RISK FACTORS FOR OESOPHAGEAL CANCER IN URUGUAY

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DECLARATION

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ABSTRACT

Introduction

There is a geographic cluster of high oesophageal cancer incidence areas in South America, which includes northeastern Argentina, southern Brazil, Paraguay and Uruguay. Data from the cancer registry in Montevideo, Uruguay has revealed age standardised incidence rates of 11.9 and 3.4 per 100 000 for males and females respectively. However in the northeast region bordering with Brazil, the age standardised incidence rate is reported to be 55.8 and 14.7 per 100 000 for males and females respectively. While alcohol drinking and tobacco smoking have been identified as the important risk factors in these populations, there has also been striking ecological correlation between the drinking of a tea-like infusion of the herb *Ilex paraguariensis*, known as maté, and the high rates of oesophageal cancer. However numerous studies on maté have yielded inconsistent results.

Objectives

The objective of this study was to evaluate maté consumption as a risk factor for oesophageal cancer and to further evaluate the role of quantity and temperature in order to assess whether the effect is related to the carcinogenicity of the plant or the high temperature at which maté is consumed. In addition the effect of diet, alcohol drinking and tobacco smoking on oesophageal cancer risk was assessed.

Design

A retrospective hospital-based case-control study was carried out at the Oncology Institute of Montevideo, Uruguay. The study included 344 cases with squamous cell carcinoma of the oesphagus and 469 controls recruited between the periods January 1988 and August 2000. A questionnaire was used to ascertain past exposure to maté, alcohol, tobacco smoking, and selected food items.

Results

Maté consumption was significantly associated with an increased risk of developing oesophageal cancer and showed a clear dose-response with a relative risk of 2.84 (95% CI 1.41 – 5.73) for those drinking more than 1L/day of maté as compared to non-drinkers. Subjects self-reporting drinking maté at a very hot temperature had an almost 2-fold increase in risk (OR=1.87, 95% CI 1.17 – 3.00 as compared to those

drinking warm to hot maté) after adjusting for age, sex, urban/rural status, education, alcohol and tobacco and cumulative consumption of maté. Maté amount and temperature were observed to have independent effects, with those drinking more than 1 L/day of maté at a very hot temperature having a 3-fold increase in risk (OR = 2.95, 95% CI 1.30 – 6.74) compared to those drinking less than 0.5 L/day of maté at a warm to hot temperature. Increased odds ratios were also observed for heavy alcohol drinking (OR=3.57, 95% CI 2.10 – 6.09 for those consuming more than 180 ml ethanol/day as compared to non-drinkers) and black tobacco smoking (OR=2.88, 95% CI 1.82 – 4.54 as compared to those smoking blonde tobacco). An increase in risk was also observed for those eating frequently barbecued meat (OR for more than 52 servings per year =2.27, 95% CI 1.44 – 3.58 as compared to less than 13 servings). A decreased risk was observed for high consumption of raw vegetables (OR=0.66, 95% CI 0.43 – 1.01 for more than 104 servings per year versus less than 25 servings per year). Subjects with high cumulative exposure to maté in the presence of low alcohol and tobacco exposures produced a lower risk estimate (OR=1.52, 95%CI 0.88 – 2.62) while those with high cumulative exposures to maté, alcohol and tobacco presented with a 7-fold increase in oesophageal cancer risk (OR=7.10, 95% CI 3.75 – 13.46). The population attributable fraction as a result of maté consumption was calculated to be 52% while that for ever having been exposed to alcohol and tobacco was calculated to be 24% and 30% respectively. This indicates that assuming a causal relationship exists between oesophageal cancer and each of these exposure variables, 52%, 24% or 30% of oesophageal cancers in the population would have been prevented if either maté, alcohol or tobacco exposures had been removed, respectively.

Conclusion

The apparent carcinogenic effect of maté is weak (aetiological effect), however the effect of drinking maté at high temperature on oesophageal cancer risk was stronger. The high population attributable fraction due to maté consumption (52%) depends mostly on the high prevalence of exposure (and would therefore be specific to this population) and it does not exclude an important effect of alcohol (24%) and tobacco (30%). The role of meat, and in particular barbecued meat also increases the risk of developing squamous cell oesophageal cancer while high consumption of fruits and vegetables confer some degree of protection.

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ABBREVIATIONS

OC	oesophageal cancer
SCC	squamous cell carcinoma
ICD-O	International classification of diseases – Oncology
RR	relative risk
CI	confidence interval
%	percent
OR	odds ratio
IARC	International Agency for Research on Cancer
km	kilometre
viz.	namely
L	litre
ml	millilitre
ilexgroup	amount of maté consumed (as a categorical variable)
tempilex	interaction term between temperature and amount of maté consumed
resi	urban/rural status
educ	years of education
amnt	number of cigarettes smoked per day
dura	smoking duration (years)
etoh	total alcohol consumed in ml ethanol per day
aldur	alcohol duration (years)
Pe	Prevalence of exposure
ilexpar	categorical term created to calculate population attributable proportion due to maté consumption
^{o}C	degrees celsius

Chapter 1: Literature Review, Study Aims and Objectives

1.1. Introduction

Oesophageal cancer (OC) is the eighth most common cancer in the world (Parkin *et al.*, 1999) with worldwide variation in incidence and mortality rates between countries, and the fourth site (in terms of mortality rates) characterised by very poor survival similar to the liver, pancreas, and lung. It has one of the lowest probabilities of cure, with 5-year survival rates estimated to be approximately 10% overall (Wong, 2000). The high mortality rate due to OC is related to the fact that symptoms first appear when the patient cannot swallow; by that time, the tumour is large, invasion of the oesophagus and surrounding tissues is advanced, and prognosis is poor (Day, 1986). It is thus a very difficult condition to treat, hence underlining the importance of understanding its aetiology for preventive purposes.

This malignancy exists in two main forms with distinct aetiological and pathological characteristics, *viz.* squamous cell carcinoma (SCC) and adenocarcinoma. However, more than 90% of oesophageal cancers worldwide are SCCs (Stoner and Rustgi, 1995; Beer and Stoner, 1998), and is defined as a malignant epithelial tumour with squamous cell differentiation, microscopically characterized by keratinocyte-like cells with intercellular bridges and/or keratinization (Gabbert *et al.*, 2000). This morphological type of carcinoma has the international classification of disease-oncology (ICD-O) code, 8070/3.

1.2. Geographical distribution

The burden of OC in terms of morbidity and mortality varies enormously accordingly to geographical area with about 80% of all cases and deaths occurring in less developed countries (Parkin *et al.*, 1985; Pisani, *et al.*, 1993). There are three regions in the world where incidence rates are particularly high. These are (A) southeast Africa (the Transkei region of the Eastern Cape Province of South Africa) (Makaula *et al.*, 1996), (B) the so-called 'Asian oesophageal cancer belt' that stretches from eastern Turkey and east of the Caspian Sea through northern Iran, northern Afghanistan, southern areas of the former Soviet Union, such as Turkmenistan,

Uzbekistan and Tajikistan to northern China (Schottenfeld, 1984; Sons, 1987; Yang, 1980) and (C) southeast South America (Vassallo *et al.*, 1985).

In South America, there is a geographic cluster of high incidence areas (Figure 1) which includes northeastern Argentina, southern Brazil, Paraguay and Uruguay (Parkin *et al.*, 1999). Age-standardized incidence rates in these regions range from 1.8 and 0.6 per 100 000 to 18.9 and 4.1 per 100 000 for males and females respectively (Parkin *et al.*, 1997) (Table 1).

Figure 1: Geographical cluster of high incidence areas of oesophageal cancer in South America

Table 1: Age standardised incidence rates (per 100 000) for men/women in South America (Parkin, 1997)

High Risk areas	Medium risk areas	Low risk countries
Porto Alegre, Brazil (18.9/4.1)	Montevideo, Uruguay (11.9/3.4)	Belem, Brazil (6.3,1.5)
Concordia, Argentina (17.5/3.7)	Goiânia, Brazil (9.3/1.9)	Lima, Peru, (1.8/0.6)
		Quito, Ecuador (3.1/1.1)
		Cali, Columbia (3.9/2.3)

Data from the cancer registry in Montevideo, Uruguay has revealed age standardised incidence rates of 11.9 and 3.4 per 100 000 for males and females respectively (Parkin *et al.*, 1997). However in the northeast region bordering with Brazil, the age standardised incidence rate is reported to be 55.8 and 14.7 per 100 000 for males and females respectively (De Stefani *et al.*, 1999a). The mortality rates for this cancer in Uruguay are one of the highest in America and range from 40/100 000 for males in the northeastern region that borders Brazil to 10 per 100 000 in the capital city of Montevideo (Vassallo *et al.*, 1985). The death rates are lower for females, with male/female ratio of 3.8 for the whole country. The most important histology type in this region is SCC (De Stefani *et al.*, 1994a).

1.3. Profile of Risk Factors in Latin America

While oesophageal carcinogenesis is a complex multistep process, and certainly has a multifactorial aetiology, some factors may be important in the initiation of the neoplastic state, whereas others may act in the promotion and progression of the lesion. Correa (1982) postulates that all the stages of oesophageal carcinogenesis require the action of three kinds of carcinogenic force: predisposing forces (vitamin deficiencies, such as those of A, B, C, E, riboflavin and niacin; oesophagitis, with small erosions and ulcerations), mutagens (nitroso compounds, tannin from cigarettes, alcoholic beverages, etc.), and promoters (phorbol esters present in some alcoholic beverages). For several factors, there is a clear dose-response effect that persists even after adjustment for other possible risk factors as discussed below.

1.3.1. Alcohol and Tobacco

With regard to the aetiology, numerous case-control studies conducted in Latin America have consistently identified tobacco smoking and alcohol drinking as the most important risk factors for OC in these populations. In a study conducted in Rio Grande do Sul, Brazil, smokers were found to be 6 times more likely to develop OC than non-smokers, while ex-smokers presented with a 2-fold increased risk (Victora *et. al.*, 1987). With regards to alcohol, the most common alcoholic drink being *cachaca* (sugar cane spirit), accounted for approximately 80% of alcohol consumption. Strong positive associations were observed both with daily intake of *cachaca* and with duration of exposure.

In a case-control study conducted by Vassallo *et al.* (1985) among patients admitted to the Oncology Institute of Montevideo, Uruguay, males showed elevated risks of OC with heavy tobacco (≥20 cigarettes/day) [relative risk (RR) = 10.8, 95% CI 6.4 – 19.1] and alcohol (≥100 ml/day) (RR=10.3, 95% CI 7.0 – 18.0) exposures after adjusting for age, while among females, the independent effects of tobacco and alcohol were non-significant. The age adjusted relative risk, resulting from joint exposure to tobacco and alcohol showed a multiplicative effect with a relative risk of 43.1 (95% CI 19.7 – 94.2) for those consuming more than 20 cigarettes/day and more than 100 ml/day of alcohol. In a subsequent study, De Stefani *et. al.* (1990) also observed strong associations with tobacco smoking and alcohol consumption. Male and female smokers of more than 25 cigarettes/day showed a relative risk of 4.62 (95% CI 1.9 – 11.1) and 3.22 (95% 1.1 – 1.93) respectively. The risk associated with the use of black tobacco was about three times higher than that associated with blond tobacco use (RR=2.6, 95% CI 1.7 – 3.9). The risk increased significantly after an average consumption of 50 ml pure alcohol/day in males and earlier in females (≥25 ml/day). Among the females, however, the estimates were unstable due to smaller number of drinkers. The relative risk for those who smoked more than 25 cigarettes/day and drank more than 350 ml alcohol/day was 22.6 compared to that of light smokers and drinkers. The risk of oesophageal cancer decreased significantly among male ex-drinkers (P=0.01) and there was a significant trend with years since quitting (P=0.001), with those quitting more than 10 years having a 54% reduced risk of developing OC (RR=0.46, 95% CI 0.23 – 0.92) compared to regular drinkers.

In Argentina, on the other hand, tobacco smoking was only moderately associated with oesophageal cancer risk (Castelletto *et. al.*, 1994). After adjusting for the effects age, sex, hospital, education and alcohol consumption, ex-smokers and current smokers were about three times more likely than controls to develop oesophageal cancer. Furthermore, smokers of black tobacco presented a statistically significant 2-fold increased risk of developing oesophageal cancer compared to smokers of blond tobacco. A strong dose-response relationship was observed with the amount of alcohol consumed daily but not with the number of cigarettes smoked. The strongest positive association was observed with the amount of pure ethanol consumed. Drinkers of more than 200 ml of ethanol/day were 8 times more likely to develop OC

(RR=8.0, 95% CI 3.0 – 21.2) compared to non-drinkers. As expected, lower alcohol categories presented lower magnitudes or risk estimates. The only type of drink that showed a statistically significant effect on cancer risk compared to non-drinkers was wine (RR=2.3, 95% CI 1.2 – 4.4). Other drinks such as beer and liquor were also associated with increased risk however these estimates were not statistically significant due to the low prevalence since only 3 – 6% of the population consumes other alcoholic beverages and 80 % of the subjects studied were wine drinkers. The joint effects of alcohol and tobacco were also estimated but were found not to be statistically significant (P=0.45) although higher risks were associated with higher exposures to both factors. Hence those drinking more than 200 ml alcohol/day and smoking over 15 cigarettes/day were observed to have a risk 19 times higher than non-exposed subjects.

