparison.

Descriptive statistics were computed overall and separately for cases and controls. These consisted of either standard parametric statistics for continuous variables (e.g. the nutrient scores) or non-parametric frequency statistics for all variables measured on an ordinal or nominal scale or as counts. The 25th, 50th and 75th percentile values for each of the nutrient scores were computed based on the entire dataset. Multivariable analysis was conducted using logistic regression. Because of the strength of association between specific types of tobacco use and oral cancer and precancer, some designation of tobacco habit was considered in specifying all statistical models. Two indicator variables describing the three major categories of tobacco use in this population (reverse *chutta* smoking, smoking in the conventional manner and chewing tobacco in any form (referent group)) was conceptually the simplest scheme and had the largest explanatory ability of any alternative. Duration of use was closely associated with age and no measure of intensity appeared to affect estimates of risk after accounting for type of tobacco use.

Social and economic variables often serve as proxies for potentially important risk factors for cancer and therefore are frequently included in analyses. As the vast majority (93%) of the population was illiterate, it was not possible to use education, one of our two indicators, in analyses. For reasons of multicollinearity, it also was not possible to include economic status (described as either higher – a brick house with tiled or corrugated tin roof; or lower – a mud house with thatched roof) because it was strongly related to smoking; e.g. for overall smoking (including reverse smoking) the Mantel Haenzel chi-square was 4.24 ($P = 0.04$), whereas for conventional smoking the chi-square was 52.36 ($P < 0.0001$). Nutrient scores were included both as continuous variables and quartiles, in separate models, because dietary nutrients are highly correlated with one another. Because dietary exposure estimates may be biased by overall errors in reporting[36,37] and some nutrients have a stoichiometric relation with total energy utilisation[38], we controlled for total energy intake by fitting it as a covariant in each model. For nutrients evincing linear effects, we computed the effect across the interquartile range of its distribution, thus standardising the effect for the distributions of nutrient exposure reported in this population.

The primary analyses were conducted on the main study data for all types of lesions combined. Additional analyses were conducted by gender and by lesion type. All analyses were conducted using the personal computer version of SAS[39,40].

Follow-up study

After one year, all 6007 tobacco users were re-examined. Among those found to be lesion-free at the first survey, 39 had a new incident lesion. For each case thus identified, a control was selected. These data were analysed separately in the same manner as for the main case–control study dataset. In order to assess whether the expected wide confidence interval (CI) was simply due to sample size (and not other factors affecting precision), we adjusted the 95% CI for the ratio of the sample sizes of the prevalent and incident case series. The 'sample-size-adjusted' 95% CI

is obtained by the formula antilog $[b \pm 1.96\mathrm{SE}_b/\sqrt{n_\mathrm{p}/n_\mathrm{i}}]$, where b = log odds ratio, SE_b = standard error of b, n_p = number of prevalent cases and n_i = number of incident cases.

Results

Table 1 shows the results from the external validation study. These consist of correlation coefficients for each of the nutrients of interest plus total energy intake, a control variable fit in all logistic regression models. Correlation coefficients for total fat and fat as percentage of energy also are shown. With the exception of sodium, ascorbic acid and β-carotene, the correlation coefficients were moderately high, comparing very favourably with those of other studies[41].

The descriptive statistics of the study population, including the reported daily nutrient intakes as estimated by the FFQ, are shown in Table 2. In both the validation study and the case–control study, there was an apparent miscalibration for rice preparations (rice, rice with starch water, and rice with buttermilk). These preparations represented 78% of total caloric intake reported in this population; about three times higher than expected based on estimates from other rice-eating populations[16] including a group we had studied in Kerala[33]. As presented, energy intake represents the total from the remaining 77 foods, but with a re-calibration of rice intake based on measurements from the predominantly rice-eating study population in Kerala[33]. This was done by computing the metabolic need per kg body weight by sex in Kerala (i.e. kcal kg^{-1} day^{-1}) and applying that rate to an individual's consumption of rice in *this* population. In all analyses of study data, the intake of energy actually reported (and not the adjusted value shown in the table) was used as a control variable. This was done to avoid using imputed data in the regression analyses.

Due to miscalibration of rice intake, the intake of many nutrients was overestimated because of the amounts involved (even though rice normally is only a minor

Table 1 Results of correlation analyses – Food-Frequency Questionnaire External Validation Study, Srikakulam District, Andhra Pradesh, India, 1993–94*

	Pearson product moment correlation†		Spearman rank correlation‡	
Nutritional variable	Pre	Post	Pre	Post
Total energy (kcal day^{-1})	0.55	0.55	0.64	0.50
Total fat (g day^{-1})	0.68	0.67	0.65	0.56
Fat (% energy)	0.81	0.81	0.72	0.70
Fibre (g day^{-1})	0.70	0.62	0.71	0.53
Iron (mg day^{-1})	0.44	0.38	0.53	0.33
Sodium (mg day^{-1})	0.29	0.14	0.34	0.31
Copper (mg day^{-1})	0.62	0.48	0.61	0.36
Zinc (mg day^{-1})	0.62	0.69	0.69	0.65
Calcium (mg day^{-1})	0.56	0.32	0.65	0.37
Ascorbic acid (mg day^{-1})	0.08	0.30	−0.005	0.31
β-Carotene (μg day^{-1})	0.10	0.15	0.18	0.26
Thiamine (mg day^{-1})	0.61	0.57	0.65	0.43
Riboflavin (mg day^{-1})	0.55	0.51	0.60	0.45

* In all instances $P < 0.05$ if $|r| > 0.25$, total $n = 60$.
† This is the parametric coefficient obtained in correlating the FFQ-derived nutrient score with the equivalent 24HR-derived nutrient score.
‡ This is based on the rank order (non-parametric) correlation.

