Squamous cell carcinoma of the lip and the oral cavity

An epidemiological study

A. Jovanovic

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An epidemiological study

VRIJE UNIVERSITEIT

SQUAMOUS CELL CARCINOMA OF THE LIP AND THE ORAL CAVITY

An epidemiological study

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geboren te Isny, Duitsland.

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Referent : prof.dr C. Scully

Aan Heleen en mijn ouders

The study described in this thesis was performed in the Departments of Oral and Maxillofacial Surgery / Oral Pathology and Otorhinolaryngology / Head and Neck Surgery, Free University Hospital / Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, the Netherlands.

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Chapter 1

GENERAL INTRODUCTION

INTRODUCTION

Cancer of the lip and the oral cavity accounts for about 1-2% of all kinds of cancer in the Netherlands.[1] Over 90% of these malignancies are squamous cell carcinomas (SCC). According to the Netherlands Cancer Registry about 550 cases of SCC of the lip and the oral cavity are registered annually.[2] Although SCC of the oral cavity comprises only a small percentage of all new cancers, the profound morbidity and rather poor survival rates add to its importance.

The incidence of SCC of the lip and the oral cavity is strongly related to the environmental exposure to carcinogens, such as the use of tobacco and alcohol.[3,4] Other factors such as sun exposure,[5] nutrition,[6] occupation,[7] dental status,[8] and (viral) infection [9] may also play a role in the carcinogenesis of SCC of the lip and the oral cavity. The possible endogenous factors in the etiology of SCC have recently been emphasized.[11] The fact that not all smokers and alcohol drinkers develop cancer of the oral cavity points to an individual susceptibility to these carcinogens. With regard to the endogenous factors, the mutagen sensitivity, presumably the result of a DNA repair deficiency, might be associated with an increased risk of developing malignancies which are related to the use of tobacco and/or alcohol.[11]

Anatomy of the lip and the oral cavity

The lip and the oral cavity extend from the skin-vermilion border of the lips to the junction of the hard and soft palate above and to the line of circumvallate papillae of the tongue below. They are divided according to the International Union Against Cancer (UICC) [12] into specific sites and subsites which are presented in Table 1. Although the anatomical sites are well defined, the oropharynx,[13] the major salivary glands and even the nasopharynx [14] are often included in reports on "oral cancer". Throughout this thesis the definitions according to the criteria of the UICC are being used (Table 1).

Registration of cancer of the lip and the oral cavity

In 1976 the World Health Organisation (WHO) introduced the International Classification of Diseases for Oncology (ICD-O),[15] in which both the primary site of the tumor and the histological type of the tumor are coded. According to this system the sites and subsites are specified by using three and four digit codes, respectively (Table 1). Although, for example, the base of tongue and the anterior two-thirds of the tongue have the same three digit code (i.e. 141), the former belongs to the oropharynx, whereas the latter belongs to the oral cavity. Recently, a revised version has been published in which the base of tongue has been given a different

three digit code than the anterior two-thirds of the tongue.[16] Regional and national cancer incidence studies have often been expressed by three digit codes. Due to the inclusion of some oropharyngeal subsites within the codes of the oral cavity (such as the base of tongue, soft palate and uvula), the incidence rates of cancer of the oral cavity are not easily accessible.

A (large) tumor may overlap the boundaries of two or more (sub)sites. This might aggravate the definition of the origin of the tumor. In general, the midpoint or the bulk of the tumor is being defined as the origin.[17] If no point of origin can be established, the WHO recommends to classify it to the subcategory "overlapping site".[15]

Table 1. Anatomical sites and subsites of the lip and the oral cavity.[12]

Lip
- Upper lip, vermilion surface (140.0)
- Lower lip, vermilion surface (140.1)
- Commissures (140.6)
Oral cavity
- Buccal mucosa
- Mucosal surfaces of upper and lower lip (140.3,4)
- Mucosal surfaces of cheeks (145.0)
- Retromolar areas (145.6)
- Bucco-alveolar sulci, upper and lower (145.1)
- Upper alveolus and gingiva (143.0)
- Lower alveolus and gingiva (143.1)
- Hard palate (145.2)
- Tongue
- Dorsal surface and lateral borders anterior to vallate papillae (anterior two-thirds) (141.1,2)
- Inferior surface (141.3)
- Floor of mouth (144)

The numbers in parenthesis refer to the codes of the International Classification of Diseases for Oncology, ICD-O, issued by the WHO in 1976.[15]

The extension and the spread of the disease are defined by the TNM classification system according to the UICC.[12] The classification system is based on the extension of the primary tumor (T), the absence or presence and the extent of regional lymph node metastasis (N) and the absence or presence of distant metastasis (M). The T classification according to the UICC is shown in Table 2.

Table 2. T classification according to the International Union Against Cancer (UICC).[12,18,22]

Tx	Primary tumor cannot be assessed
T1	Tumor 2 cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension
T4	*Lip*: Tumor invades adjacent structures, e.g. through cortical bone, tongue, skin of neck *Oral cavity*: Tumor invades adjacent structures, e.g. through cortical bone, into deep (extrinsic) muscle of tongue, maxillary sinus, skin of neck

The latest edition of the TNM classification, published in 1992, is unchanged with regard to the lip and the oral cavity.[18] The TNM classification is used to make international studies accessible for comparison and to predict prognosis. Several proposals have been made to improve the value of the grading system as a prognostic tool.[19,20] In the United States, the American Joint Committee on Cancer (AJCC) has had its own TNM classification, which is identical to that of the UICC since 1987.[21]

EPIDEMIOLOGY

Global variation of cancer incidence rates of the lip and the oral cavity

The incidence rates of cancer for 166 population groups in 50 countries have been published by the International Agency for Research on Cancer (IARC) in "Cancer Incidence in Five Continents, volume VI".[23] The annual incidence rates are expressed per 100.000 and based on the period 1983-1987. The SEER (Surveillance, Epidemiology, and End Results) represents about 10% of the United States population. To outline the geographic variation the incidence rates of cancer of the lip and the oral cavity are shown in Figures 1 and 2, respectively. In Figure 1 it is shown that habitants from Southern Australia, Granada (Spain) and Newfoundland (Canada) have the highest risk of developing lip cancer, which can be explained by the fair-skinned races and the high occurrence of out-door professions, especially in men.[24] High incidence rates of cancer of the oral cavity are seen in parts of France and India (Figure 2). In India this can be explained by local habits, such as the use of smokeless tobacco and reverse smoking (i.e. with the burning end inside the mouth), whereas in France a high consumption of tobacco and alcohol mainly contribute to the high incidence rates.

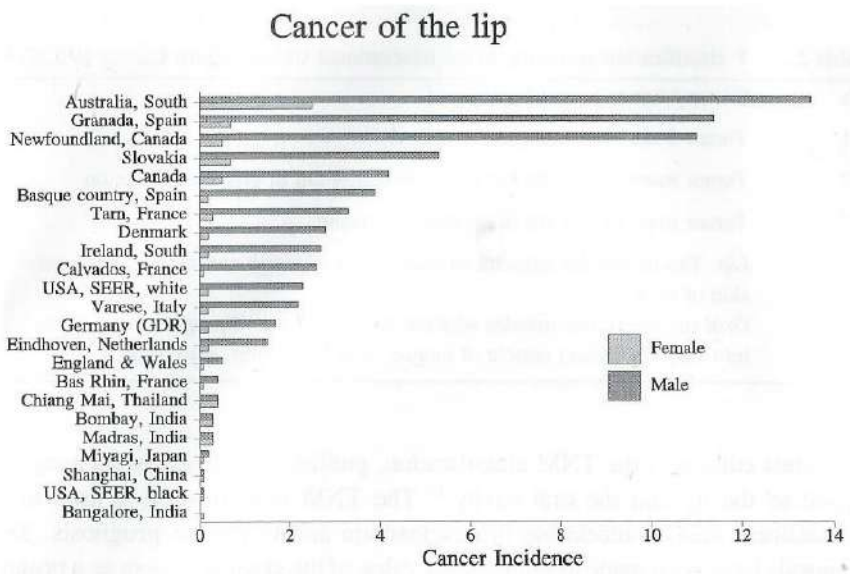

Figure 1. Worldwide variation of age-adjusted incidence rates per 100.000 for cancer of the lip, ICD-O codes 140.[23]

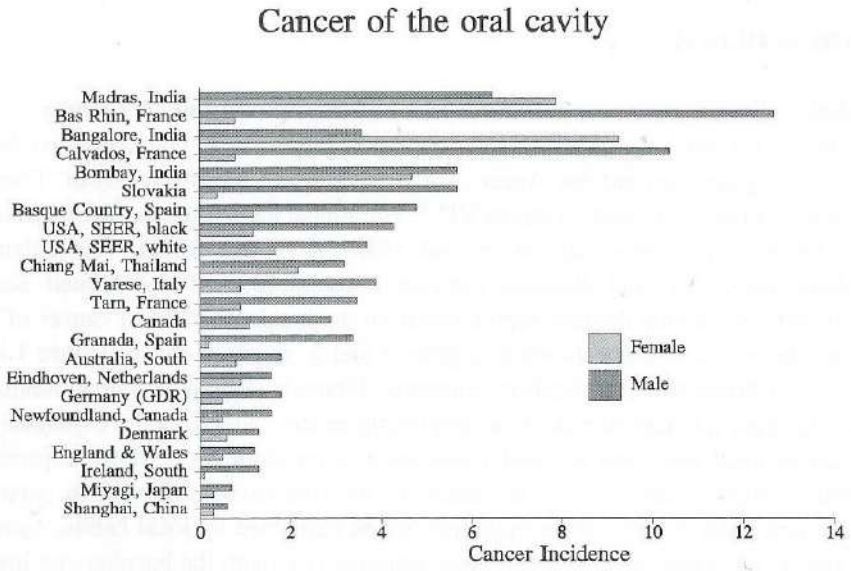

Figure 2. Worldwide variation of age-adjusted incidence rates per 100.000 for cancer of the oral cavity, ICD-O code 141.1-4, 143, 144, 145.0,1,6.[23]

Cancer incidence rates of the lip and oral cavity in the Netherlands

The regional Eindhoven Cancer Registry, which covers almost 7% of the Dutch population, has recorded all forms of cancer since 1955. In the last decade, preparations were made to develop a national cancer registry system. In 1992 these efforts resulted in the first report of the Netherlands Cancer Registry on the cancer incidence rate of 1989.[1] In the Netherlands, cancer of the lip and the oral cavity is, compared to all cancers, relatively rare. Annually about 600 patients with cancer of the lip and the oral cavity are registered, whereas over 8000 patients with lung cancer are diagnosed.[2] The majority of the oral cancers is located in the lower lip, the tongue and the floor of mouth (Table 3). Ninety percent of the cancers of the lip and the oral cavity is a histopathologically proven SCC. The remaining tumors consist of salivary gland tumors, sarcomas, non-Hodgkin's lymphomas, melanomas and other types.