In Paraguay, a study conducted by Rolón *et. al.* (1995), revealed that tobacco smoking variables such as smoking status, daily number of cigarettes, lifetime number of cigarettes, years of smoking, years since quitting and type of tobacco were all independently and strongly associated with OC risk, with a clear dose-response relationship. After adjusting for age, sex, hospital group and lifetime ethanol consumption, current smokers showed a 4.5-fold increased risk compared to non-smokers. A significant trend (P<0.00001) was observed with lifetime number of cigarettes, with those in the highest exposure category (≥300 000 cigarettes) having a 10-fold increased risk of developing OC (95% CI 3.9 – 25.8). Once again, black tobacco smoking was associated with a 2-fold increased risk in comparison to blond tobacco use. Alcohol, however, was found to be the strongest risk factor in this population. Even moderate drinkers of ethanol (<1/week) had a large excess in risk compared to non-drinkers (RR=8.0, 95% CI 3.7 – 17.3). The lifetime consumption of ethanol produced a significant dose-response relationship (P<0.00001) with those in the highest exposure category (≥1000 lifetime litres) having a relative risk of 27.2 (95% CI 11.2 – 65.9). The joint effect of tobacco and alcohol was, however not statistically significant (P=0.15) suggesting no departure from the usual multiplicative model, although increasing exposure to both factors was associated with higher risks. Hence subjects exposed to 15 – 40 cigarettes/day and consuming more than 150 ml/day of ethanol had a relative risk of 284 (95% CI 62.5 – 1291).

In a pooled analysis of 5 studies discussed above, Castellsagué *et. al.* (1999) observed a similar pattern in risks associated with smoking and alcohol exposures although estimates of risk for women were generally lower than the corresponding ones for men. Once again male smokers of black tobacco had a 2-fold increased risk as compared to blond tobacco users. Men quitting more than 5 years had a 50% reduction in risk (RR=0.5, 95% CI 0.3 – 0.8). In an attempt to determine the joint effects of amount of cigarettes smoked and selected smoking characteristics, it was observed that for a given level of consumption, smoking duration had a strong and statistically significant dose-response relationship with risk. Thus smoking 8 – 14 cigarettes/day for 50 years or more exhibited a higher risk [odds ratio (OR)=7.2, 95% CI 3.9 – 13.4) than smoking larger amounts for a shorter period of time (OR 3.0, 95% CI 1.6 – 5.8, for smoking 25 or more cigarettes for 1 – 29 years). With regards to alcohol, men showed a statistically significant decrease in risk with years since stopping the habit (P=0.02). The data further suggested that low-to-moderate daily consumption of alcohol for many years (i.e., 1 – 24 ml/day for 50 or more years) carried a lower risk (OR=2.8, 95% CI 1.5 – 5.2) than drinking larger amounts of alcohol for a shorter period of time (i.e., ≥250 ml/day for 1 to 29 years, OR=8.7, 95% CI 4.1 – 18.3). With regard to the type of alcohol, the effects of amount consumed on the risk of the disease were highest for consumption of spirits (OR for highest versus lowest level of consumption: 22.7), followed by that of wine only (OR=9.6) and that of spirits with beer and/or wine (OR=8.6). With regards to the joint exposure to tobacco and alcohol, the gender specific interaction term was statistically significant only among females (P=0.01). The overall interaction term was also statistically significant (P=0.00035) with ever smokers and ever drinkers having an 8-fold increased risk (95% CI 5.67 – 11.27) of developing OC. Since in these populations women were much less exposed than men to heavy cigarette smoking and alcohol drinking, the authors explored the hypothesis that the synergistic effect observed among women would also be detectable among men who were moderately exposed to these habits. Hence by excluding male subjects in the 2 highest consumption categories, they found a marginally significant (P=0.08) interaction between amounts of cigarette smoking and of alcohol drinking. It was also observed that combination of heavy drinking (>249 ml ethanol/day) with black tobacco smoking boosted the risk more than 100-fold (OR=106.89, 95% CI 44.91 – 254.41).

1.3.2. Diet

Epidemiologic evidence on the relation between OC and nutrition is discussed below. The protective effect of fruit and vegetables is supported by a large body of evidence, especially from case-control studies. The effects of food groups and nutrients other than fruits and vegetables are also examined. De Stefani *et. al.* (1990) studied 10 food groups, namely, fresh meat, preserved meat, barbecued meat, fat, dairy products, eggs cereals, potatoes, vegetables and fresh fruits among patients attending four hospitals in Montevideo. Analyses were performed on 261 cases and 522 controls after adjustment for age, sex, region, alcohol, smoking duration and type of tobacco. A clear protective effect and a significant dose-response relationship was found with the consumption of fresh fruits, with those who consume daily presenting a 67 % reduction in risk (OR=0.33, 95% CI 0.2 – 0.5). A reduction in risk was also observed with the consumption of vegetables (OR=0.56, 95% CI 0.3 – 1.0) but without a significant dose response. On the other hand however, a significant increase in risk for those eating barbecued meat daily was observed (OR=2.66, 95% CI 1.3 – 5.5) but without a significant dose-response relationship. The increase in risk for those who had eaten barbecued meat daily persisted even after adjusting for meat consumption. No clear effect for fresh meat and fat and no significant associations with other food groups were observed. A further study on meat intake revealed once again that high intake of salted meat (OR=2.5, 95% CI 1.1 – 5.4) and lamb (OR=2.1, 95% CI 1.1 – 4.2) were associated with increased risk of OC (De Stefani *et. al.*, 1999a).

An increased risk was observed in Argentina (Castelletto *et. al.*, 1994) for those eating barbecued meat more than once a week (OR=2.4, 95% CI 1.2 – 4.8) as compared to those eating it less than once a week. Frequent consumption of raw vegetables however was associated with slight decrease in OC risk but the effect was not significant. Consumption of oil, poultry, dairy products, citrus and non-citrus fruits, sausage, eggs, cereals and sweets was not associated with OC after adjustment for age, sex, hospital, education, number of cigarettes/day and alcohol consumption.

In Paraguay, Rolón *et. al.* (1995) also observed strong positive associations with increasing consumption of fats and red meats and, to a lesser extent, with fish and milk, after adjusting for age, sex, hospital, cigarette smoking and alcohol consumption. However, in an attempt to control for other dietary confounders, a

second model was fitted, which included the four food groups above. Strong effects were still evident with red meats and fat although the risk estimates for fish and milk were closer to the null value and not statistically significant. High consumption of cheese, cereals, and cereal products were all associated with a moderately decreased risk. High consumption of vegetables and citrus fruits, assessed as food groups, were associated with a slight but non-significant decrease in risk. Of the citrus fruits, daily consumption of lemons showed a statistically significant protective effect (OR=0.3, 95% CI 0.2 – 0.8), and daily consumption of oranges was associated with a non-significant protective effect (OR=0.6, 95% CI 0.2 – 1.4). Non-significant moderately increased risks were once again detected for high consumption of barbecued meat (OR=1.4, 95% CI 0.7 – 2.8) and for always adding salt to foods (OR=1.4, 95% CI 0.5 – 3.3).

Castellsagué *et al.* (2000) also observed statistically significant protective associations with high consumption of vegetables, fruits, cereals and tea. Daily/almost daily consumption of fruits was associated with a 63% risk reduction (OR=0.37, 95% CI 0.27 – 0.51) compared with subjects rarely consuming fruits, a protective association that followed a strong-dose response relationship ($P<0.00001$). In contrast, frequent consumption of meat (OR=1.28, 95% CI 1.02 – 1.61), animal fats (OR=1.42, 95% CI 1.03 – 1.94) and salt (OR=1.94, 95% CI 1.40 – 2.69) was associated with a moderately increased risk. No associations were found for consumption of milk, sausage, eggs or potatoes.

In a more recent study among the Uruguayan population, vegetables and more markedly, fruits, were associated with strong reductions in risk (De Stefani *et al.*, 2000). High intake of vegetables produced a 36% reduction in risk (OR=0.64, 95% CI 0.34 – 1.20) while fruits displayed a strong inverse association (OR=0.18, 95% CI 0.09 – 0.39). Nutrients were calculated using local tables of chemical composition in foods and were adjusted for total energy intake using the residuals method. It was found that 12 of 15 dietary antioxidants displayed significant inverse associations with OC risk with the strongest effect being observed for high intake of β-cryptoxanthin (OR=0.16, 95% CI 0.08 – 0.36) followed by total phytosterols (OR=0.21, 95% CI 0.10 – 0.50). The antioxidants, α-carotene, lycopene, and β-

sitosterol, were associated with significant reductions in risk. However, most antioxidants lost their effect when they were further adjusted for a term for all vegetables and fruits. Only β-cryptoxanthin intake remained significant (OR for high intake=0.29, 95% CI 0.11 – 0.74) while β-carotene showed an increased risk with high intakes (OR=2.24, 95% CI 1.12 – 4.49). On the other hand, vegetables and fruits remained as significant variables after adjustment for each antioxidant, suggesting that other micronutrients and bioactive substances could better explain the effect of vegetables and fruits and that the antioxidants studied may act synergistically as observed in experimental studies (Shklar, 1993). Although there exist the possibility that controls might have altered their diets to include more vegetables and fruits, resulting in spurious inverse associations, this may not be of high likelihood. Since dysphagia is a prominent symptom of esophageal carcinoma, it is possible that this symptom could be related to changes in the diet of patients with advanced esophageal cancer. Thus the authors concluded that it may have been conceivable that patients with OC reported a low intake of most foods, among them vegetables and fruits. However, queries of the interviewers were related to intake five years before the first symptom. Therefore it appears that the answers precluded differential misclassification.

1.3.3. Maté drinking

The drinking of maté (pronounced "ma-tay"), which is a tea-like infusion of the herb *Ilex paraguariensis* (Aquifoliaceae) is particularly prevalent in southern Brazil and Uruguay and this gave rise to a striking ecological correlation between the distribution of this habit and the high rates of OC. This ecological association led to the hypothesis that maté drinking may be an aetiological factor for OC in these high-risk areas (Muñoz *et al.*, 1987). This tea, known by the folk names of "maté" in Argentina and Uruguay and "chimarrão" in Brazil is normally drunk very hot or very hot from a gourd (the hard shell of a local fruit) or other container through a straw with a filtering tip, which places very hot fluid at the oropharynx and oesophagus. In Uruguay, the prevalence of maté drinking is greater than 80%. It has thus long been hypothesized that maté drinking may play an etiological role in the high incidence of oesophageal cancer in these areas. However, results from studies in Paraguay (Rolón *et al.*, 1995),

Brazil (Victora, 1987), Argentina (Castelletto, 2000) and Uruguay (De Stefani, 1990, De Stefani, 1991) have yielded inconsistent results.

Vasallo *et. al.* (1985) studied 226 incident cases (185 males and 41 females) of histopathologically confirmed SCC of the oesophagus treated at the Oncology Institute of Montevideo, Uruguay, between 1979 and 1984. A total of 469 unmatched controls (386 men, 83 women) with cancers at other sites, namely, skin (24%), colon or rectum (14%) and prostate (11%) were obtained from the same institute. Information on demographic variables and on consumption of tobacco, alcohol and maté was obtained during routine interviews prior to diagnostic evaluation and it was shown that maté consumption had an independent effect in both males and females, with age-adjusted relative risks for developing OC of 6.5 (95% CI 4.0 – 11.0) and 34.6 (95% CI 4.9 – 246.5) respectively, for heavy users (≥1.0 litre/day). For men and women together, a clear dose-response relationship was observed and the joint effects of maté and alcohol appeared to be multiplicative. In this study, however, the issue of information bias, reflecting the assumption among health professionals that maté drinking is involved in the aetiology of OC was not adequately addressed.

However in a subsequent study in southern Brazil (Victora *et al.*, 1987) the evidence of a strong association between maté consumption and OC was not convincing. In this study, 171 cases of OC treated in 8 main hospitals and 3 radiotherapy units in the State of Rio Grand do Sul were included in this study. For each case, two age- and sex-matched controls were selected, who did not include patients with diseases of the upper gastrointestinal tract or with conditions associated with the use of tobacco and alcohol. Alcohol drinking, tobacco smoking and rural residence were seen to be the main risk factors for OC in this population, while fruit consumption conferred some degree of protection. In the crude matched analysis, daily maté drinkers were 1.9 times more likely to have OC than non-daily drinkers (P=0.006). However, after adjustment for sugar cane spirit (*cachaca*), smoking, residence status, fruit consumption and meat intake, the odds ratio associated with daily maté drinking was reduced to 1.47 (90% CI = 0.87 – 2.50).