Table 2 Descriptive statistics – Diet and Oral Precancer Study, Srikakulam District, Andhra Pradesh, India, 1993–95*

	Cases		Controls	
Categorical variable*	*n*	(%)	*n*	(%)
Males	104	(21.4)	108	(22.2)
Females	381	(78.6)	379	(77.8)
Occupation				
Business/Professionals	3	(0.6)	2	(0.4)
Farming/Merchandise	25	(5.2)	39	(8.0)
Skilled labour	32	(6.6)	17	(3.5)
Secretarial/Clerical	9	(1.9)	8	(1.6)
Unskilled/Self-employed	229	(47.2)	257	(52.8)
Householder	187	(38.6)	164	(33.7)
Education				
Illiterate	451	(93.0)	451	(92.6)
Primary	21	(4.3)	24	(4.9)
Middle	12	(2.5)	6	(1.2)
High school	1	(0.2)	3	(0.6)
College	0	–	3	(0.6)
Social category				
Forward	206	(42.5)	222	(45.6)
Backward	196	(40.4)	171	(35.1)
Schedule	83	(17.1)	94	(19.3)
Socio-economic status				
Low	442	(91.1)	457	(93.8)
Medium	43	(8.9)	30	(6.2)
Tobacco use				
Chewing	3	(0.6)	18	(3.7)
Smoking	56	(11.5)	72	(14.8)
Smoking and chewing	29	(6.0)	39	(8.0)
Reverse smoking	397	(81.9)	358	(73.5)
Continuous variable†	Mean	(SD)	Mean	(SD)
Age (years)	52.1	(10.4)	51.3	(10.4)
Nutrients†‡				
Total energy (kcal day^{-1})§	1981	(408)	1998	(404)
Total fat (g day^{-1})	33.4	(22.4)	33.4	(14.2)
Fat (% energy)	15.4	(9.8)	15.2	(6.0)
Fibre (g day^{-1})	12.3	(5.0)	13.3	(5.9)
Iron (mg day^{-1})	23.3	(8.2)	24.5	(8.8)
Sodium (mg day^{-1})	83.2	(62.1)	88.7	(51.5)
Copper (mg day^{-1})	2.05	(0.70)	2.15	(0.82)
Zinc (mg day^{-1})	18.3	(5.5)	18.9	(6.1)
Calcium (mg day^{-1})	939	(452)	1046	(495.4)
Ascorbic acid (mg day^{-1})¶	4.3	(0.4)	4.3	(0.4)
β-Carotene (μg day^{-1})¶	7.4	(0.6)	7.5	(0.5)
Thiamine (mg day^{-1})	1.51	(0.56)	1.62	(0.64)
Riboflavin (mg day^{-1})	1.33	(0.38)	1.38	(0.40)

* Values presented are the number and percentages of all cases and controls with the attribute.
† Value is the mean and standard deviation (SD) by case and control status.
‡ Nutrients are daily amounts as calculated from the food-frequency questionnaire, as described in the text.
§ Energy intake is adjusted to account for overreporting of rice intake, as reported in the text.
¶ Values of these nutrients are log-transformed to normalise the distribution.

Table 3 Distribution of lesion types – Diet and Oral Precancer Study, Srikakulam District, Andhra Pradesh, India, 1993–95*

	Male		Female	
	n	(%)	*n*	(%)
Palatal changes (patches)	54	(51.9)	332	(87.1)
Palatal changes (red areas)	5	(4.8)	43	(11.3)
Leukoplakia	46	(44.2)	14	(3.7)
Submucous fibrosis	2	(1.9)	–	–
Carcinoma	–	–	2	(0.5)
Total number of subjects with qualifying lesions	104		381	

*Tabulated values are the number of subjects (cases) with each lesion. The value in parentheses is the proportion of males or females having the lesion. Because of multiple lesions, the total will add to a number greater than 100%.

contributor to intake of most of these nutrients). Exceptions were ascorbic acid and β-carotene, to which the contribution of rice is nil. It must be emphasised that the miscalibration in reporting rice intake appeared to be uniform across the whole study and it affected the intake values only through change of origin and scale. Correlation, however, is not affected by any change in origin or scale, and for most nutrients the correlation coefficients were relatively high, as they were for total energy (Table 1).

The distribution of lesions among the 485 cases is presented in Table 3. Among women, almost all lesions were located on the palate; whereas among men, slightly over half of all lesions were located on the palate. Smoking, in any form, was associated with elevated risk. Odds ratios (ORs) for smoking were consistent, irrespective of what control variables were fit in the model. In the model with no dietary or economic variables included, relative to chewing only, the OR for reverse *chutta* was 5.19 (95% confidence interval (CI)=1.35, 19.9) and for conventional smoking it was 3.63 (95% CI = 0.96, 13.74). As with results based on analyses of other data from these same study areas, alcohol intake was minimally associated with the presence of these lesions[42]. Inclusion of any other predictor, including alcohol, did not materially affect the size or significance of these relationships. When restricting the analysis to females, information on tobacco habit was omitted from the model because virtually all (98%) women tobacco users were reverse *chutta* smokers.