Table 3. Mean annual numbers of SCC of the lip and the oral cavity in the Netherlands (1989-1990).[2]

Anatomical site	ICD-O code 1976	Male (N)	Female (N)
Upper lip	140.0,3	10	11
Lower lip	140.1,4	159	21
Commissure	140.6	1	-
Cheek mucosa	145.0	14	11
Retromolar area	145.6	22	7
Bucco-alveolar sulcus	145.1	4	2
Upper alveolar ridge	143.0	4	4
Lower alveolar ridge	143.1	19	16
Hard palate	145.2	2	5
Tongue	141.1-3	72	57
Floor of mouth	144	87	39
Lip and oral cavity	-	394	173

Age

Far the majority of SCC of the lip and the oral cavity is related to the use of tobacco and alcohol.[25,26] In the developed countries, the occurrence of SCC of the lip and the oral cavity points to a gradual rise with advancing age with a peak in the seventh decade.[13,27-31] As in developing countries proportionally fewer people reach the age of fifty and sixty, the onset of oral SCC is relatively at a younger age.[14,32,33] Among patients with a SCC of the oral cavity are the patients with a history of cigarette

smoking in average 10 years younger compared to the patients without a history of smoking.[34-36] Lately, several reports have been published in which the population of patients seems to get younger.[37,38] In the United States this is explained, by the fact that the use of smokeless tobacco has become more popular among young men.[39]

Gender

In general, SCC of the lip and the oral cavity affects more men than women. However, due to the increase in tobacco and alcohol consumption among women since World War II, the male-to-female ratio declined.[31,35,40,41] In India, however, the incidence rate of cancer of the oral cavity for females is larger than or equal to that for men (Figure 2), since smokeless tobacco and (reverse) smoking are also very common among women.[42] In the recent literature the male-to-female ratio varies from 1.2 to 3.8.[13,27,30,31,43] SCC of the lip is mainly seen in males. The out-door profession among men and the protective function of lipstick among women are considered to contribute to this high male-to-female ratio.[24,44]

Site

The distribution of oral SCC in the various subsites of the oral cavity may vary with geographic latitude, race, or habits. Environmental carcinogens, actinic exposure, and local habits probably all contribute to the site distribution pattern of the disease for any given population. Involvement of the lip, for instance, is relatively rare among blacks,[14] whereas the lip is more often involved among fair-skinned races, particularly the ones living in rural areas, such as parts of Australia and Spain (Figure 1).[5,45]

The use of smokeless tobacco, being very common in South East Asia,[46] and nowadays also in parts of the western world, is related to SCC of the buccal mucosa (where the smokeless tobacco is usually held).[47] In the Netherlands this habit is quite rare.

Mashberg et al. suggested that all oral carcinomas related to the use of tobacco and alcohol, primarily arise from either the floor of mouth, the soft palate-anterior pillar-retromolar complex, or the ventrolateral portion of the tongue.[17,48] The absence of keratin in these sites is thought to result in a higher susceptibility to carcinogens than the keratinized sites, such as the hard palate, the dorsal surface of the tongue and the gingiva. In addition, the local effect of carcinogens can be enforced through the fact that saliva dissolves carcinogens. This solution acts as a reservoir in the floor of mouth, resulting in a prolonged contact with the mucosa of the floor of mouth.[49]

ETIOLOGY

Tobacco

Both the use of tobacco and the use of alcohol have been accepted as independent risk factors in cancer of the oral cavity.[3,4]

Besides smoking cigarettes, a variety of other forms of using tobacco, such as smoking cigar or pipe, reverse smoking and the use of smokeless tobacco, is associated with SCC of the lip and the oral cavity.[26,50-55] Tobacco-specific nitrosamines are thought to play a major role in the etiology.[56] The risk of developing lip cancer may be higher in pipe smokers than in cigarette smokers.[44] In developing countries, especially in countries in south east Asia, such as India, reverse smoking and the use of smokeless tobacco are very common.[46] Although the composition of smokeless tobacco varies regionally, areca nut, lime and tobacco flakes are usually one of the ingredients. The product is kept in the mouth in contact with the buccal mucosa or is chewed on. Recently, the use of new types of smokeless tobacco, such as chewing tobacco and snuff, has grown in popularity among young people in the Western World.[39,58] However, in 1992 it was decided to prohibit the sale of these types of oral tobacco in the countries of the European Community.[57] The association between the various aspects of cigarette smoking and oral cancer, such as the average consumption, the duration of exposure, quitting smoking, the use of filter cigarettes and the color of the tobacco are continuously studied.[3] The results of these studies with respect to the use of filter cigarettes, the concentration of tar and black tobacco as compared with blond tobacco are not consistent.[59,60] However, it is accepted that quitting smoking is associated with a sharply reduced risk of these cancers, with no higher risk detected among those who have quit for 10 years or more.[52,61] Furthermore, an increasing relative risk is accompanied by an increasing intensity and prolongation of habits.[51,61] Despite the known carcinogenic effect of cigarettes and the doctor's advice to stop smoking, by far not all patients with oral cancer quit smoking. Spitz and coworkers reported that in their study 66% of the patients with oral cancer continued to smoke. Patients of older age, with a college education and light smoking habits quit smoking more readily.[62]

Alcohol

With regard to the carcinogenic effect of alcohol, both cohort studies and case-control studies have demonstrated that drinking alcohol is a risk factor for developing oral SCC.[4] Several reports have been published on the risk of different types of alcohol, such as hard liquor, wine and beer, related to the relative risk of developing SCC of the oral cavity.[61,63-65] No consistent findings have been found, however.

Tobacco and alcohol

There is convincing evidence that a synergistic effect is present when both tobacco and alcohol are used. This means that the combined effect of the use of tobacco and alcohol is greater than the sum of the two effects independently.[53,61,66,67] The theory of the synergistic effect of the use of tobacco and alcohol in oral SCC is explained by the fact that the oxidation of ethanol by the contacted epithelial cells creates a more favorable environment for metabolic activation of procarcinogens such as nitrosamines.[68]

Occupation

With respect to occupational hazards, the long time-lapse between the exposure to the carcinogen and the actual occurrence of cancer on the one hand and the change of job on the other hand makes a reliable risk analysis very difficult. It is stated that in general "blue collar" workers, who often inhale chemical agents run a higher risk of developing cancer.[7,69]

Nutrition

There are several epidemiological indicators that nutritional factors, and especially dietary deficiencies, are associated with the risk of developing oral cancer. A statistically significant inverse association is found between oral cancer and the consumption of fruit and/or vegetables.[6,70-73] In other words, these products have a protective effect. Since animal studies showed that the administration of vitamins, such as vitamin A and its precursors, can inhibit and regress chemically induced oral cancer,[74,75] clinical chemoprevention trials have started for patients with premalignant oral lesions and for patients treated for oral SCC.[76-78]

Dental status, oral hygiene and mouth washes

In general, the dental status is reflected by oral hygiene and missing teeth. Several reports have demonstrated that poor dentition as well as poor oral hygiene are risk factors for oral cancer, independently of the known risks of smoking and alcohol drinking.[8,50,53,79] On the other hand, denture wearing does not per se increase the risk of developing oral cancer.[8] The use of mouthwash has also been an issue of interest as a possible risk factor of oral cancer.[64,80,81] As only a mouthwash of a high alcohol content seems to be associated with an increased risk, it appears that the effect of alcohol in a mouthwash is similar to alcohol in drinks.[81]

Host factors

The fact that only a minority of heavy smokers and drinkers develop cancer of the oral cavity points to a - possibly endogenous - individual susceptibility to these

carcinogens.[82] Several of such endogenous factors that may play a role in the etiology of oral cancer, such as HLA antigens, have been reported.[83] With regard to the endogenous factors, the mutagen sensitivity, presumably the result of a DNA repair deficiency, might be associated with an increased risk of developing malignancies.[10] Schantz et al. reported that patients with a decreased DNA repair capacity, indeed, experienced over four times the risk of developing a malignancy, after controlling the use of tobacco and alcohol, compared to patients with a normal DNA repair capacity.[11]

Viral infections

Increasing evidence suggests that viral infections contribute to the development of oral cancer. Human papilloma virus (HPV) infection, particularly infection with HPV genotypes 16 and 18, has been associated with SCC of the oral cavity.[9,84,85] Infection with the herpes simplex virus is reported in association with SCC of the lip.[86,87] The role of human immunodeficiency virus (HIV) in the etiology of oral cancer is not quite clear. Although Silverman et al. [88] suggested such an association, this has only occasionally been reported.[89]

Precancerous lesions

According to the WHO a precancerous lesion is defined as "a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart".[90] According to the present point of view, leukoplakia and erythroplakia are considered precancerous lesions.

Leukoplakia is defined as a white patch that cannot be removed by rubbing and that cannot be characterized as any other disease and which is not associated with any physical or chemical causative agent except with the use of tobacco.[91] The average rate of malignant transformation is 6%.[92-95] The malignant potential is related to the clinical aspect, the site and the histopathological findings. Patients with non-homogeneous leukoplakia are more liable to develop SCC than patients with homogeneous leukoplakia.[92] The tongue and the floor of mouth are high risk sites.[92,96] The presence of epithelial dysplasia on histopathological examination is also a high risk factor.

Erythroplakia is a red lesion and is defined analogous to leukoplakia. Its malignant potential, however, is much higher. Microscopically, erythroplakia is often associated with epithelial dysplasia, carcinoma in situ or even SCC.[97]

With respect to the role of oral lichen planus in the etiology of oral cancer, there is still no consensus. Reports have been published that suggest that patients with oral lichen planus have an increased risk of developing oral cancer,[98-100] others, however, do not support this theory.[101,102]

Finally, it is stated that the most precancerous condition in oral cancer is oral cancer itself.[103] The percentages of second primary tumors after oral SCC vary from 10 to 27%.[104-109] Most likely due to risk factors such as the use of tobacco and alcohol, a great majority of the second primary tumors occurs in the respiratory and upper digestive tract (RUDT) [107,108,110,111] with high occurrence rates in the oral cavity itself.[105,108,110,111] Traditionally, this was explained by the susceptibility of the epithelium of the oral cavity - field cancerization [112] - to carcinogens such as tobacco and alcohol. Recently, however, experimental evidence for this concept has been found.[113] Nowadays, it is well accepted, that the whole RUDT is susceptible to common exogenous influences.[114] The development of second malignancies remains the most obvious expression of field cancerization.

3. AIM OF STUDY

The present study is focused on the epidemiology of SCC of the lip and the oral cavity. In this respect little is known about the situation in the Netherlands.[115-117] The study comprises a retrospective analysis of a selected population with SCC of the lip and oral cavity.