Following on this, De Stefani *et al.* (1990) carried out a case-control study at four main hospitals in Montevideo on 261 patients histologically confirmed with SCC of the oesophagus and 522 sex and age-matched hospital controls who did not have a diagnosis of tobacco- or alcohol-related disease. Maté was drunk by 98% of the cases and 91% of controls. There was a strong association with a clear dose-response relationship between the daily amount of mate drunk and the risk of OC. It was found that the relative risk of those drinking over 2.5 litres of maté per day was 12.2 (95% CI 3.8 – 39.6) after adjustment for the effects of age, area of residence, alcohol and tobacco. The authors reported no consistent association, however, between the reported temperature at which maté was consumed and risk of OC.

In Le Plata, Argentina, however, a multicentre hospital-based case-control study with 131 cases and 262 controls showed that none of the maté drinking variables, *i.e.*, maté drinking status, maté amount and maté duration, were associated with OC. Drinkers of more than 1.5 litres/day had a non-significant increase in risk (OR=1.6, 95% CI 0.5 – 4.5) which disappeared after adjusting for smoking. Although there was a marginally significant positive effect of maté temperature of consumption, the association was moderate, with those drinking maté hot or very hot presenting with an almost 2-fold increased risk (OR=1.7, 95% CI 1.0 – 2.9) as compared to those drinking it warm. The authors concluded that there was no strong evidence for the role of maté in oesophageal carcinogenesis.

Similarly, in Asunción, Paraguay, a case-control study with 131 incident cases of OC and 381 controls identified the very hot temperature at which maté was drunk as an important risk factor for OC and not amount or duration of maté consumption (Rolón *et. al.*, 1995). As compared to drinkers of warm or hot mate, drinkers of very hot maté had an increased risk for OC even after adjusting for the strong effects of alcohol and tobacco consumption (OR=2.4, 95% CI 1.3 – 4.3). Thermal injury has been postulated as one of the mechanisms for the carcinogenic action of maté, considering the hot temperature with which this beverage is drunk (Victora *et al.*, 1987). There is both laboratory (Yioris, 1984) and epidemiologic evidence that high drinking temperature may produce precancerous oesophageal lesions (Muñoz *et al.*, 1987).

A review conducted by the International Agency for Research on Cancer (IARC) concluded that there was limited evidence for the carcinogenicity of hot maté drinking (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1991), although most studies have found moderate (Victora *et al.*, 1987; Franco *et al.*, 1989) to high (Vassallo *et al.*, 1985; De Stefani *et al.*, 1990) elevations in upper areodigestive tract cancer risk.

In a pooled analysis of data from five studies conducted in Argentina, Brazil, Paraguay and Uruguay discussed above, Castellsagué *et al.* (1999, 2000) reported that the main risk factors for OC were tobacco and alcohol consumption. However after adjusting for the strong effects of these exposures, heavy maté drinking (>1 litre/day) and self-reported very hot maté drinking were significantly associated with OC risk in men and women. The joint effects of maté amount and maté temperature were more than multiplicative, following a statistically significant synergistic interaction (OR=4.14, 95% CI 2.24 – 7.67) indicating that those who drank very hot maté were at four times greater risk of developing OC.

The main difficulty in evaluating the effect of maté consumption is the appropriate control of confounders, namely tobacco smoking, alcohol drinking, and dietary factors. Smoking and alcohol drinking, the most important risk factors for upper aerodigestive tract cancers, are not only mutually correlated but also strongly associated with other lifestyle characteristics. Incomplete adjustment for the former variables may leave residual confounding, manifested as spuriously elevated relative risks attributable to maté drinking. Although hot mate drinking has been classified as "probably carcinogenic to humans", it is not clear whether its potential carcinogenic effect is due to the components of the herb, to the temperature at which it is consumed or to both (IARC, 1991). Hence, in the light of current data, a study was proposed to further determine the association between maté consumption and the risk of developing OC in Uruguay and in particular to disentangle the effects of maté amount and the temperature at which maté is consumed.

1.4. Aim

In the light of the data presented, and due to the inconsistent epidemiological evidence, a further retrospective hospital based case-control study was undertaken in Uruguay to investigate the role of maté consumption and in particular the effect of quantity and temperature on the risk of developing oesophageal cancer. In addition, the influence tobacco smoking, alcohol drinking and diet on the risk of developing OC was also investigated.

1.5. Hypothesis

Exposure to maté, alcohol, tobacco and selected dietary items increase the risk of developing OC, while high intake of fruits and vegetables displays an inverse association.

1.6. Objectives

The specific objectives of this study were:

1. To identify all incident cases of histologically confirmed SCC of the oesohagus admitted to the Oncology Institute of Montevideo between January 1988 and August 2000.
2. To select appropriate controls with diseases not related to alcohol drinking, tobacco smoking or the upper gastrointestinal tract.
3. To establish if any association exists between the consumption of maté and OC.
4. To disentangle the effect of maté amount and temperature in order to assess whether the potential effect is related to the plant itself (due to the presence of carcinogenic compounds), to the high temperature at which it is consumed (chronic thermal injury), or to a combination of both.
5. To determine the association between alcohol and OC in this population and further investigation the joint effects of alcohol and maté.
6. To determine the association between tobacco smoking and OC and further investigate the joint effects between smoking duration and type of tobacco
7. To investigate the joint effects of cumulative exposure to maté, alcohol and tobacco smoking on OC risk.

Chapter 2: Subjects and Methods

2.1. Study design

This study is a retrospective hospital-based case-control study.

2.2. Study population

The target population was patients attending the Oncology Institute of Montevideo. While the name of the institute is misleading and suggests that only cancer cases are attended to, this Institute functions as a hospital also catering for other diseases as well, and thus also served as a source for controls between the period January 1988 to August 2000. This Institute has a catchment area that covers 45% of the population of Montevideo and about 55% for the rest of the country (Figure 2).

2.3. Data Source

The data source was the National Cancer Registry housed within the National Institute of Oncology, Montevideo, which belongs to the Ministry of Health. It is the first population-based cancer registry in the country. Although initial objectives of the registry were to develop and maintain a source of information on cancer incidence, mortality and prevalence in the country, mainly descriptive, it expanded its field of interest and began to undertake analytical and descriptive epidemiological studies on the Uruguayan population. The county of Montevideo (530 km^2) is the area covered by the registry. It includes the capital city of Uruguay, Montevideo city, which contains almost half of the national population, and a small rural area, which accounts for 4.6% of the population of the county. Figure 3 illustrates the data collection procedure.

Figure 2: Map of Uruguay

2.4. Sample size calculation

The study was designed to have a power of 90% for detecting a relative risk of 2 as significant at the 0,05 level, assuming that 70% of the controls would be regular maté drinkers. Sample size was calculated according to the method by Lwanga and Lemeshow (21) as follows:

Anticipated probability of "exposure" for people with the disease (P1*): ?

Anticipated probability of "exposure" for people without disease (P2*): 70%

Anticipated odds ratio: 2

Level of significance (α): 5%

Power of the test ($1-\beta$): 90%

Then, the above for a 2-sided test:

$$n = \{ Z_{1-\alpha/2} [2\, P2^* (1 - P2^*)]1/2 + Z_{1-\beta} [P1^* (1 - P1^*) + P2^* (1 - P2^*)]1/2 \}2 / (P1^* - P2^*)2$$

Therefore sample size required in case group = 178

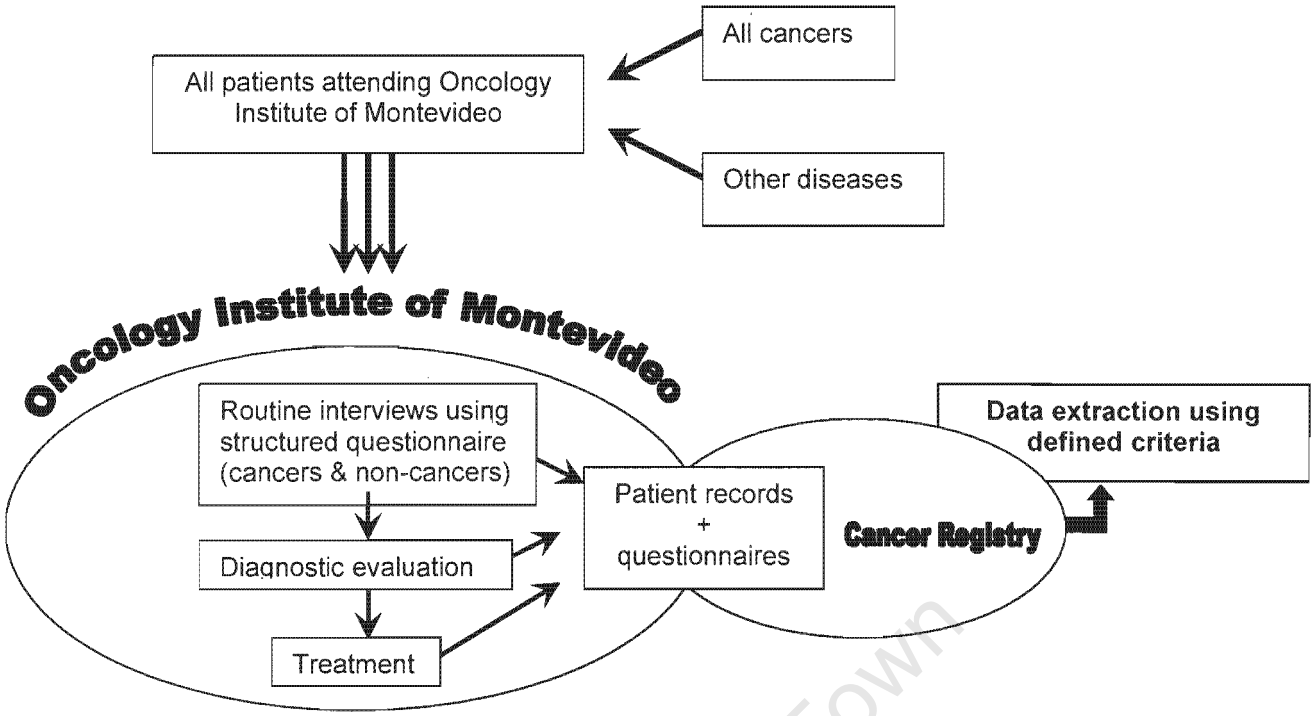

Figure 3: Patient recruitment and data collection procedure for this study

2.5. Patient recruitment period

Patients were recruited retrospectively between January 1988 and August 2000 from a datafile of patient records of the Oncology Institute's cancer registry, as data between this period had not been analysed.

2.6. Selection of Cases

In the time period between January 1988 and August 2000, all patients between 35 and 85 years old, with incident cases of histopathologically confirmed squamous cell carcinoma of the oesophagus, diagnosed at the Oncology Institute of Montevideo were considered eligible for this study. During data collection, patients were required to be of sufficiently good physical and mental health to give reliable answers to the questionnaire. Recruitment into this study further required that patients lived in Uruguay for at least 10 years and had been diagnosed within the previous 4 months at the time of the interview. A total of 344 patients (259 males and 85 females) were identified with the largest proportion (32%) from Montevideo while the remaining cases were from 20 other counties. During this period, the response rate was 96.9%.

2.7. Selection of controls

In the same Institute and in the same time period, 469 patients also aged between 35 and 85 years with conditions unrelated to tobacco smoking and alcohol drinking and without recent changes in the diet, were recruited. The controls were not matched to cases on either of the sociodemographic variables. For each eligible male case, at least one control was selected while for the female cases at least two controls were selected. The main diagnostic categories among controls are listed in Table 2: about 30% of them had an undefined or unknown disease. The response rate for controls was 92.8%.