Table 4 presents the OR and 95% CI for each of the eight nutrients found to be related to oral precancerous lesions. Six were found to have linear protective effects and two were found to be associated with reduced risk at any level above the lowest quartile of intake. For those linearly related, we show the effect of the nutrient across the interquartile range of its distribution as a way of standardising their effects. In all models, virtually identical results were observed for all control variables. For all six nutrients fit as continuous variables, the model had higher overall explanatory ability than did the quartile alternative.

Models not shown analogous to those in Table 4 but fit for women separately showed very similar results, owing to the preponderance of women in this study. Among men, except for zinc (OR = 0.87, or a 13% reduction in risk per gram of zinc consumed per day, $P = 0.06$), the results did not approach statistical significance. However, the point estimates of the ORs were similar for men and women.

Table 4 Adjusted odds ratios for nutrients in relation to overall lesions – Diet and Oral Precancer Study, Srikakulam District, Andhra Pradesh, India, 1993–95*

	OR (95% CI)	*P*-value	Effect across interquartile range (%)†
Nutrients best fit as a continuous variable‡			
Iron (10 mg day^{-1})	0.82 (0.68, 0.99)	0.04	(16.6)
Zinc (mg day^{-1})	0.91 (0.85, 0.98)	0.02	(70.2)
Copper (mg day^{-1})	0.83 (0.67, 1.03)	0.09	(16.0)
Calcium (100 mg day^{-1})	0.95 (0.92, 0.98)	0.001	(33.6)
Riboflavin (mg day^{-1})	0.51 (0.28, 0.93)	0.03	(22.1)
Fibre (g day^{-1})	0.96 (0.94, 0.99)	0.007	(29.6)
Nutrients exerting non-linear effects§			
β-Carotene (highest 3 quartiles)	0.78 (0.58, 1.05)	>0.10	
Ascorbic acid (highest 3 quartiles, females only)	0.82 (0.59, 1.13)	>0.10	

*Nutrients shown are ones hypothesised to be related to risk of oral cancer or precancer. Odds ratios (ORs) and their 95% confidence intervals (CIs) are based on seven separate logistic regression models, one for each of the seven nutrients shown (excluding ascorbic acid). Each model controlled for type of tobacco habit and total energy consumption (kcal day^{-1}). For ascorbic acid, type of tobacco habit was omitted because virtually all women were reverse *chutta* smokers.

† For each nutrient fit as a continuous variable, the effect was standardised by computing the difference of effect at the 75th percentile value (OR×nutrient$_{75}$) and its effect at the 25th percentile value (OR×nutrient$_{25}$). The value shown represents the percentage reduction across the interquartile range. The respective 25th, 50th and 75th percentile values for each nutrient shown are as follows: iron (mg day^{-1}) – 18.5, 23.1 and 27.7; zinc (mg day^{-1}) – 14.7, 19.4 and 22.5; copper (mg day^{-1}) – 1.55, 2.04 and 2.49; calcium (mg day^{-1}) – 583, 974 and 1255; riboflavin (mg day^{-1}) – 1.11, 1.36 and 1.56; fibre (g day^{-1}) – 8.5, 12.1 and 15.9; β-carotene (μg day^{-1}) – 1180, 1675 and 2405; ascorbic acid (mg day^{-1}) – 57.2, 74.4 and 93.7.

‡ Each nutritional variable is fit as a continuous variable. The units are modified to permit easier interpretation of the odds ratio (e.g. the OR shown for calcium represents the fraction of risk with each 100 mg consumed per day).

§ These variables were found to have an effect, which was clearly non-linear. In each instance the referent is the lowest quartile of reported intake.

Table 5 Adjusted odds ratios for nutrients in relation to overall newly incident lesions – Diet and Oral Precancer Study, Srikakulam District, Andhra Pradesh, India, 1993–95*

	OR (95% CI)	Sample-size-adjusted 95% CI†
Nutrients best fit as a continuous variable†		
Iron (10 mg day^{-1})	0.82 (0.39, 1.69)	(0.67, 1.00)
Zinc (mg day^{-1})	0.88 (0.63, 1.22)	(0.80, 0.96)
Copper (mg day^{-1})	0.77 (0.35, 1.73)	(0.61, 0.97)
Calcium (100 mg day^{-1})	0.98 (0.87, 1.10)	(0.95, 1.01)
Riboflavin (mg day^{-1})	0.39 (0.03, 4.53)	(0.20, 0.77)
Fibre (g day^{-1})	0.97 (0.88, 1.06)	(0.94, 1.00)

* Nutrients shown are ones hypothesised to be related to risk of oral cancer or precancer. Odds ratios (ORs) and their 95% confidence interval (CIs) are based on six separate logistic regression models, one for each of the nutrients shown. Each model controlled for type of tobacco habit and total energy consumption (kcal day^{-1}).
† This is the 95% confidence interval adjusted for the sample size observed in the main study (based on 485 eligible lesions).