As cure rates are highly associated with local and regional extension of the tumor,[118] early detection and adequate referral is of vital importance. In chapter 2 the referral pattern of patients with oral mucosal lesions is described, while in chapter 3 the patients' and doctors' delay of patients with SCC of the oral cavity is discussed.

All new patients with a malignancy of the lip and the oral cavity entered the registration system, developed at the Department of Oral & Maxillofacial Surgery and Oral Pathology at the Free University Hospital, Amsterdam. Apart from the registration form at the time of diagnosis, follow-up forms have been used at every event or at least annually. Patients with SCC of the lip and the oral cavity, diagnosed in the period between January 1, 1971 and January 1, 1991 were used for the current analysis. The epidemiologic data of 740 patients with SCC of the lip and the oral cavity, derived from this data-bank, are outlined in chapter 4.

Tobacco and alcohol are regarded as the most important etiological factors in the development of oral SCC.[3,4] Little is known, however, whether these agents are equally associated with the various (sub)sites of oral cancer.[26,61,119] In chapter 5 it is evaluated whether or not the role of tobacco smoking and alcohol drinking as a determinant of the risk of oral SCC varies by the anatomical site of the tumor.

In chapter 6 the expression of the tumor suppressor protein p53 in relation to the tobacco and alcohol habits in patients with SCC of the tongue has been examined.

In chapter 7 the possible genetic factors in the etiology of head and neck SCC are discussed. For this purpose a study has been undertaken in which the occurrence of cancer in parents and siblings of the patients and their spouses is analyzed.

In chapter 8 it was investigated, whether the incidence of second primary tumors in the respiratory and upper digestive tract following SCC of the lip and the oral cavity is related to 1) the anatomical site of the index tumor, and 2) tobacco and alcohol habits.

Finally, in chapter 9 the risk of an additional primary tumor after SCC of the lip and the oral cavity is compared to the risk of a malignancy in the general population.

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Chapter 2

REFERRAL PATTERN OF PATIENTS WITH ORAL MUCOSAL LESIONS

This chapter is based on the following publication:
Jovanovic A, Schulten EAJM, van der Waal I. Referral pattern of patients with oral mucosal lesions in the Netherlands. Community Dent Oral Epidemiol 1992, 20, 94-6.

INTRODUCTION

In the Dutch health care system referral to a specialist can only take place through a dentist or a general practitioner (GP). Studies from the literature indicate that patients with oral squamous cell carcinoma tend to consult the GP more frequently as the first source of information than the dentist.[1-4] With regard to benign lesions of the oral mucosa, no comparable reports are available.

In the present study, the referral pattern of a group of 140 patients with lesions of the oral mucosa, either benign or malignant, has been analyzed. The aim of this study was to obtain a better understanding of the referral pattern of patients with oral lesions in order to be able to take the appropriate steps in the diagnostic education of the medical and dental profession in the Netherlands.

MATERIAL AND METHODS

During a six-month period on one arbitrary chosen day a week a group of 530 consecutive patients, referred to the out-patients' clinic of the Department of Oral & Maxillofacial Surgery and Oral Pathology of the Free University Hospital in Amsterdam, has been examined. Of this group, 140 patients had been referred because of a disorder of the oral mucosa. The majority of the remaining 390 patients had been referred for dento-alveolar surgery, such as an extraction/ surgical removal of third molars and apicoectomy. The group of 140 patients was the scope of this study. The clinical and demographic data of this group have been summarized in Table 1. Over 90% of the patients were Dutch natives.

Table 1. Clinical and demographic data of 140 patients, correlated with the initially consulted health care worker.

	Dentist	GP
Total	71	69*
Male-to-female ratio	0.68:1	0.69:1
Mean age and range (in years)	49.3 (10-83)	52.7 (19-83)
Dentition: (partial) dentulous	53	38
edentulous	18	31 **

* Twenty patients did not have a dentist

** Significant for $p < 0,05$ (chi-square test)

The patient was asked whether he or she had visited the dentist or the GP as the first source of help or information. Inquiries were made with regard to the further referral pattern, including all possible consultations with other health care workers that might

have taken place before the consultation at our department. The information given by the patient was presumed to be correct.
An attempt has been made to correlate the referral pattern and the type of the various oral lesions. A registration form was used to collect all the variables. The registration form was a modified version of a form used in a previous study.[5]
The final diagnosis of the mucosal lesions was made by at least two of the authors. This diagnosis was based on the clinical criteria described by Axéll [6] and on the histopathological findings, if applicable. If no conclusive diagnosis could be made, the term "unclassified" was used.

Statistical analysis

The chi-square test was used for the statistical analysis of the present data. The results were considered to be of statistical significance if the p-value was less than 0.05.

Table 2. Outline of the specialists who had been consulted immediately after the first visit to the dentist or GP.

	Dentist (n=71)	GP (n=69)
Oral and maxillofacial surgeon	68	36
Dermatologist	-	19
Otorhinolaryngologist	-	6
General surgeon	-	3
Internist	-	2
Neurologist	-	1
Dentist	-	2
GP	3	-

RESULTS

In Table 2 an outline of the specialists initially consulted after the first visit to the dentist or the GP is presented. The dentist as well as the GP predominantly stayed within their dental and medical profession, respectively.
In Table 3 it is shown whether the dentist or the GP was consulted first for various oral disorders. It appeared that 20 cases of oral mucosal lesions referred by the dentist had been coincidentally detected during a routine dental check-up.
Patients with fibro-epithelial disorders consulted the dentist as the first source of help significantly more often than the GP. On the other hand, the GP was more frequently consulted as the first health care worker in case of squamous cell carcinoma (SCC).
Table 4 shows the distribution of the particular oral mucosal lesions referred by the GP to the various consultants. In nine cases other specialists, up to three in two

cases, were consulted in the period between the first consulted medical specialist and the referral to our department (six with glossodynia, two with aphthae and one with an unclassified lesion). Only three patients had to be referred from our department to another clinic for treatment (i.e. one patient with Langerhans' cell granulomatosis and two with non-Hodgkin's lymphoma).

Table 3. Outline of oral mucosal disorders correlated with health care worker initially visited, either the dentist or the GP.

	Dentist (n=71)	GP (n=69)
Lichen planus	12	10
Glossodynia	6	11
Fibro-epithelial disorder	11	3*
Squamous cell carcinoma	2	10*
Leukoplakia	7	5
Disorder of the minor salivary glands	6	5
Aphthae	1	5
Pigmentation	5	1
Others**	15	14
Unclassified	6	5

* Significant for $p < 0,05$ (chi-square test)
** Not more than two patients for each disorder

DISCUSSION

The results of this study show that in case of oral mucosal lesions, other than fibro-epithelial disorder and SCC, the dentist and the GP are equally often consulted as the first source of help. Edentulous patients consulted the GP significantly more often than the dentist. This can be explained by the fact that edentulous people do not regularly visit the dental office.[7] Of the 20 patients mentioned in Table 1 as "having no dentist", 16 were, indeed, edentulous. Furthermore, older and, therefore, often edentulous patients may have a different perception of the dentist's role in the diagnosis and treatment of oral mucosal lesions. And, as older patients visit their GP more often than young patients, they may have more opportunities to raise questions about a concomitant oral disorder.
Patients with fibro-epithelial lesions consulted the dentist first significantly more often than the GP. In contrast, patients with oral SCC were significantly more often seen by the GP first. Possibly, the GP is consulted first in case of symptoms which, in the patient's view, do not seem to be related to the dentition or dentures.
Thus, the patients' reason to consult a dentist or a GP for an oral mucosal lesion

depends on several variables, such as having a dentist, the dental status, dentition/denture-related lesions, and/or age. Analysis of these variables has been made, but no uniform pattern could be found.

Table 4. Outline of the 69 patients with oral mucosal lesions, who have been referred by the GP to the various specialists.

	Oral surgeon	Dermatologist	Otolar.	General surgeon	Internist	Neurologist	Dentist
Lichen planus	7	1	1	1	-	-	-
Glossodynia	3	4	1	-	2	1	-
Fibro-epithelial disorder	3	-	-	-	-	-	-
Squamous cell carcinoma	5	4	1	-	-	-	-
Leukoplakia	3	2	-	-	-	-	-
Disorder of the minor salivary glands	3	-	-	2	-	-	-
Aphthae	2	1	2	-	-	-	-
Pigmentation	-	1	-	-	-	-	-
Others*	8	5	-	-	-	-	1
Unclassified	2	1	1	-	-	-	1

* Not more than two patients for each disorder
Otolar. = Otorhinolaryngologist.

In the Netherlands the medical curriculum contains a very few lectures on the subject of oral diseases. This, however, is not known to the lay. On the other hand, it raises the question why almost half of the patients who first went to the GP, were referred to a medical specialist rather than to a dentist or an oral and maxillofacial surgeon. In fact, in this study only two patients have been referred directly to the dentist. Perhaps most GPs assume that dentists have not been trained adequately in diagnosing and treating the lesions of the oral mucosa. However, in view of the curriculum of Dutch dental schools, dentists in the Netherlands should be able to diagnose most oral mucosal lesions adequately merely based on clinical observation. The fact that dentists are, indeed, trained in the oral examination procedure is further elucidated by their coincidental discovery of 20 oral lesions. It is noteworthy that the dentist had a more direct referral pattern. This can diminish the overall costs of referring and it can decrease the doctors' delay. A further increase of "cross-referring" by the GP to the other profession might accomplish the mentioned decreased doctors' delay. Regarding the referral routes, the dentist and the GP predominantly stayed within their dental and medical profession, respectively.

Almost half of the patients had been referred by the GP. This might be explained by the fact that this study took place in a university clinic and a center for head and neck oncology.
The referral behavior of the dentist and the GP concerning oral mucosal lesions has hardly been studied. The number of visits per health care worker and the duration of time from the initial visit to the eventual referral to our department have not been investigated. This would contribute to the understanding of the behavior of the dentist and the GP and to the objective measuring of the difference in delay between the two routes.
Possibly, the dentist does not often refer patients with, for example, aphthae, because of the familiarity with the diagnosis and the known limitations for treatment. For the GP the diagnosis of such lesions may be more difficult to make, which will probably result in a referral to a specialist. One should anticipate, that the GP would by preference refer a patient with oral lichen planus, a lesion which may also occur in the skin, to the dermatologist. In this study, however, just one out of ten patients with oral lichen planus was referred by the GP (Table 4). In most cases oral lichen planus is probably not recognized as such by the GP.
The several referrals in case of glossodynia and aphthae could be explained by the inconvenience of these disorders and the lack of satisfying treatment which may induce "medical shopping".
As it was not recorded whether the final diagnosis corresponded to the referring diagnosis of the dentist or the GP, it is not possible to comment on the percentage of correct diagnoses by either group of health care workers. As a consequence, the present study is not reliable when it comes to showing whether there is a difference between dentists and GPs as to the quality of their referral behavior with regard to oral mucosal lesions. Furthermore, it is unknown whether the referral pattern and the patient's need for care are the same in large cities and rural areas.
Since this study is based on referred patients, no information is available on the percentage of patients with oral mucosal lesions who have not been referred by dentists and GPs. In fact, the patients with such lesions may be dealt with by those health care workers without the additional help of consulted specialists.