Table 2: Diagnostic categories among control patients

ICD-9	DIAGNOSIS	No.	%
210 – 229	Benign neoplasms	56	11.9
240 – 279	Endocrine, metabolic and immunity disorders	7	1.5
280 – 289	Diseases of the blood and blood-forming organs	10	2.1
345	Epilepsy	1	0.2
363 – 374	Disorders of the eye and adnexa	4	0.9
382 – 388	Diseases of the ear and mastoid process	2	0.4
454	Varicose veins of lower extremities	1	0.2
486	Pneumonia (organism unspecified)	3	0.6
523 – 529	Diseases of oral cavity, salivary glands, and jaws	26	5.6
550 – 551	Hernia of abdominal cavity	3	0.6
555 – 558	Noninfectious enteritis and colitis	2	0.4
562 – 569	Other diseases of intestines and peritoneum	2	0.4
592 – 599	Other diseases of the urinary system	9	1.9
600 – 608	Diseases of male genital organs	53	11.3
610 – 611	Disorders of breast	36	7.7
614 – 616	Inflammatory disease of female pelvic organs	3	0.6
617 – 627	Other disorders of female genital tract	8	1.7
682 – 709	Diseases of the skin and subcutaneous tissue	44	9.4
713 – 719	Arthropathies and related disorders	6	1.3
721 – 724	Dorsopathies	6	1.3
726 – 729	Rheumatism (excluding the back)	2	0.4
730 – 739	Osteopathies, chondropathies, and acquired musculoskeletal deformities	10	2.1
752 – 759	Congenital anomalies	2	0.4
777	Perinatal disorders of digestive system	18	3.8
782 – 789	Symptoms of ill-defined conditions	7	1.5
799	Other ill-defined and unknown causes of morbidity and mortality	140	29.9
802 – 872	Injury	4	0.9
927 – 959	Late effects of injuries, poisonings, toxic effects, and other external causes	4	0.9

2.8. Interviews and data collection

All patients admitted to the Oncology Institute are routinely interviewed, before the work-up diagnostic evaluation, by trained social workers who are unaware of the hypothesis being tested. The interviewers use a routine questionnaire designed to elicit information on the following sections:

1. demographic and socioeconomic characteristics (residence, income, education, family size and occupation)
2. family history of cancer in first degree relatives,
3. self reported height and weight five years before the interview,
4. history of maté drinking (amount consumed, age started, age quit, duration of consumption, maté temperature during consumption),
5. history of alcohol drinking (type of alcoholic beverage and amount of each consumed, duration of consumption, total alcohol consumption),
6. history of tobacco smoking (age started, age quit, smoking duration, number of cigarettes per day, type of tobacco, type of cigarette), and
7. a food-frequency questionnaire which assessed the dietary habits of selected food items which are representative of the usual diet of the Uruguayan population, 5 years before the interview. The food items were:
    i. total red meat,
    ii. salted meat,
    iii. barbecued meat,
    iv. processed meat,
    v. whole milk,
    vi. raw vegetables
    vii. all fruits

Total vegetables and fruits were calculated as the sum of raw vegetables and fruits. Total alcohol consumption, in ml ethanol/day, was calculated as the sum of the different alcoholic beverage types, *viz.* beer, wine and hard liquor. The frequency of consumption of each food item was recorded in servings per day, week, and month and finally converted to servings per year.

2.9. Data cleaning

Records were removed from the database if they did not comply with the selection criteria for the study. Each of the variables under study was checked for range and consistency in order to identify possible inadmissible values. For example, males were coded "1" and females "2", therefore for the variable "sex", values other than 1 or 2 were flagged as errors and cross-checked against other data, not necessarily relevant to this study, e.g., age at first birth or age at menopause would imply female. The data was checked using the "tab" and "summarize" command in STATA.

2.10. Statistical analysis

The association between the different exposures and oesophageal cancer was obtained using unconditional multiple logistic regression models (Breslow and Day, 1980) to obtain the odds ratios (ORs) as an estimate of relative risk and their 95% confidence intervals using STATA statistical software package (Version 7.0, STATA Corp., College Station, Texas). All logistic regression models included age, sex, urban/rural status, and years of education (as a proxy for social class). Potential risk factors for OC were divided into 4 groups, *viz*, maté consumption, alcohol consumption, tobacco smoking and consumption of dietary items as listed in Table 3. The regression models for evaluating mate, alcohol and tobacco exposures included an intensity measure (amount), duration measure (years) and an integrated measure (cumulative). Duration of maté consumption was calculated up to 1-year before the interview since symptoms for OC present fairly rapidly, while time since quitting maté drinking was calculated for all those who quit drinking at least 2-years before the interview. Those subjects who quit within two years of the interview were considered as current drinkers in the analysis. In addition, lifetime consumption of maté (L.years), alcohol (L.years) and lifetime habit of smoking (pack-years) were calculated to obtain cumulative exposure estimates. Food consumption among controls was used as a basis for categorising the consumption of dietary items prior to analysis.

Table 3: Risk factors investigated in this study

Maté drinking	Alcohol drinking	Tobacco smoking	Diet
Maté drinking status	Lifetime consumption (L.years)	Smoking status	Total meat (red)
Lifetime consumption (L.years)	Total alcohol consumption (ml ethanol per day	Lifetime exposure (pack years)	Salted meat at adolescence
Amount consumed (L/day)	Beer (mL ethanol per day)	Smoking duration (years)	Salted meat at present
Temperature	Wine (ml ethanol per day)	Number of cigarettes per day	Barbecued meat
Duration of consumption (years)	Spirits (ml ethanol per day)	Type of tobacco	Processed meat
Time since quitting (years)	Alcohol duration (years)		Whole milk
			Raw vegetables
			Fruits
			Total fruits + Vegetables

Regression models for maté, alcohol and smoking-related variables were adjusted for smoking and alcohol consumption, smoking and maté consumption, and alcohol and maté consumption, respectively as indicated in table 4 below.

Table 4: Risk factors included in each of the logistic regression models for maté, alcohol and tobacco smoking

Model for maté drinking	Model for alcohol drinking	Model for tobacco smoking
Age	Age	Age
Sex	Sex	Sex
Urban/rural status	Urban/rural status	Urban/rural status
Education	Education	Education
Number of cigarettes smoked/day	Number of cigarettes smoked/day	Total alcohol/day
Smoking duration	Smoking duration	Duration of alcohol consumption
Total alcohol/day	Type of tobacco	Amount of maté consumed
Duration of alcohol consumption	Amount of maté consumed	**Tobacco-related variables**
Maté-related variables	**Alcohol-related variables**	

The estimates for dietary exposures were obtained after adjustment for tobacco smoking, alcohol and maté consumption. None of the exposure-related variables were analysed as continuous variables but either as dichotomous or polytomous variables. However, tests for linear trend were performed by using the midpoint of each category of the variable in the model as a continuous variable so as to obtain the *P*-value for linear trend.

2.11. Determination of effect modification

Tests for effect modification were performed between:

1. maté amount and temperature,
2. smoking duration and type of tobacco, and
3. cumulative exposures to maté, alcohol and tobacco smoking.

2.11.1. Maté amount and temperature

One interaction term (tempilex), with six levels, was created between each category of maté amount and each category of maté temperature as shown in Table 5, with the lowest category of maté amount and maté temperature being used as the reference category.

Table 5: Cells created to show the joint effects of maté amount and temperature

Maté temperature	Maté amount		
	category 1	category 2	category 3
Warm/Hot	1 (reference)	2	3
Very Hot	4	5	6

The following stata commands were used:

```
. drop if ilexgroup==1
(79 observations deleted)
. generate tempilex=1 if temp==2 & ilexgroup==2
(539 missing values generated)
. replace tempilex=2 if temp==2 & ilexgroup==3
(261 real changes made)
. replace tempilex=3 if temp==2 & ilexgroup==4
(131 real changes made)
. replace tempilex=4 if temp==3 & ilexgroup==2
(19 real changes made)
. replace tempilex=5 if temp==3 & ilexgroup==3
(45 real changes made)
. replace tempilex=6 if temp==3 & ilexgroup==4
(33 real changes made)

. tab tempilex

   tempilex |      Freq.     Percent        Cum.
------------+-----------------------------------
          1 |        195       28.51 .     28.51
          2 |        261       38.16       66.67
          3 |        131       19.15       85.82
          4 |         19        2.78       88.60
          5 |         45        6.58       95.18
          6 |         33        4.82      100.00
------------+-----------------------------------
      Total |        684      100.00
```

Dummy variables were thereafter created in STATA, using the "xi" command.

```
. xi: logistic outcome age sex resi educ amnt dura etoh aldur i.tempilex
i.tempilex        _Itempilex_1-6      (naturally coded; _Itempilex_1 omitted)

Logit estimates                                   Number of obs   =        679
                                                  LR chi2(13)     =     104.66
                                                  Prob > chi2     =     0.0000
Log likelihood = -411.07258                       Pseudo R2       =     0.1129

------------------------------------------------------------------------------
     outcome | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
         age |   1.009179   .0090709     1.02   0.309     .9915566    1.027116
         sex |   .7294696   .1909908    -1.20   0.228     .4366609    1.218625
        resi |   1.564438   .3146083     2.23   0.026     1.054828     2.32025
        educ |   .8965642   .0308503    -3.17   0.002     .8380925    .9591153
        amnt |   .9925511   .0059711    -1.24   0.214     .9809166    1.004324
        dura |   1.018054   .0057035     3.19   0.001     1.006937    1.029295
        etoh |   1.001695   .0006932     2.45   0.014     1.000337    1.003054
       aldur |   1.009629   .0051445     1.88   0.060     .9995965    1.019763
_Itempilex_2 |   1.596925   .3454268     2.16   0.030     1.045113     2.44009
_Itempilex_3 |   1.801334    .469711     2.26   0.024     1.080531    3.002972
_Itempilex_4 |    2.84495   1.437821     2.07   0.039     1.056532    7.660673
_Itempilex_5 |   2.799576   1.019143     2.83   0.005     1.371593    5.714252
_Itempilex_6 |   2.960692   1.243538     2.58   0.010     1.299795    6.743907
------------------------------------------------------------------------------
```

Effect modification was determined by assessing the likelihood ratio test statistic between the model containing the main terms and the interaction terms.

Similarly, as per above, interaction terms with different levels were created to assess interaction between (A) smoking duration and type of tobacco, and (B) cumulative exposures to maté, alcohol and tobacco smoking. An α-value of 0.05 was used as the indicator of statistical significance, and accordingly 95% CIs are reported. All *P*-values were derived from 2-sided statistical tests.

2.12. Population attributable proportion

Population attributable proportion due to maté consumption, alcohol drinking and tobacco smoking was calculated from logistic regression data. Firstly, the prevalence of exposure to each of the three risk factors was first calculated in the controls, on the assumption that these would give a reasonable distribution of the prevalence of a given exposure. This was done using the following equation:

$$Pe = \frac{number\ exposed}{total\ number} x100 \qquad (1)$$

Using the relative risk obtained from the logistic regression model, the following equation was used:

$$Population\ attributable\ proportion = \frac{Pe(RR-1)}{Pe(RR-1)+1} \qquad (2)$$

With regards to maté drinking, population attributable proportion was further calculated for high maté consumption (>1L/day), high temperature and finally for those consuming high amounts of maté at high temp. It was also possible to obtain the population attributable proportion from stata by using the "aflogit" command.

2.13. Ethical considerations

This study recognised the need to obtain information from patient records and was done in good faith and in accordance with four basic ethical principles, namely respect for persons, beneficence, non-maleficence, and justice. The interviews for the study had been conducted following informed consent from the participants who were informed of the purpose and nature of the study and the benefits intended to result from it. During data entry, all personal identification was removed so that there was no violation of confidentiality.

Chapter 3: Results

3.1. Summary statistics

Data from 344 cases and 469 controls were analysed in this study. The control-to-case ratio was 1.1 for males and 2.1 for females. The prevalence of maté drinking, alcohol drinking and tobacco smoking among the cases and controls is presented in Table 6.

Table 6: Prevalence of mate drinking, alcohol drinking and tobacco smoking between cases and controls.

Risk factors	Prevalence (%)	
	Cases	Controls
Maté drinking	96	86
Alcohol drinking	65	42
Tobacco smoking	79	61

From basic summary statistics computed, the mean age of the cases was 66 years while that of the control group was 64 years. The mean duration of smoking for cases and controls was 35 and 23 years respectively. The cases were higher drinkers of wine and hard liquor than the control group and consumed maté for a longer duration (mean = 50 years) than the controls (mean = 40 years). In addition, the cases consumed on average, 443 servings per year of red meat in comparison to the control group, which consumed 308 servings per year.

Categorical data, which included the study characteristics and distribution of cases and controls according to sociodemographic and selected exposure variables were summarized using frequency tables, the results of which are presented in Table 6. Cases smoked and consumed alcohol in a greater proportion, when compared with controls and were more likely to come from rural areas having received less education. Furthermore, the cases tended to be heavier drinkers of maté (>1.01 L/day) and consumed less fruits and vegetables (<417 servings/year) than the controls. The Pearson chi^2 statistic indicated a significant *P*-value for the relationship between the cases and each of the variables in Table 7.

Table 7: Study characteristics and distribution of cases and control according to sociodemographic and selected exposure variables.