Analyses based on palatal changes consisting of patches were similar to those based on overall lesions, with significant protective effects for calcium (OR = 0.95; 95% CI = 0.92, 0.98), riboflavin (OR = 0.46; 95% CI = 0.23, 0.93) and fibre (OR = 0.97; 95% CI = 0.94, 0.99). Point estimates of the OR in women and in men were virtually identical (though none were statistically significant in men). Results from analyses of palatal changes consisting of red areas showed a protective effect of zinc in the higher quartiles of intake: for quartile 2, OR = 0.11 (95% CI = 0.02, 0.57); for quartile 3, OR = 0.09 (95% CI = 0.01, 0.74); and for quartile 4, OR = 0.05 (95% CI = 0.003, 0.80). Results in women were identical to the overall results. There also was a larger decrease in risk from calcium (OR = 0.92, 95% CI = 0.82, 0.99) for red areas, as compared with patches. Leukoplakia-specific results were unremarkable, with only suggestions of protective effects in women for zinc (OR = 0.38; 95% CI = 0.14, 1.08), fibre (OR = 0.80; 95% CI = 0.61, 1.05) and calcium (OR = 0.73; 95% CI = 0.52, 1.02).

Analyses focusing on newly incident cases (Table 5) were meant to corroborate the results of the main case–control study shown in Table 4. Due to the small sample size and the confirmatory nature of that portion of the study, there was neither an intention of formal hypothesis testing nor one of examining effects in any subset of the data. Among individuals who were originally lesion-free, 39 were found to have one or more lesions after one year (cases). One such person was included as a control in the main case–control study, but was classified as a case in this follow-up dataset. All female cases were reverse *chutta* smokers. Of the 32 women with lesions, 30 had palatal patches and two had red areas. Among seven new male incident cases, five were diagnosed with leukoplakia, one had palatal changes, and one had lichenplanus. Despite very wide confidence limits, as expected, the point estimates of the ORs were similar to those presented in Table 4. When we 'adjusted' the 95% CI to the size of sample in the main study, they were very similar to those shown in Table 4.

Discussion

Studies attempting to relate diet with oral cancer must confront two major obstacles, one inherent in the relationships among relevant risk factors and the other a consequence of the distribution of oral cancer in human populations. In most populations, oral cancer is strongly related to either tobacco use or alcohol consumption or both[11]. Typically, these two risk factors are related to diet, with tobacco users consuming diets that are otherwise less healthy than diets of non-tobacco users[43,44]. As such, these risk behaviours have the potential to confound the apparent effect of dietary factors. Besides relationships among risk behaviours, there are organic relationships among dietary constituents and those related to the use of tobacco. For example, products of tobacco combustion will create a demand for antioxidants, such as β-carotene, whose only source (at least in a population such as this) is dietary. Thus, smoking is an important determinant of serum β-carotene levels, even in subjects who are apparently healthy[45,46]. This demand might be increased in subjects with cancers or precancerous conditions, especially in those who continue to smoke. So, while the use of biomarkers of dietary exposure may have conceptual appeal, tissue levels may not be an adequate reflection of dietary intake (although it may have relevance to tissue-level exposure to the nutrient or its metabolite). In studies using serum levels of β-carotene as a biomarker[47], unless smoking is carefully measured and controlled in analyses, some of the variability in β-carotene levels will be explained by tobacco smoking, and inferences regarding dietary β-carotene almost certainly will be confounded, even in cohort studies of subjects who are apparently healthy when recruited[45,46].

The second obstacle in the design and execution of epidemiological studies is the fact that oral cancer is a rare disease in most populations. Therefore, it has been amenable to study mainly using case–control designs. Such designs are subject to biases in self-report, arising either directly or indirectly from changes in exposure to risk factors, especially diet, concomitant with the onset of disease symptoms[11,19] or to beliefs held by research subjects regarding the causes of disease or disease progression[48]. Because oral cancer is likely to affect the diets of oral cancer patients and diet–cancer hypotheses have been popularised in many populations, such studies are limited by the potential for biased dietary recall among the cases as compared with the controls[11]. Apparently, there is no specific scientific literature on beliefs or attitudes about diet in relation to cancer in India, although there are widely held beliefs about diet and health more generally[49].

In this study, we were careful to enrol only users of

tobacco and then to measure their exposure to tobacco products very carefully using methods that had been developed and refined through years of study in this population[12,50]. In designing this study, a decision was made to focus on precancerous lesions. This was done to increase outcome yield and to reduce the probability of biased dietary exposure estimates due to the presence of a condition that could affect the physical sensation and palatability of food among the cases. Our prior research had indicated a high relative risk of the precancerous lesions seen in this population progressing to frank cancer[14]. By studying these conditions earlier on in the natural history of the disease, there would be a better chance of measuring diet during the more aetiologically relevant period. Finally, in order to reduce further the probability of bias, we chose to withhold the diagnosis of the condition from both the subject and the interviewer until the diet interview was completed (<5 days from the exam).

Oral precancerous lesions included in this study, with the exception of oral submucous fibrosis, produced no symptom that would materially affect the usual diet of the affected individual. Oral submucous fibrosis almost invariably causes a burning sensation on intake of spicy food and since the food in this part of India is especially spicy, that could cause some changes in usual diet. Following the study protocol, oral submucous fibrosis cases were included in the case group even though there were only two and they would not have materially affected findings. It was not feasible to conduct a separate analysis for oral submucous fibrosis, as was done for the Gujarat study[10].

Study findings in context

As expected, the strongest relationship observed was that between reverse *chutta* and palatal lesions, which represented the most common tobacco habit and most common lesion type, respectively. As with other studies in India, there was no affect of reported alcohol exposure[42]. This may be due to the dominance of tobacco use in causing these lesions or to relatively low rates of exposure to alcohol.