CONCLUSIONS

This paper indicates that there is no uniform referral pattern in the Netherlands for patients with oral mucosal lesions referred to a university out-patients' clinic. Apparently, the dentist and the GP are equally often consulted by patients as the first source of help. This finding stresses the need for incorporating sufficient education of oral diseases in the medical curriculum.

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Chapter 3

DELAY IN DIAGNOSIS OF ORAL SQUAMOUS CELL CARCINOMA

This chapter is based on the following publication:
Jovanovic A, Kostense PJ, Schulten EAJM, Snow GB, van der Waal I. Delay in diagnosis of oral squamous cell carcinoma; a report from the Netherlands. Oral Oncology, Eur J Cancer 1992, 28B, 37-8.

INTRODUCTION

In the Netherlands, the majority of patients with oral cancer is treated in specialized centers. Departments of Otolaryngology, Oral and Maxillofacial Surgery, and Radiotherapy usually are involved in the treatment of such patients. The referral to such a center can either be made by a general practitioner (GP), a dentist or a specialist. The purpose of this study was to evaluate the patients' and doctors' delay of 50 patients suffering from a squamous cell carcinoma (SCC) of the oral cavity. The delay has been related to the referral pattern and patient variables, such as age, gender, dental status, and tumor size and site.

MATERIAL AND METHODS

Population of study

In the period June 1990 to July 1991, fifty consecutive patients with a primary SCC of the oral cavity have been examined. The patients were referred to the Department of Oral & Maxillofacial Surgery and Oral Pathology of the Free University Hospital in Amsterdam, the Netherlands. The patients had been asked whether they had initially sought advice from a dentist or a GP. Furthermore, the referral pattern was followed until the visit to this department. The population of study consisted of 32 male and 18 female patients. The mean age was 62 years (range 23 - 78 years). The dental status of the patients was divided in edentulous (n= 29) and (partial) dentulous (n= 21). All but two patients were Dutch natives. Twenty-five patients did not regularly visit the dentist. The T-stage of the SCC has been staged according to the TNM classification.[1]

In seven of the 50 patients studied, an asymptomatic lesion was detected by the dentist at a routine dental examination and consequently referred to the department. After clinical and histo-pathological examination these lesions proved to be SCC (three T1, three T2 and one T3 carcinoma). The oral and maxillofacial surgeon detected SCC in two other patients, who had been referred for extraction of one or more teeth (a T2 and T4 carcinoma, respectively). These nine patients have been excluded from the further analysis.

For the purpose of the study the patients' delay was defined as the time period between the point of noticing a discomfort in the mouth and the first visit, mostly to the dentist or the GP. Doctors' delay was defined as the time period which elapsed from the first consultation until the final diagnosis. The patients' delay and doctors' delay together was defined as overall delay.

Table 1. Patients' delay of 41 patients with oral squamous cell carcinoma.

	0-4 weeks	5-16 weeks	>16 weeks
Number of patients	19	12	10

Table 2. Doctors' delay of 41 patients with oral squamous cell carcinoma correlated with the professional source first consulted.

	0-4 weeks	5-16 weeks	>16 weeks
Dentist	8	3	1
GP	22	4	1
Specialist *	1	1	-

* Two patients consulted the dermatologist to whom they were familiar directly because of unrelated skin lesions.

Statistical analysis

Fisher's exact test for the statistical analysis has been used in case of a fourfold table. In case the columns of a 2xk-contingency table represent an ordinal classification, the test for trend in proportions has been used.[2] The results were considered to be of statistical significance, if the p-value was less than 0.05.

RESULTS

Patients with oral SCC consulted the GP more often than the dentist (27 vs.12). The patients' and doctors' delay have been summarized in Tables 1 and 2, respectively. The patients' delay varied from one week to two years, with a mean of 103 days and a median of 35 days. The doctors' delay ranged from one day to six months with a mean of 22 days and a median of 11 days. The overall delay ranged from fourteen days to two years, with a mean of 125 days and a median of 46 days.

There was no significant difference in doctors' delay when comparing the initially consulted dentists and GPs. A doctors' delay of more than 5 weeks occurred significantly more often in patients younger than 40 years of age (Table 3). The age, gender, dental status, tumor site and size did not significantly correlate with the overall delay. There was also no significant correlation between the tumor size and the first source of help. However, lesions of the tongue had a significantly smaller size than tumors of the floor of mouth (Table 4).

Table 3. Distribution of 41 patients with oral squamous cell carcinoma by the doctors' delay and age.

	≤40 years	>40 years
0-4 weeks	-	33
5-16 weeks	3	4
>16 weeks	-	1

Table 4. Distribution of 41 patients with oral squamous cell carcinoma by T classification and localization.

	T1	T2	T3	T4
Tongue	8	5	-	1
Floor of mouth	4	2	5	1
Lower alveolar ridge	2	2	2	2
Buccal Mucosa including retromolar area	2	3	1	-
Upper alveolar ridge	-	-	-	1

DISCUSSION

The importance of a regular dental check-up is well demonstrated by the fact that the dentist detected an asymptomatic lesion in seven cases during a routine intraoral examination. Although the referring diagnosis of the dentist is not known, these lesions turned out to be a SCC. However, in two other patients who had been referred by the dentist for extraction of one or more teeth, the oral carcinoma had apparently remained unnoticed to the dentist.

Patients under the age of 40 had a rather long doctors' delay, which is in a way understandable because of the scarcity of oral cancers in young persons. In this study, the mean overall delay is in agreement with the findings in the literature.[3,4,5] The fact that no significant correlation was found between the overall delay and age, gender, size of the tumor and site of the tumor is also in agreement with the results from most other studies.[4,5] Only Mashberg et al. found that patients with T1 cancers had a shorter overall delay than patients with larger lesions.[6]

The overall delay was not significantly correlated with tumor size. This suggests that the difference of tumor size at the time of diagnosis is due to the intrinsic difference in tumor aggressiveness.[4,7] This hypothesis is also suggested for tumors outside the oral cavity.[8,9] Nevertheless, in our study, lesions of the tongue had a significantly

smaller size than tumors of the floor of mouth in spite of the similar overall delay in both groups. The mobility of the tongue may result in earlier symptoms and therefore in smaller tumor size at the time of diagnosis. This theory has been confirmed in a study carried out by Mashberg et al.[6]

The majority of the patients with symptomatic oral carcinoma consulted the GP. first rather than the dentist, which is in agreement with the literature.[3,10,11] Apparently, patients are not aware that dentists are also trained in handling symptoms which do not seem to be related to the dentition or dentures, such as oral mucosal lesions. Furthermore, 50 % of the patients in the present study had no dentist, which also explains the preference for the GP. There is no difference in the size of the tumors between patients visiting the dentist or the ones visiting the GP. This in contrast with previous studies, in which patients consulting the dentist had smaller lesions.[3,7] Probably due to the good accessibility of the oral cavity for examination, the doctors' delay is generally shorter than when it concerns sites that are not directly visible, such as the stomach, where the mean doctors' delay is three months.[12]
Due to the limited number of patients in this study, the outcome of the statistical analysis should be interpreted carefully. Although in some cases no significance could be shown, there might still be differences between the groups compared.

CONCLUSIONS

The majority of the patients with a SCC of the oral cavity consult the GP rather than the dentist. On the other hand, in 14 % of the cases the SCC had been initially detected by the dentist during a routine dental examination. In general, the aim is to improve the detection of cancer in an early stage, which generally results in a better prognosis. Considering the delay in diagnosis in this study, one should especially try to reduce the patients' delay. Therefore, public and professional education must be stimulated. This should eventually result in an improvement of the patients' attitude with regard to oral health. In particular, patients older than fifty years with a history of smoking and alcohol drinking should be stimulated to visit the dentist regularly, as they are known to have a higher risk to develop oral cancer.[13,14]

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Chapter 4

SQUAMOUS CELL CARCINOMA OF THE LIP AND THE ORAL CAVITY: AN EPIDEMIOLOGICAL STUDY OF 740 PATIENTS

This chapter is based on the following publication:
Jovanovic A, Schulten EAJM, Kostense PJ, Snow GB, van der Waal I. Squamous cell carcinoma of the lip and the oral cavity in the Netherlands: an epidemiological study of 740 patients. J Craniomax-Fac Surg 1993, 21, 149-52.

INTRODUCTION

One to two percent of all malignancies in the U.S.A. is located in the oral cavity.[1] Squamous cell carcinoma (SCC) accounts for about 80 % of all oral malignancies. There is, however, a worldwide geographic variation in the incidence of oral cancer. For instance, in Madras, India, the annual age-standardized incidence rate for oral cancer is estimated to be 6.5 and 7.9 per 100.000 for males and females, respectively.[2] In the Netherlands, however, this rate is estimated to be 1.6 and 0,9 per 100.000, respectively.[2]

This paper provides epidemiological information on 740 patients suffering from SCC of the lip and the oral cavity treated in a head and neck oncology center in the Netherlands.

MATERIAL AND METHODS

Population of study

Data were gathered of 740 consecutive patients suffering from a primary SCC of the lip and the oral cavity and who had not been treated before. The primary SCC was diagnosed at the Free University Hospital, Amsterdam, the Netherlands in the period between January 1, 1971 and January 1, 1991. Apart from demographic data such as age and gender, the medical history concerning previous malignancies, the anatomical site of the tumor, and TNM classification were registered, for which standardized forms were used.

In case tumors involved more than one (sub)site, the (sub)site in which the bulk of the tumor was localized, was chosen. Patients' ages were grouped into 10-year-intervals. The sites and subsites of the lip and the oral cavity were defined according to criteria of the International Union Against Cancer (UICC) and the International Classification of Diseases for Oncology (ICD-O).[3,4] Synchronous multiple carcinomas of the lip and the oral cavity were recorded using the criteria provided by Warren and Gates.[5] Regarding the tumor size (T), all tumors were coded according to the recommendations of the latest UICC TNM classification.[4] Tumors of the alveolar ridge and retromolar area were classified as T4 only if gross bone destruction was present, based on clinical and radiographic examination. The N stages were grouped in clinically negative and clinically positive neck node(s).