VARIABLE	CATEGORIES	CASES (344)	CONTROLS (469)	Chi^2 P-value
		No. (%)	No. (%)	
Age (years)	≤ 40	2 (0.6)	18 (3.8)	
	41 – 55	46 (13.4)	101 (21.6)	
	56 – 70	165 (48.0)	197 (42.0)	
	70+	131 (38.1)	153 (32.6)	
Sex	Males	259 (75.3)	294 (62.7)	
	Females	85 (24.7)	175 (37.3)	
Residence	Urban	234 (68.0)	383 (81.8)	
	Rural	110 (32.0)	85 (18.2)	<0.0001
	Data unknown		1	
Years of study	None	42 (12.3)	38 (8.1)	
	1 – 4	208 (60.8)	203 (43.3)	
	5 – 8	84 (24.6)	167 (35.6)	
	9+	8 (2.3)	61 (13.0)	<0.0001
	Data unknown	2		
Smoking status	Non-smoker	73 (21.2)	181 (38.6)	
	Ex-smoker	83 (24.1)	126 (26.9)	
	Current smoker	188 (54.7)	162 (34.5)	<0.0001
Type of tobacco	Non-smokers	73 (21.3)	181 (38.6)	
	Blond	92 (26.8)	190 (40.5)	
	Black	151 (44.0)	73 (15.6)	
	Mixed	27 (7.9)	25 (5.3)	<0.0001
	Data unknown	1		
Total alcohol (ml ethanol per day)	Non-drinker	120 (34.9)	270 (57.6)	
	1 – 60	58 (16.9)	93 (19.8)	
	61 – 180	82 (23.8)	66 (14.1)	
	181+	84 (24.4)	40 (8.5)	<0.0001
All fruits and vegetables (servings/year)	<105	106 (30.8)	105 (22.4)	
	105 – 416	165 (48.0)	228 (48.7)	
	417+	73 (21.2)	135 (28.9)	0.007
	Data unknown		1	

3.2. Effect of maté consumption

Table 8 shows the estimated effects of maté consumption on oesophageal cancer risk. All the aspects of maté drinking were analysed as polychotomous exposure variables and were found to be significantly related to risk of developing OC. Ever drinkers of maté were observed to have a 2-fold increase in risk compared to non-drinkers (OR=2.26, 95% CI 1.19 – 4.27).

3.2.1. Lifetime exposure

The cumulative (lifetime) consumption of maté also presented an increased risk with increasing exposure. There was a significant trend among drinkers (P=0.009) particularly those having a lifetime consumption greater than 8000 L.years. Maté consumers in the highest category (>24001 L.years) presented a highly significant 3-fold increase in risk of developing OC compared to non-drinkers (OR=3.07, 95% CI 1.53 – 6.16). In a second model, the analysis was restricted only to maté drinkers and cumulative exposure was further adjusted for maté temperature. This resulted in a change in the reference category (1 – 8000 L.years) however the relative risk estimate only decreased moderately (OR=2.16, 95% CI 1.23 – 3.79 for highest exposure category).

3.2.2. Amount consumed

An increase in maté amount consumed, increased the risk from 1.69 (95% CI 0.85 – 3.35) to 2.84 (95% CI 1.41 – 5.73). A statistically significant dose-response relationship (P=0.017) was evident particularly for drinkers of more than 0.50 L/day. Hence those consuming more than 1 L/day of maté had a 2.8-fold increased risk of developing squamous cell OC compared to non-drinkers. An effect of amount of maté consumed was still evident, even after further adjustment for maté temperature and duration of maté consumption, with those drinking more than 1 L/day having a 1.6-fold increased risk (OR=1.62, 95% CI 1.01 – 2.62) compared to those drinking less than 0.51 L/day.

3.2.3. Maté temperature

Compared to non-drinkers, subjects reporting drinking maté at warm to hot temperatures had a 2-fold increased risk while consumers of very hot maté had an almost 4-fold increased risk. A highly significant trend (P=0.004) was observed

among drinkers only. After further adjustment for cumulative (lifetime) consumption, consumers of very hot maté presented an almost 2-fold increase in risk (OR=1.87, 95% CI 1.17 – 3.00) as compared to consumers at lower temperature.

3.2.4. Duration of consumption

Duration of maté consumption was calculated up to one year before the interview and an increase in the estimate was observed with increasing years of maté consumption (P=0.005). The increased risk was particularly evident (4-fold) for consumers of maté for more than 59 years (OR = 4.31, 95% CI 1.99 – 9.34). The trend remained significant (P=0.04) even after further adjustment for maté amount and temperature.

3.2.5. Time since quitting

A decrease in risk was observed with years since quitting maté drinking, although the analysis was hampered by the small number of quitters.

3.2.6. Stratification by gender

Upon stratification of the data by gender, females ever having consumed maté presented a 1.5 times greater risk (OR=2.83, 95% CI 0.79 – 10.13) compared to males (OR=1.97, 95% CI 0.93 – 4.16). The risk estimates also increased in both sexes with increasing cumulative exposure with males in the highest exposure category (>24000 L.years) presenting a 2-fold increased risk (OR=2.43, 95% CI 1.07 – 5.50) while females presented a 5-fold increase in risk (OR=4.96, 95% CI 1.22 – 20.18). Compared to non-drinkers, males self-reporting drinking maté at a very hot temperature had a 3-fold increased risk (OR=3.13, 95% CI 1.29 – 7.59) while females presented with a 6-fold increase in risk (OR=6.52, 95%CI 1.49 – 28.46). This evidence suggests that the association between maté exposure and OC is stronger in females than in males.

VARIABLES	CASES		CONTROLS		OR_1[a]	(95% CI)	OR_2[b]	(95% CI)
	N	(%)	*N*	(%)				
Maté drinking								
Never	15	(4.4)	64	(13.7)	1.00*			
Ever	327	(95.6)	405	(86.3)	2.26	(1.19 – 4.27)		
Lifetime consumption (L.years)								
Non-drinkers	15	(4.4)	64	(13.7)	1.00*			
1 – 8000	31	(9.1)	89	(19.0)	1.43	(0.68 – 3.01)	1.00*	
8001 – 16000	86	(25.2)	127	(27.1)	2.21	(1.12 – 4.35)	1.64	(0.95 – 2.81)
16001 – 24000	93	(27.3)	104	(22.2)	2.43	(1.22 – 4.83)	1.70	(0.98 – 2.95)
24001+	116	(34.0)	84	(18.0)	3.07	(1.53 – 6.16)	2.16	(1.23 – 3.79)
P for trend (drinkers only)					0.009		0.2	
Amount consumed (L/day)								
Non-drinkers	15	(4.4)	64	(13.6)	1.00*			
0.01 – 0.50	73	(21.4)	149	(31.8)	1.69	(0.85 – 3.35)	1.00*	
0.51 – 1.00	152	(44.4)	172	(36.7)	2.47	(1.28 – 4.77)	1.49	(1.00 – 2.23)
1.01+	102	(29.8)	84	(17.9)	2.84	(1.41 – 5.73)	1.62	(1.01 – 2.62)
P for trend (drinkers only)					0.02		0.3	
Temperature								
Non-drinkers	15	(4.8)	64	(14.1)	1.00*			
Warm/hot	241	(77.8)	347	(76.4)	2.00	(1.05 – 3.81)	1.00*	
Very hot	54	(17.4)	43	(9.5)	3.98	(1.98 – 8.44)	1.87	(1.17 – 3.00)
P for trend (drinkers only)					0.004			
Duration of consumption (years)								
Non-drinkers	15	(4.4)	67	(14.3)	1.00*			
1 – 35	33	(9.6)	92	(19.7)	1.31	(0.61 – 2.81)	1.00*	
36 – 49	98	(28.6)	117	(25.0)	2.29	(1.16 – 4.52)	1.62	(0.91 – 2.88)
50 – 58	92	(26.9)	98	(20.9)	2.58	(1.27 – 5.24)	1.90	(0.96 – 3.73)
59+	104	(30.4)	94	(20.1)	4.31	(1.99 – 9.34)	3.06	(1.35 – 6.94)
P for trend (drinkers only)					0.005		0.04	
Time since quitting (years)								
Non-drinkers	15	(4.8)	64	(14.1)	1.00*			
Current drinkers**	280	(89.7)	359	(78.9)	2.31	(1.22 – 4.37)		
3 – 10	11	(3.5)	14	(3.1)	1.84	(0.63 – 5.36)		
11+	6	(1.9)	18	(3.9)	1.13	(0.35 – 3.62)		
P for trend (quitters only)					0.1			

[a] OR_1, adjusted for age, sex,urban/rural status, years of education, number of cigarettes smoked per day, smoking duration, total alcohol per day and duration of alcohol consumption.
[b] OR_2, Analysis restricted to ever maté drinkers, Lifetimetime consumption further adjusted for temperature; Amount consumed, maté temperature and duration of maté consumption further adjusted for each other.
*reference category; **includes quitters of up to 2 years

3.2.7. Joint effect of maté consumption and drinking temperature

Maté amount and temperature were found to have independent effects on risk. The interaction between these two variables was subsequently explored by fitting appropriate logistic regression models with interaction terms for these variables to estimate the effect of amount in each stratum of temperature and the effect of temperature in each stratum of amount (Table 9). Although the interaction between these two variables was not statistically significant (P=0.7), higher risks were associated with increased temperature across all amount strata, while the risk was fairly constant for all levels of amount consumed, across the temperature strata. Those drinking more than 1 L/day of maté at a very hot temperature had a 3-fold increase in risk (OR = 2.95, 95% CI 1.30 – 6.74) compared to those drinking less than 0.5 L/day of maté at a warm to hot temperature.

Table 9: Odds ratio of oesophageal cancer for the joint effects of amount and temperature of maté drinking[1]

Maté drinking temperature	Maté drinking amount (L/day)		
	0.01 – 0.50	0.51 – 1.0	1.01+
	OR (95% CI) Cases/Controls	OR (95% CI) Cases/Controls	OR (95% CI) Cases/Controls
Warm/hot	1.0 (reference) 60/135	1.60 (1.05 – 2.44) 115/146	1.80 (1.08 – 3.00) 65/66
Very hot	2.84 (1.06 – 7.66) 9/10	2.80 (1.37 – 5.71) 27/18	2.95 (1.30 – 6.74) 18/15

[1]Odds ratios (ORs) adjusted for age, sex,urban/rural status, years of education, number of cigarettes smoked per day, smoking duration, total alcohol per day, and duration of alcohol consumption.
P-value for the interaction between maté amount and maté temperature = 0.7.

3.2.8. Population attributable proportion due to maté consumption

The population attributable proportion due to maté consumption was calculated from logistic regression data. This was done by dichotomising maté exposure (ilexgroup) into "ever" or "never" exposed to maté (ilexpar) as indicated below ("0" = never; "1" = ever) .

Maté consumption (all users vs never users)

```
. tab ilexgroup

  ilexgroup |      Freq.     Percent        Cum.
------------+-----------------------------------
          0 |         79        9.74        9.74
  0.01-0.50 |        222       27.37       37.11
   0.51-1.0 |        324       39.95       77.07
      1.01+ |        186       22.93      100.00
------------+-----------------------------------
      Total |        811      100.00

. generate ilexpar=0
. replace ilexpar=1 if ilex>0
(734 real changes made)
. replace ilexpar=. if ilex==.
(2 real changes made, 2 to missing)

. tab ilexpar

    ilexpar |      Freq.     Percent        Cum.
------------+-----------------------------------
          0 |         79        9.74        9.74
          1 |        732       90.26      100.00
------------+-----------------------------------
      Total |        811      100.00
```


The prevalence of exposure to maté was subsequently calculated in the control group using equation 1 and found to be 86%. Based on a relative risk of 2.26 (95% CI 1.19 – 4.27) for ever exposure to maté, a population attributable risk of 52% was obtained (95% CI 14.8% - 74.3%) using equation 2. Alternatively, obtaining the logistic regression model, the attributable fraction can be obtained using stata as follows:

```
. aflogit ilexpar,cc
Population attributable fraction from logistic regression
Case-control data (n=770)

Term       Ref.     A.F.        s.e.      [95% Conf. Int.]*
-------------------------------------------------------------
ilexpar    0       0.5220      0.1432      0.1476   0.7430
-------------------------------------------------------------
TOTAL              0.5220      0.1432      0.1476   0.7430

* CI calculated on log(1-AF) scale
```

Thus assuming a causal relationship exists, 52% of the oesophageal cancers in the population would have been prevented if this exposure had been removed. The high population attributable risk for maté is due to the high prevalence of maté exposure in the population. With emphasis on maté-related variables, the population attributable fraction for high maté consumption (>1L/day) versus non-drinkers was calculated to be 24.8%, from a relative risk of 2.84, while high temperature of maté consumption attributed to 22.1% of the cases (RR=3.98).

3.3. Effects of alcohol consumption

The adjusted risk estimates for alcohol-related variables are presented in Table 10.