Judging by the size of the effect across the interquartile range of exposure (Table 4), the strongest dietary relationships observed in this study were the protective effects of zinc, calcium and fibre. The observed effect of zinc is consistent with that reported in another study in reverse *chutta* smokers[22,25]. Zinc is a necessary component of over 200 enzyme systems necessary for the proper differentiation and growth of cells and as a structural constituent of many proteins, hormones, neuropeptides, hormone receptors and probably polynucleotides[51]. Like zinc, iron showed a linear (though weaker) effect in these data. Also like zinc, iron may be important for proper differentiation of epithelial tissue and other potential mechanisms of carcinogenesis[52–55].

In a hospital-based case–control study in China, it was found that dietary fibre derived from fruits and vegetables showed a strong negative association with oral cancer risk[47]. These results were similar to those from a population-based case–control study in which risks decreased with increasing intake of fruits and some vegetables[27]. In another case–control study in the USA, it was observed that dietary fibre was associated with decreased risk[29]. Calcium, however, had not been observed to have a strong relationship with oral cancer previously. There is some suggestion that Ca^{2+} release affects cell rounding and retraction in human oral cavity epidermoid carcinoma cells[56]. There is one case–control study that reports higher nail concentrations of iron and calcium in oesophageal cancer cases than in controls[57]. Still, these findings pertain to a different site and histological type and, in frank cases of cancer, there may be metabolic alterations that further obfuscate the relationship between diet and disease.

Results of a survey of a population with a high risk of oral and oesophageal cancer (in Uzbekistan) indicated that blood levels of retinal, carotene and riboflavin were lower among individuals with these conditions[28,58]. The use of blood measures in people with frank disease may lead to biased estimates of exposure relative to typical diet in the aetiological period of interest, irrespective of the effect of smoking on tissue levels of antioxidants. Analysing data collected before disease onset, a nested case–control study in Washington County, MD showed that serum levels of carotenoids and α-tocopherol were lower among subjects who developed oral and pharyngeal cancer than in matched controls who were free of disease[59]. Because of its design, that study was able to circumvent problems with disease-related biases[59].

Sodium, ascorbic acid and β-carotene showed some of the lowest correlation and regression coefficients in comparing the FFQ- and 24HR-derived dietary data. Also, these three nutrients were only weakly associated with the lesions, if at all. It may be that these two observations are related; i.e. to some extent imprecision in estimating intake may explain the lack of strong relationship with disease status. In our data, there was a suggestion that β-carotene intake in the highest quartile (here estimated to be $>2.4\,\text{mg day}^{-1}$) may be protective. That this is still far below pharmacological range is consistent with findings from other studies on the effect of β-carotene in the physiological range[23,60–63].

In this study, riboflavin was found to be protective. In one case–control study conducted in Western New York State, riboflavin was associated with increased risk[29]. However, in another case–control study from Italy, an increased maize intake among cases with cancers of the oral cavity, pharynx and oesophagus was reported[30]. Because maize can cause deficiencies of riboflavin, this result is consistent with a broad range of evidence indicating a protective effect of this B vitamin from

studies conducted in Africa, China, the United States and Italy[30].

In an intervention trial of reverse *chutta* smokers from Srikakulam District, using the frequency of micronucleated cells and DNA adducts as indicators of DNA damage, it was reported that supplementation with four nutrients (vitamin A, riboflavin, zinc and selenium) reduced micronuclei and DNA adducts in subjects both with and without precancerous lesions at the beginning of the study[25]. It also was found that these same nutrients were related to a reduced incidence of oral precancerous lesions[22]. In a randomised, double-blind intervention trial conducted in a population with a high incidence of disease in Huixian, People's Republic of China, there was only a weak suggestion of protective effects of riboflavin and zinc[64].

The incident oral precancerous lesions diagnosed during follow-up after one year (39 cases) and an equal number of matched controls examined and interviewed exactly in the same manner as in the case–control study provided a built-in check for the results obtained in the main case–control study. Although the one-year dataset afforded little statistical power, it did provide a unique opportunity to compare point estimates of the OR with those from the main case–control study. When we adjusted the 95% CI for the sample size in the main case–control study we found that they were remarkably similar, indicating that the wide confidence limits were due to small sample size and not heterogeneity of effect. In the main case–control study, no estimate of the duration of the presence of the lesion was possible and there could have been some undetermined heterogeneity with regard to that in the case group. Analysis of these 39 incident cases addressed that problem and it was reassuring that the results were very similar.

Weaknesses and recommendations for future study

Because of the uniformly low level of education in this population, it was not possible to control for it in analyses or to examine covariance in other factors (e.g. dietary calcium) with which it may be related. Future work in this population should aim to enrol subjects with a wider range of educational attainment.

Except for two studies on which we reported from Gujarat[10] and Kerala[9], studies of diet and cancer previously reported from India have used simple diet checklists and FFQs inadequate for the purposes of nutrient estimation. In Andhra Pradesh, a large portion of the adult population is illiterate. This fact, as well as our need to standardise collection methods to the extent possible, compelled us to use the interviewer-administered FFQ. Testing of this instrument was conducted in an external validation study in a population similar to that used as the basis of the case–control study. Results indicated a relatively high level of agreement between nutrient consumption data derived from this FFQ and data derived from eight days of 24HR administered over a one-year period. This was true even for total caloric intake to which rice was a major contributor and occurred despite an obvious miscalibration in reporting intake of rice preparations. The overestimate in rice intake was similar in direction to the social approval bias that we have observed among men in the USA[48,65], but of somewhat larger magnitude. Unlike results in both Gujarat[34] and Kerala[33], the overestimate affected both the 24HR-derived and the FFQ-derived estimates. Given the high level of importance attached to food in India, future work should focus on understanding the source of the bias and methods developed to minimise its effect.