Statistical analysis

For the statistical analysis chi-square tests were used. In case the columns of a 2xk-contingency table represented an ordinal classification, the test for trend in proportions was used.[6] The results were considered to be of statistical significance if the p-value was less than 0.05.

Table 1. Distribution of 740 patients with SCC of the lip and oral cavity by gender and (sub)site.

(sub)site	ICD-O code [3]	Male	Female	M-F ratio
Lower lip	140.1	50	6	8.3
Upper lip	140.0	1	-	-
Commissure	140.6	1	1	1
Tongue	141.1,2,3	138	108	1.3
Upper alveolar ridge	143.0	10	11	0.9
Lower alveolar ridge	143.1	31	25	1.2
Floor of mouth	144	140	59	2.4
Cheek mucosa	145.0	26	27	1.0
Retromolar area	145.6	60	20	3.0
Hard palate	145.2	8	5	1.6
Multiple tumors of lip and oral cavity	-	8	5	1.6
All (sub)sites	-	473	267	1.8

The numbers in the ICD-O column refer to the codes of the International Classification of Diseases for Oncology, ICD-O.[3]

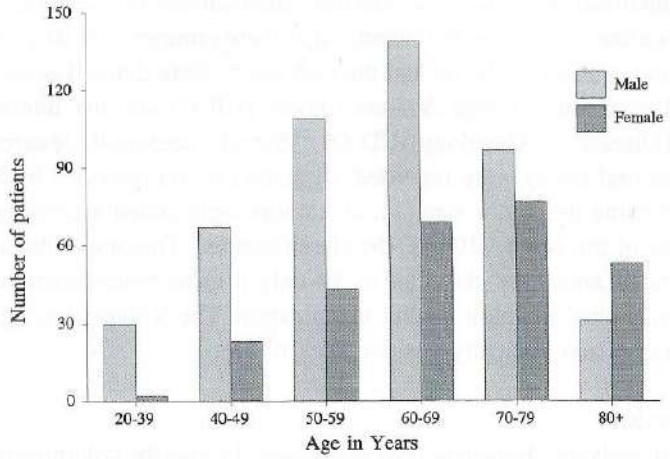

Figure 1. Distribution of 740 patients with SCC of the lip and the oral cavity by age.

RESULTS

The group of 740 patients with SCC of the oral cavity consisted of 473 men and 267 women. The age ranged from 23 to 99 years with a mean age of 63 years and a median of 64 years. The mean age for men was 61 years and for women 68 years, with a median of 62 years and 69 years respectively.

The distribution by gender and age is shown in Figure 1. In this study more men than women were observed, but women dominated the age group of 80 years and older. Far more men than women were involved in the age group below 40 years (95% confidence interval: 79.2- 99.2% male).

The distribution of the oral (sub)sites in relation to gender is given in Table 1. The overall male-to-female ratio was 1.8, with a range from 8.3 for the lower lip to 0.9 for the upper alveolar ridge. Of the 13 patients with multiple oral SCCs, the tongue was affected in ten patients, the lower lip and lower alveolar ridge in four patients, the floor of mouth in three patients, the retromolar area and cheek mucosa in two patients and the hard palate in one patient, respectively.

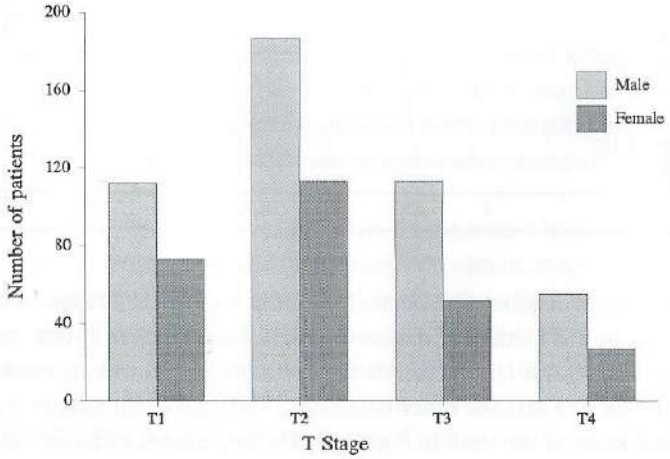

Figure 2. Distribution of 740 patients with SCC of the lip and the oral cavity by T stage and gender.

The distribution by age group and oral (sub)site is shown in Table 2. The peak age for both genders varied, depending on the (sub)site, from the seventh to the eighth decade. Woman's peak age was in general one decade higher except for the floor of mouth, for which both genders had the same peak age of 60 to 69 (not shown). The distribution by size of the tumor and gender is shown in Figure 2. There was no significant association between gender and the size of the tumor. Furthermore, no significant association or trend could be established between the size of the tumor and age.

Table 2. Distribution of 740 patients with SCC of the lip and the oral cavity.

(sub)site	20-	30-	40-	50-	60-	70-	80+	Total
Lower lip	-	1	8	14	15	12	6	56
Upper lip	-	-	-	-	-	1	-	1
Commissure	-	-	-	1	-	1	-	2
Tongue	3	13	33	38	60	61	38	246
Upper alveolar ridge	1	-	-	4	4	8	4	21
Lower alveolar ridge	-	1	4	6	23	16	6	56
Floor of mouth	-	8	34	53	61	34	9	199
Cheek mucosa	-	-	5	7	11	17	13	53
Retromolar area	-	3	6	24	25	18	4	80
Hard palate	-	2	-	2	6	3	-	13
Multiple tumors of lip and oral cavity	-	-	-	3	3	3	4	13
All (sub)sites	4	28	90	152	208	174	84	740

The size of the tumor has been associated with the (sub)sites. It appeared that SCC of the lower lip in a T1 stage is diagnosed significantly more often than SCC in other (sub)sites ($p<0.0001$). No significant associations, however, could be found between the tumor size and the other (sub)sites. The size of the tumor is associated with the clinical stage of the neck in Figure 3. The percentage of a clinically positive neck showed a linear trend in case of an increasing size of the primary tumor ($p<0.0001$).

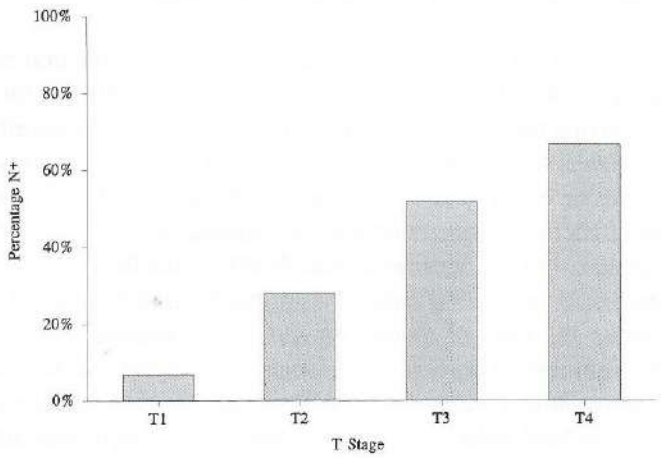

Figure 3. Distribution of 740 patients with SCC of the lip and the oral cavity by T stage and clinically positive neck node(s) (N+).

DISCUSSION

In epidemiological studies the definitions of the anatomical sites of the oral cavity proposed by the UICC,[4] is not always used in a uniform way. In many studies the oropharynx and nasopharynx are also included when reporting on "oral" cancer.[7,8] This diversity in anatomic regions results in a bias when comparing studies reported in the literature.

The male-to-female ratio in this study is 1.8. In the recent literature the ratio varies from 1.2 to 3.8.[8,9] Women appear to develop oral cancer at an older age than men. The mean age of females was 7 years higher than the mean age of males, which is in accordance with most reports in the literature.[7] Furthermore, far more men than women under the age of 40 years were observed in this study, which is confirmed by a study of Cusumano and Persky.[10] The later development of oral SCC in women may be due to the fact that proportionally more women reach the age of 80 and over. In addition, later exposure to carcinogens such as tobacco and alcohol, a lower intensity of the use of tobacco and alcohol or other, yet unknown, factors may also explain this difference.

In the present study, the tongue and the floor of mouth were the most frequently affected sites in the oral cavity, both in men and in women. These results are also in agreement with other reports.[8,11,12] Carcinomas of the lip were seen much more frequently in males. Out-door profession is considered to contribute to this high

male-to-female ratio. Furthermore, the use of lipstick is considered to be protective with regard to the development of lip carcinoma in women.[13]

The distribution of SCC in the various (sub)sites of the lip and the oral cavity may vary with geographic latitude, race, or habits. Involvement of the lip, for instance, is relatively rare among blacks,[14] whereas the lip is a frequently affected site in Israel and Finland.[15,16] Habits such as betel chewing may result in an increased involvement of the commissures or buccal mucosa.[17] However, this habit is very uncommon in the Netherlands. Lederman[18] suggested that the mucosal contact with tobacco and alcohol might explain the high occurrence rate of SCC of the floor of mouth. Mashberg et al.[7,19] suggested that all oral carcinomas primarily arise from non-keratinized mucosa from either the floor of mouth, the soft palate-anterior pillar-retromolar complex, or the ventrolateral portion of the tongue. The absence of keratin in these sites is thought to result in a higher susceptibility to carcinogens than the keratinisized sites, such as the hard palate, the dorsal surface of the tongue and the gingiva. In the present material, SCC of the hard palate, the dorsal surface of the tongue and the gingiva all together occurred, indeed, in less than five percent of the patients. The 13 multiple oral SCCs may have developed due to field cancerization or to multiple leukoplakias associated with SCC.[20,21] No obvious explanation can be found for the fact that the tongue was the most frequently affected site in these cases.
Apart from the association found in the lower lip, no significant association was found between the oral (sub)site and the size of the tumor. A relatively high number of small tumors (T1 and T2 size) of the tongue is reported in the literature. This is explained by the fact that lesions of the tongue are easily detected and perhaps give rise to symptoms in a relatively early clinical stage.[7]

Until 1987, two different TNM systems were used, one according to the UICC [22] and the other according to the American Joint Committee on Cancer.[23] In order to achieve standardization the criteria for classification have become the same in both systems in the latest edition of the TNM system.[4]
It should be mentioned that a T4 category defines no minimal size of the tumor, but merely indicates the destruction of underlying tissues such as the bone, the skin etc. It seems questionable, however, whether a small carcinoma of the alveolar ridge or retromolar area with a superficial, but distinct bone invasion should be staged as T4. As mentioned in the section of material and methods this category has been applied only in case of gross bone destruction.
As expected, clinically positive nodes with an increasing size of the tumor were observed more often, which significantly influences the prognosis.[24]

CONCLUSIONS

This study indicates that the epidemiological data of Dutch patients with SCC of the lip and the oral cavity are similar to data in other parts of the western world. The mean age of the patients was 63 years, whereas female patients were on average seven years older than male patients. Furthermore, most patients under the age of 40 years were men. The mean male-to-female ratio showed to be 1.8. The tongue and the floor of mouth were the most affected sites. No significant association was found between the age or gender of patients compared to the T classification of the tumor. However, a significant association was seen between the localization and the T classification. The size of the lesions of the lower lip was significantly smaller than the size of the tumors in other sites.