3.3.1. Lifetime consumption

Cumulative (lifetime) consumption of alcohol presented a 5-fold increase in risk (OR = 5.01, 95% CI 2.72 – 9.24) in the highest category compared to non-drinkers. A highly significant dose-response relationship (P=0.000) was observed.

3.3.2. Amount of alcohol

Total alcohol consumption (sum of averages of ml ethanol from beer, wine and hard liquor) was positively associated with oesophageal cancer risk with drinkers of more than 180 ml ethanol/day being 3.5 times more likely to develop OC (OR = 3.57, 95 % CI 2.10 – 6.09) than non-drinkers. A highly significant trend was observed amongst the drinkers (P=0.000). The model was further adjusted for alcohol duration (drinkers only) with the lowest consumption category (1 – 60 ml ethanol/day) being the reference category. A significant trend was observed (P=0.042), with drinkers in the highest category presenting a 3-fold increase in risk (OR=3.19, 95% CI 1.74 – 5.85) compared to those drinking less than 61 ml ethanol/day. As expected, lower alcohol consumption categories presented lower risk estimates.

3.3.3. Alcohol duration

Duration of alcohol consumption was also positively associated with oesophageal cancer risk although a plateau effect was observed amongst those consuming alcohol for more than 30 years (OR = 1.79, 95% CI 1.13 – 2.84). There was no significant monotonic dose-response observed (P=0.343) among the drinkers. The risk estimates decreased after adjusting for total alcohol consumption.

3.3.4. Effect of beer, wine and spirits

Beer was the least consumed alcoholic beverage in this study population (10 % drinkers) with the risk estimate indicating a protective effect although not statistically significant (OR = 0.78, 95% CI 0.39 – 1.56). Furthermore, not much weight can be attached to this estimate due to the small number of beer drinkers in the study population. Both wine and hard liquor consumption were associated with increased risks, with wine being the most frequently consumed alcoholic beverage. A highly significant dose-response relationship (P=0.002) was observed for wine drinkers in comparison to the hard liquor drinkers (P=0.093) although the risks were fairly similar (2.5-fold increase) and highly significant in the highest consumption categories (>60 ml ethanol/day) for both alcoholic beverages compared to non-drinkers. Hence in this population, the sum of liquor and wine consumption would be a better indicator of risk associated with alcohol exposure than a measure of total alcohol consumption.

3.3.5. Population attributable proportion due to alcohol consumption

The prevalence of exposure to alcohol was calculated as 42%. Based on a relative risk of 1.76 (95% CI 1.21 – 2.56) for ever having been exposed to alcohol, the population attributable proportion as a result of alcohol exposure was 24.2%.

Table 10: Odds ratio of oesophageal cancer for consumption of alcohol

VARIABLES	CASES		CONTROLS		OR_1[a]	(95% CI)	OR_2[b]	(95% CI)
	N	(%)	*N*	(%)				
Lifetime consumption (L.years)								
Non-drinkers	120	(37.9)	270	(58.8)	1.00			
1 – 832	47	(14.8)	76	(16.6)	1.23	(0.76 – 1.99)		
833 – 3285	83	(26.2)	90	(19.6)	1.52	(0.96 – 2.42)		
3286+	67	(21.1)	23	(5.0)	5.01	(2.72 – 9.24)		
P for trend (drinkers only)					0.000			
Average alcohol consumption (ml ethanol per day								
Non-drinkers	120	(34.9)	270	(57.6)	1.00			
1 – 60	58	(16.9)	93	(19.8)	1.14	(0.72 – 1.79)	1.00	
61 – 180	82	(23.8)	66	(14.1)	1.97	(1.22 – 3.19)	2.15	(1.24 – 3.74)
181+	84	(24.4)	40	(8.5)	3.57	(2.10 – 6.09)	3.19	(1.74 – 5.85)
P for trend (drinkers only)					0.000		0.042	
Beer (mL ethanol per day)								
Non-drinkers	326	(94.8)	445	(94.9)	1.00			
1+	18	(5.2)	24	(5.1)	0.78	(0.39– 1.56)		
Wine (ml ethanol per day)								
Non-drinkers	151	(43.9)	306	(65.3)	1.00			
1 – 60	80	(23.3)	101	(21.5)	1.19	(0.79 – 1.78)		
61+	113	(32.8)	62	(13.2)	2.51	(1.63 – 3.88)		
P for trend (drinkers only)					0.002			
Spirits (ml ethanol per day)								
Non-drinkers	204	(59.3)	370	(78.9)	1.00			
1 – 60	55	(16.0)	54	(11.5)	1.42	(0.89 – 2.27)		
61+	85	(24.7)	45	(9.6)	2.42	(1.53 – 3.85)		
P for trend (drinkers only)					0.093			
Alcohol duration (years)								
Non-drinkers	120	(37.9)	270	(58.8)	1.00			
1 – 30	41	(12.9)	59	(12.9)	1.49	(0.89 – 2.50)	1.00	
31 – 46	85	(26.8)	75	(16.3)	1.79	(1.13 – 2.84)	1.13	(0.64 – 2.00)
47+	71	(22.4)	55	(12.0)	1.73	(1.03 – 2.90)	1.25	(0.63 – 2.49
P for trend (drinkers only)					0.343		0.769	

[a] OR_1, adjusted for age, sex,urban/rural status, years of education, number of cigarettes smoked per day, smoking duration, type of tobacco and amount of maté consumed.
[b] OR_2, Average alcohol consumption and alcohol duration further adjusted for each other.

3.3.6. Joint effects of alcohol and maté consumption

The interaction between alcohol and maté consumption was investigated by fitting appropriate logistic regression models with interaction terms for these variables to estimate the effect of maté amount in each stratum of alcohol and the effect of alcohol in each stratum of maté amount (Table 11). Although the interaction between these two variables was not statistically significant (P=0.28), higher risks were associated with increased maté consumption across all alcohol amount strata above 60 ml ethanol/day. Similarly increased alcohol consumption also increased the risk estimates across the maté amount strata. Hence those drinking more than 180 ml ethanol/day and consuming more than 1 L/day of maté had a 7-fold increased risk of developing oesophageal cancer (OR = 7.05, 95% CI 2.85 – 17.40) compared to those drinking ≤0.5 L/day of maté but not consuming any alcohol.

Table 11: Joint effects of maté and alcohol consumption on oesophageal cancer risk[1]

Total alcohol consumption (ml ethanol per day)	Maté drinking amount (L/day)		
	0.01 – 0.50	0.51 – 1.0	1.01 – 1.50
	OR (95% CI) Cases/Controls	OR (95% CI) Cases/Controls	OR (95% CI) Cases/Controls
0	1.0 (reference) 36/98	1.57 (0.91 – 2.70) 53/88	1.68 (0.86 – 3.26) 27/43
1 – 60	1.60 (0.69 – 3.71) 14/21	1.63 (0.82 – 3.25) 29/39	1.26 (0.52 – 3.05) 13/20
61 – 180	1.15 (0.49 – 2.71) 14/23	2.98 (1.41 – 6.26) 37/24	5.38 (2.14 – 13.51) 28/10
181+	2.34 (0.73 – 7.54) 9/7	3.46 (1.59 – 7.55) 33/21	7.05 (2.85 – 17.40) 34/11

[1]Odds ratios (ORs) adjusted for design variables, number of cigarettes smoked per day, smoking duration and type of tobacco.
P-value for the interaction between maté and alcohol consumption = 0.2812.

3.4. Effects of tobacco smoking

The adjusted risk estimates associated with smoking-related variables are presented in Table 12. Current smokers were about 2.4 times more likely to develop OC (OR = 2.39, 95% CI 1.43 – 3.98) than non-smokers, while ex-smokers showed a mild risk that was statistically non-significant (OR = 1.12, 95% CI 0.65 – 1.91).

3.4.1. Lifetime exposure

Cumulative exposure to smoking expressed in pack years also presented with increased risks, with increasing pack years although the trend was statistically non-significant (P = 0.067). A 2-fold increase in risk was observed for those who had a cumulative exposure greater than 17156 pack years (OR=2.18, 95% CI 1.24 – 3.85). The analysis was thereafter restricted to smokers only, and the model further adjusted for type of tobacco. The increase in risk persisted, with those in the highest exposure category once again presenting with a 2-fold increased risk (OR=1.87, 95% CI 0.98 – 3.57).

3.4.2. Smoking duration

The effect of smoking duration on risk of OC presented a monotonic dose-response that was statistically significant (P=0.05). Increased risks were associated with increasing duration of exposure. Among smokers, smoking duration was further adjusted for number of cigarettes smoked per day and type of tobacco in order to measure the independent effect of smoking duration. Subjects smoking for more than 49 years had a 2-fold increase in the risk compared to those smoking for less than 25 years although the trend was statistically non-significant (P=0.211).

3.4.3. Number of cigarettes per day

The independent effect of number of cigarettes smoked per day was measured by adjusting the regression model for smoking duration and type of tobacco. Those smoking more than 11 cigarettes per day presented with a 2-fold increase in risk compared to smokers of 10 or less cigarettes per day (OR = 1.96, 95% CI 1.14 – 3.34). The higher exposure category was not associated with further elevation in risk suggesting a plateau effect.

3.4.4. Type of tobacco

Smokers of black tobacco presented a statistically significant 3-fold increased risk as compared to smokers of blond tobacco (OR = 2.88, 95% CI 1.82 – 4.54). The risk was moderately increased yet non-significant among those smoking a mixture of blond and black tobacco (OR = 1.58, 95% CI = 0.80 – 3.16). Once again, this measure was an independent effect as the model was adjusted for smoking duration and number of cigarettes smoked per day.

Table 12: Odds ratio of oesophageal cancer for tobacco smoking

VARIABLES	CASES		CONTROLS		OR_1[a]	(95% CI)	OR_2[b]	(95% CI)
	N	(%)	*N*	(%)				
Smoking status								
Non-smokers	73	(21.2)	181	(38.6)	1.00			
Ex-smokers	83	(24.1)	126	(26.9)	1.12	(0.65 – 1.91)		
Current smokers	188	(54.7)	162	(34.5)	2.39	(1.43 – 3.98)		
P for trend					0.001			
Lifetime exposure (pack years)								
Non-smokers	73	(21.3)	181	(38.6)	1.00			
1 – 5840	31	(9.0)	60	(12.8)	1.22	(0.66 – 2.26)	1.00	
5841 – 17155	98	(28.6)	118	(25.2)	1.93	(1.11 – 3.34)	1.61	(0.86 – 3.00)
17156+	141	(41.1)	110	(23.4)	2.18	(1.24 – 3.85)	1.87	(0.98 – 3.57)
P for trend (smokers only)					0.067		0.504	
Smoking duration (years)								
Non-smokers	73	(21.3)	181	(38.6)	1.00			
1 – 24	27	(7.9)	60	(12.8)	1.16	(0.60 – 2.24)	1.00	
25 – 48	131	(38.2)	150	(32.0)	1.84	(1.09 – 3.10)	1.65	(0.89 – 3.05)
49+	112	(23.6)	78	(16.6)	2.12	(1.19 – 3.78)	2.05	(0.99 – 4.18)
P for trend (smokers only)					0.050		0.211	
Number of cigarettes per day								
Non-smokers	73	(21.3)	181	(38.6)	1.00			
1 – 10	45	(13.1)	81	(17.3)	1.17	(0.67 – 2.06)	1.00	
11 – 20	114	(33.2)	99	(21.1)	2.58	(1.48 – 4.47)	1.96	(1.14 – 3.34)
21+	111	(32.4)	108	(23.0)	1.79	(1.01 – 3.19)	1.40	(0.81 – 2.42)
P for trend (smokers only)					0.936		0.142	
Type of tobacco								
Non-smokers	73	(21.3)	181	(38.6)	1.00			
Blond	92	(26.8)	190	(40.5)	1.28	(0.78 – 2.12)	1.00	
Black	151	(44.0)	73	(15.6)	3.39	(1.92 – 6.00)	2.88	(1.82 – 4.54)
Mixed	27	(7.9)	25	(5.3)	1.88	(0.87 – 4.06)	1.58	(0.80 – 3.16)

[a] OR_1, adjusted for age, sex,urban/rural status, years of education, total alcohol per day, duration of alcohol consumption and amount of maté consumed.
[b] OR_2, smoking duration, number of cigarettes and type of tobacco further adjusted for each other; lifetime exposure further adjusted for type of tobacco.

3.4.5. Population attributable proportion due to tobacco smoking

The prevalence of exposure to tobacco was calculated as 61%. Based on a relative risk of 1.71 (95% CI 1.06 – 2.75) for ever having smoked tobacco, the population attributable proportion as a result of tobacco smoking was 30.2%.