Rather than make *post hoc* adjustments to account for miscalibration, we used the actual values in all analyses. As with most epidemiological studies of diet and cancer in humans, this study produced ORs as estimates of relative risk of exposure to these nutrients and this miscalibration would not affect these estimates. By not adjusting, we have avoided adding possible error to the estimated relative risk. Still, estimating exact nutrient dose–response relationships would be problematic because of the overreporting of rice intake (i.e. real exposure levels would be lower than percentile scores shown in Table 4).

Summary

The results of this study, unencumbered by the kind of biases that normally would beset a study of nutrition and oral cancer, indicate a protective effect of several micronutrients in oral precancerous lesions in a population exposed to tobacco. In its design, we recognised the potential for intractable confounding and took advance remedial steps such as the use of blinded interviews to minimise the possibility of bias associated with diagnosis, referral and assessment procedures. A focus on oral precancerous lesions offered a particularly good opportunity for research since, unlike oral cancer, the individual was generally not aware of the lesion and had few, if any, associated symptoms that might affect dietary intake. Results from this study support consumption of a nutrient-dense, vegetable-based diet in reducing risk of oral precancerous lesions, a conclusion consistent with that reached by a variety of governmental and non-governmental agencies[66–68]. Even though a disease-related bias was unlikely, future work should focus on identifying and controlling for more generalised (i.e. non disease-related) biases in the self-reporting of dietary intake.

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References

1 Parkin DM, Pisani P, Ferlay J. Esimates of worldwide incidence of eighteen major cancers in 1985. *Int. J. Cancer* 1993; **54**: 594–606.

2 World Health Organization (WHO). Control of oral cancer in developing countries: a WHO meeting. *Bull. WHO* 1984; **62**: 817–30.

3 Mehta FS, Shroff BC, Gupta PC, Pindborg JJ. A correlative histocytological study of carcinoma and epithelial atypia of the palate among Indian reverse smokers. *Br. J. Cancer* 1972; **26**: 230–3.

4 Mehta FS, Jalnawalla PN, Daftary DK, Gupta PC, Pindborg JJ. Reverse smoking in Andhra Pradesh, India: variability of clinical and histologic appearances of palatal changes. *Int. J. Oral Surg.* 1977; **6**: 75–83.

5 Pindborg J, Mehta F, Gupta P, Daftary D, Smith C. Reverse smoking in Andhra Pradesh, India: a study of palatal lesions among 10,169 villagers. *Br. J. Cancer* 1971; **25**: 10–20.

6 Marshall J, Graham S, Mettlin C, Shedd D, Swanson M. Diet in the epidemiology of oral cancer. *Nutr. Cancer* 1982; **3**(3): 145–9.

7 Winn D, Ziegler R, Pickle L, Gridley G, Blot W, Hoover R. Diet in the etiology of oral and pharyngeal cancer among women from the Southern United States. *Cancer Res.* 1984; **44**: 1216–22.

8 Franco EL, Kowalski LP, Oliveira BV, Curado MP, Pereira RN, Silva ME, *et al.* Risk factors for oral cancer in Brazil: a case–control study. *Int. J. Cancer* 1989; **43**: 992–1000.

9 Gupta PC, Hebert JR, Bhonsle RB, Murti PR, Mehta H, Mehta FS. Influence of dietary factors on oral precancerous lesions in a population-based case–control study in Kerala, India. *Cancer* 1999; **85**: 1885–93.

10 Gupta PC, Hebert JR, Bhonsle RB, Sinor PN, Mehta H, Mehta FS. Dietary factors in oral leukoplakia and submucous fibrosis in a population-based case–control study in Gujarat, India. *Oral Dis.* 1998; **4**: 200–6.

11 Marshall JR, Boyle P. Nutrition and oral cancer. *Cancer Causes Control* 1996; **7**: 101–11.

12 Gupta PC, Mehta FS, Daftary DK, Pindborg JJ, Bhonsle RB, Jalnawalla PN, *et al.* Incidence of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. *Community Dent. Oral Epidemiol.* 1980; **8**: 287–333.

13 Pindborg JJ. *Oral Cancer and Precancer.* Bristol: John Wright & Sons, Ltd, 1980; 177.

14 Gupta PC, Bhonsle RB, Murti PR, Daftary DK, Mehta FS, Pindborg JJ. An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. *Cancer* 1989; **63**: 2247–52.

15 Murti PR, Bhonsle RB, Gupta PC, Daftary DK. Oral health consequences of tobacco use in Ernakulam District, Kerala, India. In: Gupta PC, Hamner JE, Murti PR, eds. *Proceedings of an International Symposium on Control of Tobacco-Related Cancers and Other Diseases, Bombay, India, 15–19 January 1990.* Oxford: Oxford University Press, 1992; 85–105.

16 National Institute of Nutrition (NIN). *National Nutrition Monitoring Bureau Report of Repeat Surveys (1988–90).* Hyderabad, India: NIN, Indian Council of Medical Research, 1991.

17 Rao B. Monitoring nutrient intakes in India. *Indian J. Pediatr.* 1987; **54**: 495–501.

18 Hebert JR, Gupta PC, Mehta H, Ebbeling CB, Bhonsle RB, Varghese F. Sources of variability in dietary intake in two distinct regions of rural India: implications for nutrition study design and interpretation. *Eur. J. Clin. Nutr.* 2000; **54**: 479–86.