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Chapter 5

TOBACCO AND ALCOHOL RELATED TO THE ANATOMICAL SITE OF ORAL SQUAMOUS CELL CARCINOMA

This chapter is based on the following publication:
Jovanovic A, Schulten EAJM, Kostense PJ, Snow GB, van der Waal I. Tobacco and alcohol related to the anatomical site of oral squamous cell carcinoma. J Oral Pathol Med, in press.

INTRODUCTION

Tobacco and alcohol are considered to be the most important etiological factors in the development of squamous cell carcinoma (SCC) of the lip and the oral cavity.[1,2] Little is known about the fact whether these agents are equally associated with the (sub)sites of oral cancer.[3-5]

The aim of the present study was to evaluate, whether the role of tobacco smoking and alcohol drinking as a determinant of the risk of SCC of the lip and the oral cavity varies by the anatomical site of the tumor.

MATERIAL AND METHODS

Population of study

Data were gathered of 740 consecutive patients suffering from a primary SCC of the lip and the oral cavity and who had not been treated before. The SCC was diagnosed at the Free University Hospital, Amsterdam, the Netherlands in the period between January 1, 1971 and January 1, 1991. Details on this population of study have been described elsewhere.[6]

Data such as age and gender, and the anatomical site of the tumor specified according to the International Union Against Cancer (UICC) [7] were registered, using standardized forms. In case tumors involved more than one (sub)site, the (sub)site in which the bulk of the tumor was localized, was chosen.

The use of tobacco and alcohol at the time of the diagnosis of the tumor was registered. The use of tobacco was expressed in cigarettes per day. Smokers were defined in those who smoked 1 to 20 cigarettes per day (moderate smokers) and those who smoked more than 20 cigarettes per day (heavy smokers). One cigar and one pipeful were assumed to be equal to 4 and 2 cigarette equivalents, respectively. None of the patients had a history of using smokeless tobacco. Intake of alcoholic beverages was expressed in units of alcohol per day, assuming that the amount of alcohol in a consumption of hard liquor, wine and beer is equal for all drinks (approximately 10 g of alcohol per unit).[2] Drinkers of alcohol were divided into those who consumed one to four units per day (moderate drinkers) and those who consumed more than four units per day (heavy drinkers). Patients on whom no information on habits was available were excluded from this study, their total number being 50. The remaining 690 patients were used for further analysis. Of these patients, the mean age was 63.2 years (SD $\pm$13.1) and the male-to-female ratio was 1.8.

Statistical analysis

To evaluate the role of alcohol and smoking as possible determinants of SCC in the various oral (sub)sites, a case-case study has been performed. As no reference group of persons without SCC was available as in a case-referent design, relative risks (RRs) for SCC at these (sub)sites could not be directly estimated from this data. However, the odds ratios (ORs) for the association between (sub)sites of the tumor and the putative determinants could be estimated. These ORs can be equated with ratios of RRs. The ORs were only the means to obtain estimates of *ratios* of these RRs of interest. Patients with SCC of the tongue, being the largest subgroup, have been used as the reference group in case ratios of RRs are at issue. For instance, an OR of 5 for patients with smoking habits and SCC of floor of mouth (relative to SCC of the tongue) means, that for smokers the RR of developing SCC of the floor of mouth is five times higher than the RR for SCC of the tongue.

By use of the Mantel-Haenszel method (χ^2-test for stratified 2×2 contingency tables) the OR associated with the use of tobacco and alcohol were adjusted for possible confounding by each other.[8] Dose-response relationships were assessed to be the stratified test for linear trend.[9] Moderate and heavy users of tobacco and alcohol were contrasted with non-users. Furthermore, stratification by age and gender has been performed. The possibility of a different synergistic effect of the use of tobacco and alcohol and the anatomical sites of oral SCC was explored by likelihood ratio tests of uniformity of the OR.

Table 1. Percentage of users of tobacco or alcohol with squamous cell carcinoma of the lip and the oral cavity by gender and (sub)site (N=690).

(sub)sites	N	Users of tobacco (%)		Users of alcohol (%)	
		Male	Female	Male	Female
Floor of mouth	198	95	75	91	61
Retromolar area	79	95	68	83	53
Lower alveolar ridge	53	83	44	83	35
Upper alveolar ridge	20	78	18	56	27
Lower lip	38	78	0	53	0
Tongue	230	75	33	66	29
Cheek mucosa	50	68	8	40	16
Hard palate	10	100	20	80	20
Multiple tumors of lip and oral cavity	12	100	25	75	25
Total	690	85	43	75	37

RESULTS

The distribution of patients with smoking and alcohol habits by gender and (sub)site of the tumor is shown in Table 1. With regard to SCC involving (sub)sites with at least 15 patients, the highest rate of users of tobacco was seen concerning SCC of the floor of mouth and retromolar area, being 95% for both (sub)sites in males, and 75% and 68% in females, respectively. The lowest rate of users of tobacco was noticed concerning SCC of the cheek mucosa, being 68% for males, and 8% for females, respectively. Similar findings apply to users of alcohol. Furthermore, twice as many male as female patients used tobacco and/or alcohol, i.e. 85 % vs. 43% for users of tobacco, and 75% vs. 37% for users of alcohol, respectively.

The mean age of users and non-users is shown in Table 2. The highest mean age in female patients, being 73 years of age, was observed in patients who did not use tobacco and alcohol, while the lowest mean age of 59 years was seen in users of both tobacco and alcohol.

Table 2. Number and mean age of 690 patients with squamous cell carcinoma of the lip and the oral cavity classified by gender and tobacco- and alcohol habits.

		NT and NA	T and NA	NT and A	T and A
Male:	Number	42	69	23	311
	Mean age	63	65	62	59
Female:	Number	127	28	14	76
	Mean age	73	71	71	59

NT = non users of tobacco; NA = non users of alcohol; T = users of tobacco; A = users of alcohol

The ORs for the oral (sub)sites related to the use of tobacco are adjusted for alcohol and presented in Table 3; the patients with SCC of the tongue were used as the reference group. The highest ORs were seen in the retromolar area (OR= 2.92 and 7.46 for 1-20 and >20 cigarettes per day, respectively). The ORs for SCC of the floor of mouth were 2.73 and 6.17, respectively). The lowest ORs were seen in SCC of the cheek mucosa (OR= 0.69 and 0.67, respectively).

The ORs for the oral (sub)sites related to the use of alcohol are adjusted for tobacco and shown in Table 4; the patients with SCC of the tongue were used as the reference group. SCC of the floor of mouth is significantly more associated with alcohol than SCC of the tongue (OR = 2.53 and 3.33 for 1-4 and >4 units of alcohol per day, respectively. SCC of the cheek mucosa on the other hand was associated significantly less with the use of alcohol (OR = 0.51 and 0.47,

respectively). The apparent strong association between the use of alcohol and SCC of the retromolar area (relative to SCC of the tongue) appeared to be largely explained by confounding with smoking (not shown). In addition, no difference in a possible synergistic effect of tobacco and alcohol could be demonstrated between the various (sub)sites of the oral SCC. Adjustment for age and gender hardly affected the results, as no noticeable changes of the ORs for tobacco or alcohol were observed.

Table 3. Dose-specific odds ratios (ORs) for the use of tobacco, adjusted for the use of alcohol, of squamous cell carcinoma (SCC) of the lip and the oral cavity related to various (sub)sites, with patients with SCC of the tongue as reference group.

	Moderate smokers		Heavy smokers		Trend
(sub)sites	OR	95% CI	OR	95% CI	P
Retromolar area	2.92	1.13 - 7.58	7.46	3.01 - 18.52	<0.0001
Floor of mouth	2.73	1.40 - 5.32	6.17	3.11 - 12.20	<0.0001
Lower lip	3.18	1.19 - 8.55	4.81	0.95 - 24.39	0.09
Lower alveolar ridge	0.97	0.40 - 2.39	1.48	0.60 - 3.88	0.47
Tongue	1	-	1	-	-
Cheek mucosa	0.69	0.30 - 1.59	0.67	0.23 - 1.98	0.28

CI: confidence interval

Table 4. Dose-specific odds ratios (ORs) for the use of alcohol, adjusted for the use of tobacco, of squamous cell carcinoma (SCC) of the lip and the oral cavity related to various (sub)sites, with patients with SCC of the tongue as the reference group.

	Moderate drinkers		Heavy drinkers		Trend
(sub)sites	OR	95% CI	OR	95% CI	P
Floor of mouth	2.53	1.15 - 3.66	3.33	1.77 - 6.25	0.0004
Retromolar area	1.16	0.52 - 2.56	2.24	1.08 - 4.65	0.035
Lower alveolar ridge	1.49	0.66 - 3.33	1.74	0.63 - 4.81	0.32
Tongue	1	-	1	-	-
Lower lip	0.60	0.22 - 1.62	0.68	0.25 - 1.85	0.25
Cheek mucosa	0.51	0.21 - 1.23	0.47	0.16 - 1.37	0.045

CI: confidence interval

DISCUSSION

Tobacco and alcohol are considered as the most important etiological factors in the development of oral SCC. As persons often use both tobacco and alcohol, it is difficult to distinguish the separate effects of these agents. Various authors have reported on the synergistic effect of these habits.[4,10] Some authors suggest that the use of tobacco is the major cause and that the use of alcohol is the cofactor,[11] but the opposite has also been reported on.[12,13]

In the Netherlands smoking of cigarettes is by far the most common way to use tobacco (95% of the users of tobacco smoke cigarettes, while the remainder smokes pipe or cigars).[14] Worldwide various other tobacco habits are common; like reverse smoking and using smokeless tobacco with or without lime or betelquid.[15,16] These habits may influence the distribution of SCC in the various (sub)sites of the lip and the oral cavity. Oral SCC in reverse smokers, for instance, has a predilection for the palate and among users of smokeless tobacco the sites of predilection are where the product is kept, such as the lower bucco-alveolar sulcus or gingiva.[15,16] However, these habits are very uncommon in the Netherlands. In the present study, no information was available with regard to the duration and the changes of patients' smoking and alcohol drinking habits. Furthermore, other possible etiological factors and cofactors mentioned in the literature, such as dentition,[17,18] mouthwash,[19] nutrition,[20] viral infections[21] or professional occupation,[22] have not been included in the present study.