3.4.6. Joint effect of smoking duration and type of tobacco

The risk estimates for the joint effect of smoking duration and type of tobacco is presented in Table 13. Although the interaction was not statistically significant (*P*=0.1645), increased smoking duration was associated with increased risks for black tobacco. Hence those smoking black tobacco for a period of 25 years or more had a 6.4-fold increased risk of developing OC (OR = 6.42, 95% CI 2.52 – 16.35) compared to those smoking blonde tobacco for a period of 24 years or less.

Table 13: Joint effects of smoking duration and type of tobacco on oesophageal cancer risk[1]

Smoking duration (years)	Type of tobacco		
	Blonde	Black	Mixed
	OR (95% CI) Cases/Controls	OR (95% CI) Cases/Controls	OR (95% CI) Cases/Controls
1 – 24	1.0 (reference) 12/47	2.31 (0.59 – 9.07) 12/9	3.09 (0.54 – 17.86) 3/4
25 – 48	1.54 (0.69 – 3.43) 51/1108	6.42 (2.52 – 16.35) 70/28	1.31 (0.36 – 4.68) 10/14
49+	2.39 (0.88 – 6.49) 29/35	4.89 (1.83 – 13.10) 69/36	5.23 (1.43 – 19.19) 14/7

[1]Odds ratios (ORs) adjusted for age, sex,urban/rural status, years of education, total alcohol per day, duration of alcohol consumption, number of cigarettes smoked per day and amount of maté consumed. *P*-value for the interaction between smoking duration and type of tobacco = 0.1645

3.5. Joint effects between cumulative exposures to maté, alcohol and tobacco smoking

The combined effects of lifetime exposure to maté, alcohol and tobacco smoking was assessed by fitting a multivariate model, adjusted for age, sex, urban/rural status and years of education, which included the main effect terms at each level characterised by "high" or "low" exposure and the interaction terms between the different exposure variables (Table 14). The interaction terms were not statistically significant (*P*-value 0.96). The low exposure category for the three variables was used as the reference category. The OR for high consumption of maté with low exposures to alcohol and smoking was 1.68 (95% CI 0.88 – 2.62); that for high tobacco consumption with low exposures to maté and alcohol was 2.36 (95% CI 1.23 – 4.53), and that for high exposure to alcohol in the presence of low exposures to maté and tobacco was 2.54 (95% CI 0.93 – 6.95). The OR for high cumulative exposures of all three agents was 7.10 (95% CI 3.75 – 13.46). Given the small numbers and wide confidence intervals, an examination of the RR differences shows that the effects are probably additive or perhaps more than additive but not significantly so. Examination of the multiplication of RRs shows that the effect is less than multiplicative but not significantly so (*P*-value 0.96) hence it is possible that there is no interaction and no departure from multiplicativity or from additivity on the log scale.

Table 14: Joint effects of cumulative exposure to maté, alcohol and tobacco smoking on oesophageal cancer risk[1]

Lifetime consumption of maté[a]	Lifetime drinking of alcohol[b]	Lifetime tobacco smoking[c]	Number of cases/controls	OR (95 %CI)
Low	low	low	43/148	1.00 (reference)
Low	low	high	32/67	2.36 (1.23 – 4.53)
Low	high	low	8/14	2.54 (0.93 – 6.95)
Low	high	high	45/47	4.06 (2.07 – 7.96)
high	low	low	38/68	1.52 (0.88 – 2.62)
high	low	high	51/62	3.12 (1.69 – 5.78)
high	high	low	7/6	3.40 (1.00 – 11.59)
high	high	high	89/46	7.10 (3.75 – 13.46)

3.6. Effects of Dietary variables

Table 15 summarizes the results for the frequency of consumption of the main dietary variables assessed in this study. Total red meat intake was associated with a strong positive association on oesophageal cancer risk. After further adjustment for alcohol, tobacco and maté variables, total red meat intake greater than 365 servings per year was associated with a 3-fold increase in risk (OR = 2.89, 95% CI 1.80 – 4.66) and a highly significant monotonic dose-response was observed (P=0.000). Subjects eating salted meat at adolescence (especially those eating more than 100 servings/year) were also at an increased risk (OR = 2.01, 95% CI 1.23 – 3.29). A highly significant trend (P=0.001) in risk was also observed for subjects consuming barbecued meat. Those eating more than 53 servings per year were at a 2.3-fold greater risk than those who ate below 12 servings per year. Even when allowing for the effects of other meat intake, barbecued meat, presented a high risk and remained highly significant (P=0.000) for the highest category of exposure (OR = 2.46, 95% CI 1.54 – 3.91, >53 servings/year). Frequent consumption of raw vegetables was associated with a reduction in oesophageal cancer risk. A 34% reduction in the risk was observed for those who consumed more than 105 servings per year, although this reduction was of borderline significance (OR = 0.66, 95% CI 0.43 – 1.01). The consumption of fruits was also observed to have a protective effect with those eating more than 157 servings per year having a 30% reduced risk of developing oesophageal cancer. The intake of total fruits and vegetables of more than 261 servings/year was also associated with a 28% non-significant reduction in risk (OR = 0.72, 95% CI 0.46 – 1.13).

Table 15: Odds ratio of oesophageal cancer for consumption of selected foods

VARIABLES (servings/year)	CASES		CONTROLS		OR_1[a]	(95% CI)	OR_2[b]	(95% CI)
	N	(%)	*N*	(%)				
Total meat (red)								
0 – 156	45	(13.1)	158	(33.8)	1.00		1.00	
157 – 260	39	(11.3)	93	(19.9)	1.38	(0.82 – 2.30)	1.07	(0.62 – 1.87)
261 – 364	112	(32.6)	118	(25.2)	2.70	(1.74 – 4.18)	2.34	(1.48 – 3.68)
365+	148	(43.0)	99	(21.1)	3.75	(2.41 – 5.85)	2.89	(1.80 – 4.66)
P for trend							0.000	
Salted meat at adolescence								
0	226	(65.7)	385	(82.3)	1.00		1.00	
1 – 100	53	(15.4)	46	(9.8)	1.90	(1.22 – 2.97)	1.43	(0.87 – 2.35)
101+	65	(18.9)	37	(7.9)	2.35	(1.48 – 3.73)	2.01	(1.23 – 3.29)
P for trend							0.006	
Salted meat at present								
0	279	(89.4)	429	(94.7)	1.00		1.00	
1 – 30	18	(5.8)	14	(3.1)	1.53	(0.72 – 3.24)	1.35	(0.62 – 2.96)
31+	15	(4.8)	10	(2.2)	1.58	(0.67 – 3.73)	1.21	(0.49 – 2.99)
P for trend							0.673	
Barbecued meat								
0 – 12	95	(27.6)	212	(45.3)	1.00		1.00	
13 – 24	34	(9.9)	59	(12.6)	1.10	(0.67 – 1.83)	1.18	(0.70 – 1.99)
25 – 52	108	(31.4)	131	(28.0)	1.62	(1.12 – 2.34)	1.40	(0.94 – 2.08)
53+	107	(31.1)	66	(14.1)	2.74	(1.80 – 4.16)	2.27	(1.44 – 3.58)
P for trend							0.001	
Processed meat								
0 – 12	111	(35.6)	187	(41.3)	1.00		1.00	
13 – 24	21	(6.7)	50	(11.0)	0.67	(0.37 – 1.20)	0.70	(0.38 – 1.27)
25 – 104	130	(41.7)	147	(32.5)	1.45	(1.02 – 2.06)	1.29	(0.89 – 1.87)
105+	50	(16.0)	69	(15.2)	1.23	(0.78 – 1.94)	1.08	(0.67 – 1.74)
P for trend							0.770	
Whole milk								
0 – 52	76	(22.1)	134	(28.6)	1.00		1.00	
53 – 364	103	(29.9)	159	(34.0)	1.06	(0.71 – 1.57)	1.26	(0.82 – 1.92)
365+	165	(48.0)	175	(37.4)	1.41	(0.97 – 2.05)	1.81	(1.19 – 2.73)
P for trend							0.004	

VARIABLES	CASES		CONTROLS		OR_1[a]	(95% CI)	OR_2[b]	(95% CI)
	N	(%)		(%)				
Raw vegetables								
0 – 24	88	(25.6)	105	(22.4)	1.00		1.00	
25 – 52	90	(26.2)	94	(20.1)	1.17	(0.77 – 1.79)	1.20	(0.75 – 1.84)
53 – 104	81	(23.5)	96	(20.5)	1.18	(0.77 – 1.82)	1.06	(0.67 – 1.66)
105+	85	(24.7)	173	(37.0)	0.69	(0.46 – 1.04)	0.66	(0.43 – 1.01)
P for trend							0.008	
Fruits								
0 – 52	135	(39.3)	145	(31.0)	1.00		1.00	
53 – 156	82	(23.8)	99	(21.1)	0.98	(0.66 – 1.45)	1.08	(0.71 – 1.65)
157 – 360	34	(9.9)	73	(15.6)	0.54	(0.33 – 0.89)	0.70	(0.41 – 1.19)
361+	93	(27.0)	151	(32.3)	0.83	(0.57 – 1.22)	0.94	(0.62 – 1.42)
P for trend							0.519	
Total fruits + Vegetables								
0 – 104	106	(30.8)	105	(22.4)	1.00		1.00	
105 – 260	100	(29.1)	105	(22.4)	1.04	(0.70 – 1.55)	1.05	(0.68 – 1.62)
261 – 442	69	(20.1)	130	(27.8)	0.61	(0.40 – 0.93)	0.72	(0.46 – 1.13)
443+	69	(20.1)	128	(27.8)	0.70	(0.45 – 1.07)	0.72	(0.45 – 1.15)
P for trend							0.068	

[a] OR_1, adjusted for age, sex, urban/rural status and years of education

[b] OR_2, adjusted for age, sex, urban/rural status and years of education, number of cigarettes smoked per day, smoking duration, total alcohol per day, duration of alcohol consumption and amount of maté consumed.

Chapter 4: Discussion

4.1. Key Findings

This study confirms previous findings in Uruguay of the association between maté drinking and oesophageal cancer. The evidence suggests that two independent and competitive mechanisms could be at play, firstly the possible role of carcinogens in *Ilex paraguariensis* and secondly, the role of chronic thermal injury to the oesophagus as a result of very hot maté consumption. The apparent carcinogenic effect of maté is weak (low, non-significant OR in low drinkers) however the effect of temperature is stronger. There was no evidence of interaction, however, between amount and temperature. The high attributable risk due to maté consumption depended mostly on the high prevalence of exposure (and would therefore be specific to this population). Alcohol drinking and tobacco smoking were also found to be significantly associated with OC risk. The role of meat, and in particular barbecued meat increased the risk of developing squamous cell OC, while high consumption of fruits and vegetables confered some degree of protection.

4.2. Findings in the light of previous studies

4.2.1. Maté drinking

Consistent with previous epidemiologic studies in Uruguay (Vassallo *et al.*, 1985; De Stefani *et al.*, 1990), this study once again showed that maté consumption is associated with an elevated risk of oesophageal cancer. An almost three-fold increase in risk was observed among those drinking more than 1 L/day of maté, after adjusting for the effects of age, sex, urban/rural status, education, tobacco smoking and alcohol drinking. A previous study conducted by Vassallo *et al.* (1985), showed that heavy maté consumption (≥1.0 litre/day) in males was associated with a 5-fold increase in oesophageal cancer risk (OR=4.8, 95% CI 1.9 – 12.1) after adjusting for age, tobacco and alcohol. Females who were also exposed to this level of maté however, presented with a 35-fold increased risk (OR=34.6, 95% CI 4.9 – 246.5) after adjusting for age indicating a profound interaction with sex. Subsequently, De Stefani *et al.* (1990) found that the relative risk of those drinking over 2.5 litres of maté per day was 12.2 (95% CI, 3.8-39.6) after adjustment for the effects of age, area of residence, alcohol and tobacco. Castellsagué *et al.* (2000) reported an odds ratio of 3.04 (95% CI 1.84 –

5.02) for maté amount (>1L versus <0.5L). The possible effect of maté drinking on precancerous lesions of the oesophagus has also been shown in an endoscopic survey conducted in Rio Grande do Sul (Muñoz, 1987) where a 2.2-fold excess (90% CI 1.1 – 9.8) of histologically confirmed oesophagitis was shown among maté drinkers, compared to non-drinkers.