19 Hebert JR, Miller DR. Methodologic considerations for investigating the diet–cancer link. *Am. J. Clin. Nutr.* 1988; **47**: 1068–77.

20 Kandarkar SV, Sawant SS. The effect of vitamin C on the hamster cheek pouch treated with the water soluble carcinogen 4-nitroquinoline-1-oxide (4NQO). *Eur. J. Cancer* 1996; **32B**: 230–7.

21 Shklar G, Schwartz J. Oral cancer inhibition by micronutrients. The experimental basis for clinical trials. *Eur. J. Cancer* 1993; **29B**: 9–16.

22 Krishnaswamy K, Prasad MP, Krishna TP, Annapurna VV, Reddy GA. A case study of nutrient intervention of oral precancerous lesions in India. *Eur. J. Cancer* 1995; **21B**: 41–8.

23 Garewal HS, Schantz S. Emerging role of beta-carotene and antioxidant nutrients in prevention of oral cancer. *Arch. Otolaryngol. Head Neck Surg.* 1995; **121**: 141–4.

24 Maher R, Aga P, Johnson NW, Sankaranarayanan R, Warnakulasuriya S. Evaluation of multiple micronutrient supplementation in the management of oral submucous fibrosis in Karachi, Pakistan. *Nutr. Cancer* 1997; **27**: 41–7.

25 Prasad MP, Mukundan MA, Krishnaswamy K. Micronuclei and carcinogen DNA adducts as intermediate end points in nutrient intervention trial of precancerous lesions in the oral cavity. *Eur. J. Cancer* 1995; **31B**: 155–9.

26 Tanaka T. Chemoprevention of oral carcinogenesis. *Eur. J. Cancer* 1995; **31B**: 3–15.

27 Zheng W, Blot WJ, Shu XO, Diamond EL, Gao YT, Ji BT, *et al.* Risk factors for oral and pharyngeal cancer in Shanghai, with emphasis on diet. *Cancer Epidemiol. Biomark. Prev.* 1992; **1**: 441–8.

28 Zaridze D, Blettner M, Trapeznikou N, Kuvshinou J, Matiakin E, Poljakov B. Survey of a population with a high incidence of oral and oesophageal cancer. *Int. J. Cancer* 1985; **36**: 153–8.

29 Marshall JR, Graham S, Haughey BP, Shedd D, O'Shea R, Brasure J, *et al.* Smoking, alcohol, dentition and diet in the epidemiology of oral cancer. *Eur. J. Cancer* 1992; **28B**: 9–15.

30 Franceschi S, Bidoli E, Baron AE, La Vecchia C. Maize and risk of cancers of the oral cavity, pharynx, and esophagus in northeastern Italy [see comments]. *J. Natl. Cancer Inst.* 1991; **83**: 138–9.

31 National Institute of Nutrition (NIN). *Nutritive Value of Indian Foods.* Hyderabad, India: NIN, 1993.

32 Gupta PC, Mehta FS, Pindborg JJ, Aghi MB, Bhonsle RB, Daftary DK, *et al.* Intervention study for primary prevention of oral cancer among 36,000 Indian tobacco users. *Lancet* 1986; **1**: 1235–9.

33 Hebert JR, Gupta PC, Bhonsle RB, Murti PR, Mehta H, Verghese F, *et al.* Development and testing of a quantitative food frequency questionnaire for use in Kerala, India. *Public Health Nutr.* 1998; **1**: 123–30.

34 Hebert JR, Gupta PC, Bhonsle RB, Sinor PN, Mehta H, Mehta FS. Development and testing of a quantitative food frequency questionnaire for use in Gujarat, India. *Public Health Nutr.* 1999; **2**: 39–50.

35 Bhonsle RN, Murti PR, Gupta PC. Tobacco habits in India. In: Gupta PC, Hamner JE, Murti PR, eds. *Proceedings of an International Symposium on Control of Tobacco-Related Cancers and Other Diseases, Bombay, India, 15–19 January 1990.* Oxford: Oxford University Press 1992; 25–46.

36 Willett WC. *Nutritional Epidemiology.* 2nd ed. Monographs in Epidemiology and Biostatistics, Vol. 30. New York: Oxford University Press, 1992.