Rich and Radden[23] found that patients who had never used tobacco or alcohol developed cancer particularly on the cheek mucosa and upper alveolar ridge. In the current study there is, indeed, a close relation between non-smoking and SCC of the cheek mucosa and, to a lesser extent, of the upper alveolar process. This suggests that smoking is a stronger determinant of SCC of other (sub)sites in the oral cavity than of SCC of the cheek mucosa. Our finding that patients with tobacco and/or alcohol habits were, on average, younger than patients with no such habits is also in accordance with the literature.[23] These findings suggest that the onset of the tumor is related to the habits of the patient.

The present study suggests, that the RR for smokers and alcohol drinkers to develop oral SCC varies by anatomical site. The estimated OR of 7.46 for heavy smokers with SCC of the floor of mouth (which is relative to SCC of the tongue) means that for heavy smokers the RR for the development of SCC of the floor of mouth is about 7.5 times higher than the RR for SCC of the tongue, which in turn is about 1.5 times (1:0.67) higher than the RR for SCC of the cheek mucosa (Table 3). A dose-response relationship can be demonstrated for tobacco smoking as well as for alcohol drinking, which is in accordance with the literature.[3-5] Furthermore, this study suggests that contrasts between RRs observed by the anatomical site of oral SCC are

more pronounced for tobacco smoking than for the use of alcohol.

Other studies have focused on the relation between the anatomical site of oral SCC and the use of tobacco and alcohol.[4,24,25] However, as in these studies broad anatomical sites such as SCC of the tongue vs. remaining oral sites were analyzed, it is difficult to compare these results to the present findings. Blot et al.[4] demonstrated that the relation between smoking and drinking habits were weaker for tongue cancer than for cancer of other sites of the oral cavity. In a recent study the tongue and the floor of mouth were sites which could be compared to our findings.[5] In that study, the RRs of cigarette smoking and alcohol drinking for SCC of the floor of mouth were also higher than for SCC of the tongue, although less evident than in the present study.

The assumed role of the use of tobacco and alcohol in the etiology of oral SCC in its (sub)sites can be divided into local and systemic effects.

With regard to the local effect(s) of the use of tobacco and/or alcohol, several factors may be involved, such as saliva, which may act as a reservoir for the carcinogens,[26] the degree of keratinization[27] and the permeability of the oral mucosa.[28] Furthermore, the possible synergistic effect of the use of tobacco and alcohol may be influenced by the anatomical site of oral SCC.[29] However, this could not be confirmed in the present study. One way to objectify the possible local effect(s) is to analyze the frequency of micronucleated cells at specific (sub)sites, which is a measure for the genotoxic effect.[30]

On the other hand, the use of tobacco and alcohol may have a systemic effect with a preference for specific oral (sub)sites. This hypothesis is derived from animal studies, which showed that systemically supplied tobacco constituents, or so called organ-specific carcinogens, cause cancer in specific sites of the body.[31]

The effects of contact carcinogens (e.g. local effect) have readily been observed and measured, but assessment of organ-specific carcinogens requiring metabolic activation at the target site (e.g. systemic effect) is more difficult to perform and needs further study.

CONCLUSIONS

Although no controls were available for the calculation of the RRs for oral SCC as a whole, it was possible to determine which (sub)sites of the oral cavity were more at risk than other (sub)sites due to the use of tobacco and alcohol. SCC of the floor of mouth and retromolar area were significantly more related to the use of tobacco and/or alcohol than SCC of the tongue and cheek mucosa. Due to these variations of susceptibility for the use of tobacco and alcohol within the oral cavity, one should be aware that in case-control studies of SCC of the oral cavity, the RRs may differ between the various (sub)sites.

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Chapter 6

RELATIONSHIP OF TOBACCO AND ALCOHOL USE TO p53 EXPRESSION IN PATIENTS WITH LINGUAL SQUAMOUS CELL CARCINOMA

This chapter is based on the following publication:
Matthews JB, Scully C, Jovanovic A, van der Waal I, Yeudall WA, Prime SS. Relationship of tobacco/alcohol use to p53 expression in patients with lingual squamous cell carcinomas. Oral Oncology, Eur J Cancer, in press.

INTRODUCTION

Tumor suppressor proteins function as negative regulators of cellular proliferation. Deletion and/or mutation of the p53 gene not only leads to unrestrained cellular growth,[1] but is also common in squamous cell carcinomas (SCCs) of the head and neck[2,3] and is correlated with a history of heavy smoking and drinking.[4,5]

Gene mutations may produce a more stable p53 protein than the wild-type product.[6] This observation has resulted in the use of immunocytochemical techniques to demonstrate abnormally high levels of p53 which, when detected, are assumed to reflect the presence of a mutant product. Positive reactivity has been described in 34% to 100% of SCCs of the head and neck.[2,4,7] Two recent studies, using a monoclonal antibody on frozen sections (PAb 1801[8] or a polyclonal antiserum on paraffin sections (CM1[9]), have suggested that between 35%[8] and 54%[9] of the oral SCCs are p53 positive. The majority of these studies have been carried out using tissues from patients with and without previous treatment and often without recourse to the patients' social history. It is important, therefore, to extend the current observations to a specific group of malignant oral lesions where the risk factors are relatively well-defined. The purpose of the present study was to examine p53 immunoreactivity in lingual SCCs from patients from whom a history of smoking and drinking was known and who had not undergone any previous cancer therapy.

MATERIAL AND METHODS

Patients and Tissues

Formalin-fixed paraffin embedded blocks of tissue from 49 Dutch patients diagnosed as having lingual SCC during the period 1972-1986 were studied. None of the patients had been exposed to previous radiotherapy or chemotherapy, or had had a previous history of malignancy. Data such as age and gender, information concerning habits such as tobacco smoking and alcohol intake, the anatomical site of the tumor and TNM classification were registered on standardized forms. Patients were classified as non-smokers, incidental smokers (not daily), moderate smokers (<20 cigarettes per day) or heavy smokers (>20 cigarettes per day) or equivalent quantities of pipe tobacco. With regard to the use of alcohol patients were similarly classified as non-drinkers, incidental drinkers (not daily), moderate drinkers (1-4 units of alcohol per day) or heavy drinkers (>4 units of alcohol per day). Individuals with a history of smoking tobacco and/or drinking alcohol that had stopped either/both prior to presentation with lingual SCC were not included in the study.

Immunocytochemistry

Staining for p53 was performed using an improved Biotin-Streptavidin immunoperoxidase technique (StrAviGen; Biogenex) and a monoclonal antibody (PAb 1801, Oncogene Science [10]· reactive with a denaturation resistant epitope between amino acids 32 and 79 of normal and mutant p53. This antibody has been used extensively to detect p53 in frozen [7,8,11] and formalin-fixed, paraffin-embedded tissue sections.[4,12] In brief, 5 μm deparaffinized sections were immersed in a buffer in 0.3% hydrogen peroxide for 10 minutes to abolish endogenous peroxidase activity, washed in a buffer and treated at room temperature for 1 hour with 3 μg/ml anti-p53 antibody. After washing, the sections were treated with biotinylated anti-mouse immunoglobulin (BioGenex; 1/100 dilution in a buffer containing 1% normal human serum; 1 hr at room temperature), washed again and then overlaid with peroxidase-labelled streptavidin (BioGenex; 1/100; 1 hr at room temperature). Reaction products were developed by immersing sections in 3,3'-diaminobenzidine reagent (5 min) and subsequently enhanced by treatment with 0.5% (w/v) copper sulphate in 0.01M phosphate-buffered saline (PBS), pH 7.6 for 5 min at room temperature. Stained sections were lightly counterstained in Meyer's hematoxylin and mounted in Xam. All reagent dilutions and washing were performed in PBS.

Negative controls included replacement of the primary layer with normal mouse immunoglobulin (3 and 10 μg/ml), a monoclonal antibody of irrelevant specificity (MRC OX-6; IgG_1, anti-rat I-A; 3 and 10 μg/ml) and phosphate-buffered saline. Adjacent sections of all tissues were also stained for keratin (clone LP34,[13] Dako, 1/200, 1 hr) in order to more clearly define areas of tumor epithelium and to act as a positive 'tissue' control.

Evaluation of Tissue Sections

Nuclei with a clear brown color, regardless of the staining intensity, were regarded as positive for p53. An assessment of the immunostained sections was performed by one of us (JBM) without prior knowledge of the clinical details of the patients.

Statistical Analysis

Data were analyzed using Minitab (ver 8.2). Comparisons between p53-positive and p53-negative groups have been performed by way of the χ^2 test or in case of age differences by way of the Mann Whitney U test.

Table 1. Summary of p53 staining results and patient details.

	N	Mean age(±SD)	Age range	Female(%)	Smoking and drinking habits(%)			
					None	Alcohol only	Tobacco only	Both
p53 +ve	12	62.3 (±14)	38-79	50	33.3	25	16.7	25
p53 - ve	28	56.7 (±12.7)	37-84	36	7.1	17.9	3.6	71.4
All tumors	40	58.4 (±13.2)	37-84	40	15	20	7.5	57.5

Table 2. The grading of habits and the number of individuals within p53-positive (n=12) and p53-negative (n=28) tumor groups related to the use of tobacco and alcohol.

Habit	Tumor group	None	Incidental	Moderate	Heavy
Smoking	p53 +ve	7	1	1	3
	p53 - ve	7	2	6	13
Alcohol	p53 +ve	6	2	3	1
	p53 - ve	3	5	12	8

RESULTS

Of the 49 blocks of tissue examined immunocytochemically for keratin and by routine hematoxylin and eosin staining, 9 contained very small islands of carcinoma (n=3) or no detectable carcinoma (n=6) and were excluded from the study. According to the histology, thirty-five of the remaining 40 specimens contained normal lingual epithelium in addition to invading SCC. All 40 specimens stained intensely for keratin when the LP34 monoclonal antibody was used.

In Tables 1 and 2 the results of the immunostaining for p53 and other patient details including the use of tobacco and alcohol are summarized. Smoking tobacco was significantly more common in males (21/24) than in females (5/16; $\chi^2=13.35$, $p<0.00005$). Although the level of alcohol usage was also higher in males (21/24) than in females (10/16) this was not significant ($\chi^2=3.44$, p=NS).

Twelve carcinomas (i.e.30%) demonstrated nuclear reactivity for p53. Positive cells were not evenly distributed throughout the tumors but tended to occur in foci and were most common within the basal layers. No positive cells were detected in histologically normal overlying lingual epithelium or in the 5 specimens where there were areas of obvious epithelial dysplasia.

There were no significant differences between p53-positive and p53-negative groups in respect of age (Mann Whitney U test), gender or TNM classification (χ^2test). At the time when the tissues were obtained, neck lymph node metastases were

associated with 25% (3/12) and 35.7% (10/28) of p53-positive and p53-negative tumors, respectively. In both groups well differentiated carcinomas predominated and accounted for 75% (9/12) of the p53-positive and 71.4% (20/28) of the p53-negative tumors.