4.2.2. Possible effect of carcinogenic chemical constituents of maté

In studying the association between maté consumption and oesophageal cancer, one of the challenges lies in disentangling the effects of amount and temperature in order to assess whether the potential effect is related to the plant itself (due to the presence of carcinogenic compounds), to the high temperature at which maté is consumed (as a result of thermal injury to the oesophagus), or to a combination of the two factors. It has long been suspected that herbs can cause oesophageal cancer. Studies undertaken in Curacao, Venezuela, and South Carolina resulted in the identification of numerous plant products consumed in large quantities by local populations in which oesophageal cancer is prevalent (Morton, 1970, 1973, 1979). It is possible that exposure to maté may increase the risk of oesophageal cancer through the presence of carcinogenic compounds present in the beverage. Maté infusion contains tannins in a proportion that ranges from 7% to 14% (Hilal, 1976), and experimental studies with tannins have shown the occurrences of malignant fibrous histiocytomas at the inoculation sites and of malignant tumours in rat liver (Kirby, 1960; Pradhan, 1974; Kapadia, 1976). In addition, aqueous solutions of maté have also been shown to induce mutagenisis in *Salmonella typhimurium* strains TA97, TA98, TA100 and TA 102 (Leitão and Braga, 1994) and increase the frequency of chromosomal aberrations in human peripheral lymphocytes (Fonseca *et al.*, 2000).

4.2.3. Possible effect of high temperature

In addition to a possible direct carcinogenic effect of maté, the temperature at which maté is consumed is likely to play an important role in oesophageal carcinogenesis. A population-based survey conducted in southern Brazil (Victora *et al.*, 1990) where the temperature at which maté was consumed by 1400 adults was measured, showed that the mean temperature just before consumption was 69.5 °C. This indicated that the oesophageal mucosa may be exposed to temperatures that may cause chronic thermal injury. This study showed that drinkers of very hot maté presented a 2-fold increase in

risk as compared to those consuming warm/hot maté. Hot maté drinking was a risk factor for oesophageal cancer in Paraguay (OR=2.4, 95% CI 1.3 – 4.3) after adjusting for the effects of alcohol consumption and tobacco smoking (Rolón *et al.*, 1995). In Argentina, the effect of drinking maté at hot/very hot temperatures was associated with a 70% increase in risk (OR=1.7, 95% CI 1.0 – 2.9) compared to those drinking warm maté (Castelleto *et al.*, 1994). The findings in South America are consistent with several other studies that have suggested a possible effect of hot drinks on OC incidence as a result of thermal injury to the organ. In China, preference for drinking "burning hot" beverages" was identified as the strongest risk factor for oesophagitis (Wahrendorf *et al.*, 1989), and in Hong Kong, preference for consuming drinks or soups at high temperatures was associated with OC risk accounting for 14% of this cancer in this population (Cheng *et al.*, 1992). In northeastern China, scalding temperature of meals and drinks was identified as the strongest dietary risk factor for oesophageal cancer (Hu *et al.*, 1994). In Iran, a case-control study indicated that inhabitants from high risk areas consumed larger quantities of hot tea than those from low-risk areas (Cook-Mozaffari *et al.*, 1979). De Jong *et al.* (1974) also found that oesophageal cancer cases in Singapore were more likely than controls to report drinking "burning hot" beverages, with a similar association being observed from a case-control study investigating the intake of hot coffee in Puerto Rico (Martinez, 1969). In observing the results of this study, such results, one needs to acknowledge that self-reporting of temperature at which maté was consumed may be subject to misclassification. Since there was no objective measurement, subjects may misclassify the "warm/hot" than "very hot" category. However it can be postulated that since the knowledge of the association between hot temperature and oesophageal cancer risk is practically unknown in this population, it is unlikely that misclassification was differential with regard to case-control status. If misclassification did occur, then it is likely to have been non-differential hence the reported risk estimates would be an under-estimation of the real underlying effect of temperature.

4.2.4. Population attributable proportion due to maté consumption

Based on a relative risk of 2.26 (95% CI 1.19 – 4.27) for exposure to maté, a population attributable risk of 52% was obtained (95% CI 14.8% - 74.3%). Thus assuming a causal relationship exists, 52% of the oesophageal cancers in the population would have been prevented if this exposure had been removed. The high population attributable risk for maté is due to the high prevalence of maté exposure in the population. With emphasis on maté-related variables, the population attributable fraction for high maté consumption (>1L/day) versus non-drinkers was calculated to be 24.8 %, while high temperature of maté consumption attributed to 22.1% of the cases.

4.2.5. Effect of alcohol drinking and tobacco smoking

Alcohol drinking also remains an important risk factor for oesophageal cancer in this population with a 3.6-fold increase in risk for the highest category of alcohol consumption compared to non- drinkers. The magnitude of the increase in risk associated with the consumption of spirits was similar to that associated with wine drinking, which is not surprising considering that about 84% of the drinkers are wine drinkers and 57% are drinkers of spirits. However, no increased risk associated with the consumption of beer could be detected because there are too few beer drinkers (10%). It has long been suggested that alcohol may act as a solvent facilitating the transport of carcinogens through the oesophageal mucosa (Horie *et al.*, 1965), but it may also act as a chronic irritant, raising the susceptibility to carcinogens by accelerating cell turnover and thus favouring contact between the carcinogens and the dividing target cells (Day and Muñoz, 1982).

With regards to tobacco smoking, current smokers presented a higher risk of developing oeosphageal cancer than non-smokers or ex-smokers. Moreover, the type of tobacco used was found to be an important variable. A comparison of the effects of blond (flue-cured), black (air-cured) and a mixture of both types of tobacco was undertaken. The risk in smokers of black tobacco was increased approximately 3-fold compared to those smoking blond tobacco while those smoking a mixture of the two presented an intermediate risk estimate. These findings are in agreement with the trends observed in other studies on oesophageal cancer (La Vecchia *et al.*, 1986; De Stefani *et al.*, 1990; Castelletto *et al.*, 1994), bladder cancer in Italy (Vineis *et al.*,

1984), and Argentina (Iscovich *et al.*, 1987), on laryngeal cancer in southern Europe (Tuyns *et al.*, 1988) and previous studies on cancers of the larynx and oropharynx in Uruguay (De Stefani *et al.*, 1987; 1988). Moreover these observations are reinforced by laboratory results showing that the smoke of black tobacco cigarettes contained more aromatic amines and tobacco-specific nitrosamines than that from blond tobacco cigarettes (Patrianakos and Hoffmann, 1979) and that the urine of smokers of black tobacco contained about twice as much mutagenic activity as did the urine of blond cigarette smokers (Malaveille *et al.*, 1989). Black tobacco has been shown to contain higher concentrations of *N*-nitroso compounds and higher alkalinity than blond tobacco (Hoffmann *et al.*, 1984). In, Uruguay, it has been reported that more than 50% of the smoking population used black tobacco, frequently hand-rolled and has been suggested that due to its strong taste and higher alkalinity, black tobacco is less easily inhaled and remains longer in the buccal and pharyngeal structures (De Stefani *et al.*, 1988).

4.2.6. Effect of diet

Concerning the dietary factors, a distinct increase in risk, which was highly significant, was observed for those eating total red meat and barbecued meat. This effect was observed after adjusting for age, sex, urban/rural status, education, smoking, alcohol and maté consumption. The increased risk for barbecued meat remained even after adjusting for other meat intake. This evidence is further supported by laboratory work showing the presence of animal carcinogens and mutagens in barbecued foods formed by the pyrolysis of proteins (Sugimura *et al.*, 1983). Fried or broiled meat is a rich source of heterocyclic amines, potent mutagens and carcinogens in animal studies (IARC, 1993). While the latest reviews (Cheng and Day, 1996; World Cancer Research Fund, 1997) reflected the difficulties in making a judgement about the relationship between red meat consumption and oesophageal cancer, the results of this study are consistent with previous findings in Uruguay (De Stefani *et al.* 1990, 1998). Furthermore, a study using ecological data from 59 countries, also found a highly significant association between high meat consumption and oesophageal cancer rates (Hebert *et al.* 1993). Meat intake has also been found to be positively associated with stomach cancer (Stemmermann *et al.*, 1990), and other cancers of the upper digestive tract (Winn *et al.*, 1984, De Stefani *et al.*, 1999b), although a large case-control study conducted in Calvados, France, found a protective

effect for fresh meat consumption (Tuyns *et al.*, 1987). Salted meat intake, in the present and more so in the past, presented increased risks for squamous cell oesophageal cancer in our study. Previous studies on salted meat and oesophageal cancer (Cheng *et al.*, 1992; De Stefani *et al.*, 1999a, 1999b) have also reported increased risks for this malignancy. Salted meat has been considered as a source of exogenous nitrosamines, like nitrodimethylamine (De Stefani *et al.*, 1994b) and has been considered to be biologically plausible, since oesophageal cancer has been considered as related to nitrosamine exposure (Eichholzer and Gutzwiller, 1998).

Increased consumption of whole milk also presented higher risk estimates with a highly significant trend. A previous study by Tuyns *et al.* (1987) also showed a significant increase in risk of developing oesophageal cancer associated with whole milk consumption. A multicentre case-control study of fat-rich foods suggested an effect of fat in oesophageal carcinogenisis (Launoy *et al.*, 1998) hence the effect of whole milk may be due to its fat content. Consistent with many observational studies on diet and cancer, a protective effect with a significant dose response was observed with the consumption of raw vegetables. A protective effect was also seen for higher consumption of fruit although no trend was observed. The estimates obtained for vegetable and fruit consumption are not as low as compared to previous studies in Uruguay (De Stefani *et al.*, 1990; 2000) and this may be due to the less detailed questionnaire on diet that was used in this study and misclassification.

4.3. Strengths and weaknesses of the study

This study was hospital based and hence may have been subject to various kinds of bias. Selection bias may concern cases if they are not representative with respect to all the cases in the population and this is bound to affect the external validity (generalisability) of the results. The Oncology Institute of Montevideo has a catchment area that covers 45% of the population of Montevideo and 55% of the rest of the country. Selection bias for controls arises if they are not representative of the source population of the cases and would affect the internal validity of the study results. Selection bias would be particularly relevant for hospital-based controls if their referral to participating hospitals differs from that of cases. We addressed this source of bias by selecting controls from several diagnostic categories. Lack of response is a further potential source of selection bias; however it is not likely to have

played an important role since the response rate was high among both cases and controls.

Errors in measurement may also be introduced as a result of observer (interviewer) or responder (patient) bias. Although it is rather difficult to exclude observer bias, the interviewers were blind to the hypothesis being tested and the interviews were done prior to diagnosis. Responder bias can manifest as a result of differential recall of information by cases and controls; for instance, cases may be more likely to recall past exposure, especially if its association with the disease is widely known. This type of bias can exaggerate the degree of effect associated with the exposure. In this study, this form of bias is unlikely to have generated the difference between cases and controls concerning exposure to risk factors as the patients were interviewed before the work-up diagnostic evaluation, so their diagnosis at the time of interview was unknown. Hence it is unlikely that case subjects overestimated their exposure to maté. Furthermore the association of maté with oesophageal cancer is not widely known. Hence if misclassification did occur, it is likely to be nondifferential with regard to case-control status. Thus the reported risk estimates might be an underestimation of the real underlying effect of maté exposure.

Controls with cancer or with alcohol- and tobacco-related problems were excluded. However approximately 30% of the controls had ill-defined conditions and it may have been possible that some of the controls in this category may have had other cancers or conditions related to smoking and alcohol drinking. The risk estimates for maté, alcohol and tobacco smoking did increase slightly when this group of controls was removed from the analysis indicating that some misclassification might have occurred. It is important to note that the extent to which confounding can be controlled for will depend on the accuracy of the data. Non-differential misclassification of exposure to either alcohol or tobacco will lead to an underestimation of the effect of these factors, hence the association is likely to persist even after adjustment because of residual confounding. Furthermore we must also recognise that confounding from dietary factors has not been taken into account.

4.4. Approaches to prevention of SCC of the oesophagus

Firstly, results of this research need to be circulated to the scientific community, Uruguayan population and policy makers. In view of this study and in keeping with the results from previously published literature, it is clear that maté consumption, alcohol drinking, tobacco smoking and consumption of diets low in fruits and vegetables and rich in red meat is positively associated with OC in this population. Therefore, in view of the exposures described, one approach to minimizing the incidence of OC is through primary prevention.

Primary prevention strategies can be developed through educational programs necessary to inform populations at risk of the major role of the above risk factors in development of the disease. While it is difficult to change lifestyle behaviour in the adult population, it would be best to target adolescence/teenagers and advise them on leading healthier lifestyles, including avoidance of alcohol and tobacco use. This could be done through the teaching curriculum in schools and publication of educational brochures.

Concerning secondary prevention, one approach can be early detection campaigns using abrasive cytology. These have been carried out mainly in the high-risk areas of China although with little success, probably due to the low coverage of the screening programme (Muñoz and Castellsagué, 1994). Secondary prevention can ensure that the lesions are detected and treated in the early stages of the disease. This campaign needs to have a high coverage of high-risk groups and this can be done through media advertising (radio and television interviews by health experts).

Chemoprevention, to address factors associated with the aetiology and progression of the disease, is another viable approach that may have special relevance in high incidence areas of the world where carcinogen exposure is quite high. Animal models provide an excellent opportunity to evaluate chemoprevention strategies against cancer.

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