37 Bingham SA, Nelson M. Assessment of food consumption and nutrient intake. In: Margetts BM, Nelson M, eds. *Design*

Concepts in Nutritional Epidemiology. New York: Oxford University Press, 1991; 153–67.
38 Goodhart RS, Shils ME. *Modern Nutrition in Health and Disease*. Philadelphia, PA: Lea & Febiger, 1980; 1370.
39 SAS. *SAS User's Guide*. Cary, NC: SAS Institute, Inc., 2001.
40 SAS. *SAS/STAT Software: Changes and Enhancements through Release 8.01 (Guide)*. Cary, NC: SAS Institute, Inc. 2001; 1167 pp.
41 Hebert JR, Miller DR. The inappropriateness of conventional use of the correlation coefficient in assessing validity and reliability of dietary assessment methods. *Eur. J. Epidemiol.* 1991; **7**: 339–43.
42 Gupta PC. Epidemiologic study of the association between alcohol habits and oral leukoplakia. *Oral Epidemiol.* 1984; **12**: 47–50.
43 Hebert JR, Kabat GC. Differences in dietary intake associated with smoking status. *Eur. J. Clin. Nutr.* 1990; **44**: 185–93.
44 Hebert JR, Kabat GC. Implications for cancer epidemiology of differences in dietary intake associated with alcohol consumption. *Nutr. Cancer* 1991; **15**: 107–19.
45 Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am. J. Epidemiol.* 1988; **127**: 283–96.
46 Hebert JR, Hurley TG, Hsieh J, Rogers E, Stoddard AM, Sorensen G, *et al*. Determinants of plasma vitamins and lipids: the Working Well Study. *Am. J. Epidemiol.* 1994; **140**: 132–47.
47 Zheng T, Boyle P, Willett WC, Hu H, Dan J, Evstifeeva TV, *et al*. A case–control study of oral cancer in Beijing, People's Republic of China: associations with nutrient intakes, foods and food groups. *Eur. J. Cancer* 1993; **29B**: 45–55.
48 Hebert JR, Ma Y, Clemow L, Ockene IS, Saperia G, Stanek EJ, *et al*. Gender differences in social desirability and social approval bias in dietary self report. *Am. J. Epidemiol.* 1997; **146**: 1046–55.
49 Messer E. Intra-household allocation of food and health care: current findings and understandings – introduction. *Soc. Sci. Med.* 1997; **44**: 1675–84.
50 Gupta PC, Mehta FS, Pindborg JJ, Bhonsle RB, Murti PR, Daftary DK. *et al*. Primary prevention trial on oral cancer in India: a 10-year follow-up study. *J. Oral Pathol. Med.* 1992; **21**: 433–9.
51 Fabris N, Mocchegiani E. Zinc, human diseases and aging. *Aging* 1995; **7**: 77–93.
52 Chen TS, Chen PS. Rise and fall of the Plummer–Vinson syndrome. *J. Gastroenterol. Hepatol.* 1994; **9**: 654–8.
53 Paul RR, Chatterjee J, Das AK, Dutta SK, Roy C. Zinc and iron as bioindicators of precancerous nature of oral submucous fibrosis. *Biol. Trace Elem. Res.* 1996; **54**: 213–30.
54 Stevens R, Jones Y, Micozzi M, Taylor P. Body iron stores and the risk of cancer. *N. Engl. J. Med.* 1988; **319**: 1047–52.
55 Wynder E, Hultberg S, Jacobson F, Bross I. Environmental factors in cancer of the upper alimentary tract. *Cancer* 1957; **10**(3): 470–87.
56 Bay BH, Sit KH, Liau LS. Cytosolic calcium mobilization concomitant with cell retraction induced by sulphate in oral KB carcinoma cells. *Anticancer Res.* 1996; **16**: 821–6.
57 Rogers MA, Thomas DB, Davis S, Vaughan TL, Nevissi AE. A case–control study of element levels and cancer of the upper aerodigestive tract. *Cancer Epidemol. Biomark. Prev.* 1993; **2**: 305–12.
58 Zaridze DG, Huvshinov JP, Matiakin E, Polakov BI, Boyle P, Blettner M. Chemoprevention of oral and esophageal cancer in Uzbekistan, Union of Soviet Socialist Republics. *J. Natl. Cancer Inst. Monogr.* 1985; **69**: 259–62.
59 Zheng W, Blot WJ, Diamond EL, Norkus EP, Spate V, Morris JS, *et al*. Serum micronutrients and the subsequent risk of oral and pharyngeal cancer. *Cancer Res.* 1993; **53**: 795–8.
60 Kaugars GE, Silverman SJ, Lovas JG, Thompson JS, Brandt RB, Singh VN. Use of antioxidant supplements in the treatment of human oral leukoplakia. *Oral Surg. Oral Med. Oral Pathol.* 1996; **81**: 5–14.
61 Zheng W, Sellers TA, Doyle TJ, Kushi LH, Potter JD, Folsom AR. Retinol, antioxidant vitamins, and cancers of the upper digestive tract in a prospective cohort study of postmenopausal women. *Am. J. Epidemiol.* 1995; **142**: 955–60.
62 Garewal J, Mayskens FJ, Friedman S, Alberts D, Ramsey L. Oral cancer prevention: the case for carotenoids and antioxidant nutrients. *Prev. Med.* 1993; **22**: 701–11.
63 Garewal JS. Beta-carotene and vitamin E in oral cancer prevention. *J. Cell Biochem.* 1993; **17F**: 262–9.
64 Munoz N, Hayashi M, Bang LJ, Wahrendorf J, Crespi M, Bosch FX. Effect of riboflavin, retinol, and zinc on micronuclei of buccal mucosa and of esophagus: a randomized double-blind intervention study in China. *J. Natl. Cancer Inst.* 1987; **79**: 687–91.
65 Hebert JR, Ma Y, Ebbeling CB, Matthews CE, Ockene IS. *Self-Report Data. Compliance in Healthcare and Research*. Armonk, NY: Futura; 2001; 163–79.
66 US Departments of Agriculture and Health and Human Services. *Nutrition and Your Health: Dietary Guidelines for Americans*, 4th ed. Home and Garden Bulletin No. 232. Washington, DC: US Departments of Agriculture and Health and Human Services, 1995.
67 American Institute for Cancer Research. *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. Washington, DC: American Institute for Cancer Research, 1997; 670.
68 Butrum RR, Clifford CK, Lanza E. NCI dietary guidelines: rationale. *Am. J. Clin. Nutr.* 1988; **48**: 888–95.