From the post-surgery follow-up data (between 4-11 years), it appeared that with respect to the p53-positive tumors, 25% (3/12) of the individuals died as a direct result of recurrence/metastatic spread, 25% (3/12) died of causes not related to the SCC and 50% (6/12) lived free from SCC (4-10 year follow-up; mean, 5.7 years. Similarly, it appeared that with respect to the p53-negative tumors 39.3% (11/28) of the individuals died as a direct result of recurrence/metastasis, 10.7% (3/28) died of non-cancer related causes and 46.4% (13/28) remained alive and free from carcinoma (5-11 year follow-up; mean, 6.9 years).

The use of alcohol and tobacco differed significantly when individuals within the p53-positive and p53-negative tumors groups were compared ($p<0.01$ for alcohol, $\chi^2=7.4$; $p<0.05$ for smoking, $\chi^2=4.1$; $p<0.01$ for alcohol & smoking, $\chi^2=7.5$). The biggest contributor to this difference was the high percentage of individuals in the p53-positive group who did not drink (50%; 6/12), smoke (58.3%; 7/12) or even abstained from both habits (33.3%; 4/12). In comparison, 75% (21/28) of the individuals in the p53-negative group smoked tobacco (19 at moderate to heavy levels), 89.3% (25/28) drank alcohol (20 at moderate to heavy levels) and 71.4% (20/28) used both tobacco and alcohol (16 at moderate to heavy levels for both habits).

DISCUSSION

Heavy smoking and drinking are well recognized risk factors for the development of upper aerodigestive tract malignancies, including lingual SCC.[14-16] In such neoplasms, mutations in the p53 gene may be induced by specific carcinogens which are present in tobacco smoke.[2,4,5,17-19] The most common mutation is G to T transversion which are found in head and neck,[2] non-small cell lung [17,19] and oesophageal [18] carcinomas.

From the present immunocytochemical study which used the monoclonal antibody PAb1801, it appeared that 12/40 (30%) lingual carcinomas contained detectable amounts of the p53 gene product. This figure is consistent with previous data on oral carcinomas.[8,9] However, the surprising feature of these results was the apparent negative association between the immunocytochemical detection of p53 and tobacco smoking and/or alcohol intake. No alcohol or tobacco risk factors were evident in 33.3% of p53-positive carcinomas, whereas only 7.1% of the p53-negative cases showed an absence of these risk factors. Furthermore, 25% of the p53-positive and 71.4% of the p53-negative carcinomas were found in patients who had been exposed

to both alcohol and tobacco. This suggests that overexpression/stabilization of p53 is not necessarily associated with the two risk factors. These results are in direct contrast to the previously reported positive link between p53 immunoreactivity and smoking [4,9] and smoking and drinking[5] concerning SCC of the head and neck [4,5] and the oral cavity.[9]

Apart from the specific oral site of the carcinomas studied, there are two major differences between our studies and those of Field et al. [4] and Ogden et al.[9] Firstly, our material was obtained from a Dutch population whereas the other studies were based on UK patients. The age standardized cumulative incidence rates of mouth and lingual cancer in the Netherlands and the UK are similar. This indicates that there are no obvious population differences.[20] However, the incidence rate of bronchial cancer is higher in the Netherlands and shows a much greater difference between men (85.5 per 100,000) and women (8.5 per 100,000) than in England and Wales (65.4 and 20.5 per 100,000 for men and women respectively) suggesting possible differences in smoking habits between these populations.

Secondly, three different antibodies to p53 have been used. The initial studies on head and neck cancer of Field et al. [4] are based on combined data using two monoclonal antibodies which, individually, were only used on a proportion of the total number of specimens (PAb1801 on 40/73; PAb421 on 53/73). As one of the monoclonal antibodies (PAb-421) is thought to cross-react with keratin,[21] and as the published results do not allow determination of p53 positivity with the p53-specific PAb1801 alone, interpretation of this data requires caution. This may be important as only 7 of the 73 carcinomas investigated were from non-smokers. By contrast, Ogden and co-workers[9] used a p53-specific polyclonal antibody (CM1) in their study on oral cancer and precancer. CM1, apparently able to detect smaller amounts of p53 than monoclonal antibodies,[22] gave a higher rate of positives (54%) on formalin-fixed paraffin embedded oral carcinomas compared to PAb1801 on either frozen (35% [8]) or routinely processed tissues (30%; this study). Their data clearly suggest a relationship between smoking and p53 expression in a group of 26 oral carcinomas, including 10 from non-smokers, but the data are dependent on the specificity of the polyclonal antibody. Our contradictory data, based on the exclusive use of PAb1801 on paraffin sections, are dependent on the sensitivity of p53 detection, which, when compared to the results of Warnakulasuriya and Johnson [8] using the same antibody on frozen sections, appears satisfactory.

In this study it was not possible to determine whether the positive immunoreactivity for p53 found in 30% of carcinomas reflects the presence of stable mutant p53 species or the stabilization of normal p53 by way of binding to the products of cellular genes. For example, it has recently been shown that p53 protein is stabilized by cdc2 protein kinase,[23] casein kinase II,[24] the mdm2 gene product [25] and heat shock

proteins.[26] Furthermore, the viral status of the lingual carcinomas in the present study is unknown, which is a significant factor as oncoproteins (SV40 large T antigen and adenovirus E1b) of DNA tumor viruses may stabilize cellular p53.[27,28] Ignoring technical problems, there are a number of potential explanations for the absence of immunocytochemical reactivity for the p53 protein in 70% of carcinomas, including those from patients with a background of tobacco and/or alcohol use. Negative carcinomas may have arisen independently of the mutation of the p53 gene or, alterations in this locus may have resulted in either deletion of both alleles or an alteration in transcription. Such changes have been detected in some lung tumor cell lines.[29,30] Another possibility is that point mutations of the p53 gene, which do not result in the production of a sufficiently stable product that can be detected immunocytochemically, are present. Such mutations have been detected in thyroid[31] and lung cancer cell lines.[30] Also in one study of breast carcinoma, 7/10 specimens containing missense mutations were negative by frozen section immunocytochemistry.[32] We have shown that variable stability of the p53 protein in human oral carcinoma cell lines harboring a variety of p53 mutations (Yeudall et al., submitted), which suggest that there may be alternative mechanisms of p53 stabilization or regulation of expression in oral cancer. Finally, human papillomavirus (HPV) infection of oral carcinomas is common [33] and the HPV E6 protein is shown to target the degradation of p53 via a ubiquitin-dependent protease system.[34] The HPV status of the carcinomas is unknown in the present study, but, possibly, this could account for many of the p53-negative cancers.

There are no data available on the direct effects of the use of tobacco and alcohol on steady state levels of the p53 protein in the presence or absence of gene mutation. The interaction of viral and chemical carcinogens in cellular transformation is a significant area of cancer research at the present time and is likely to assume more importance in the future. Although the numbers are small, our data could indicate that oral cancer in patients with a background of tobacco and/or alcohol use is more likely to be immunocytochemically negative for p53 because of one or a combination of these factors. The corollary would be that an oral carcinoma arising in an individual not exposed to these risk factors would be more likely to have gene mutations leading to a stabilization of the p53 protein.

CONCLUSIONS

The results of this study indicate that the purported correlation of tobacco/alcohol and increased steady state levels of p53 expression is questionable. Further investigations, combining p53 gene sequencing studies and immunocytochemistry, need to define more clearly the relationship between this tumor suppressor gene product, oral cancer and the use of tobacco and alcohol.

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Chapter 7

EVIDENCE FOR A MAJOR ROLE OF GENETIC FACTORS IN THE ETIOLOGY OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

This chapter is based on the following publication:
Copper MP, Jovanovic A, Nauta JJP, Braakhuis BJM, de Vries N, van der Waal I and Snow GB. Evidence for a major role of genetic factors in the etiology of head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg, in press.

INTRODUCTION

Cigarette smoking and alcohol consumption have been established as major etiologic factors for head and neck squamous cell carcinoma (SCC). Approximately 90% of this type of cancer in the oral cavity, the pharynx and the larynx occurs in smokers[1,2] and the vast majority of the patients is a moderate to heavy drinker.[1,2,3] Some types of malignancy including carcinoma of the breast,[4] colon,[5] lung[6] and oesophagus[7] show a familial clustering whereas other tumors, such as retinoblastoma[8] and glomus tumors[9] provide unequivocal evidence of a relationship between heredity and carcinogenesis. Although attention has also been paid to other potential etiological factors of head and neck SCC, such as viral infections[10] and occupational factors,[11] little is known about the possible role of hereditary factors in this type of cancer. It seems very likely that a genetically determined susceptibility to external carcinogens is important in the etiology of head and neck SCC, because so many individuals have been and are using tobacco and alcohol, whereas only few actually develop cancer in the respiratory and upper digestive tract (RUDT). Only a few case-reports have been published on the familial clustering of oral[12,13] and laryngeal cancer[14,15] describing the simultaneous incidence of a similar type of a SCC in two or three members of a single family.

The importance of identifying persons at high risk of developing SCC of the RUDT is increasingly recognized.[16] The identification of these individuals could be of great value to decrease the incidence of SCC of the RUDT, now that preventive modalities have become available. Not only by means of passive prevention, such as stopping smoking and excessive alcohol consumption, but also by means of active prevention, since chemopreventive treatment seems to be effective in reducing the incidence of cancer of the RUDT.[17,18] This study was performed in order to assess the influence of genetic factors as an individual risk factor for head and neck SCC. The study has been based on the hypothesis that if hereditary factors play a role in the etiology of head and neck SCC, it can be expected that the occurrence of cancer of the RUDT will be higher among relatives of head and neck SCC patients than among relatives of a comparable group of references.

MATERIAL AND METHODS

Population of study

All new patients who presented themselves with their first head and neck SCC at the Department of Otorhinolaryngology / Head and Neck Surgery or at the Department of Oral and Maxillofacial Surgery of the Free University Hospital, Amsterdam, the Netherlands between December 1991 and January 1993, and who were at presentation accompanied by their spouses were asked to collaborate in this study. The population of study consisted of first-degree relatives of the patients

(fathers, mothers, brothers and sisters). As a reference-group the first-degree relatives of the patients' spouses were used, which had the following advantages. Firstly, these relatives were assumed to share a similar environment as well as a similar socio-economic status as the patients' relatives, which makes a bias by different socio-economic classes unlikely.
Secondly, the spouses' relatives were of about the same age as the patients' relatives. In total 105 patients collaborated in the study (56 laryngeal SCC patients, 29 oral SCC patients, 19 pharyngeal SCC patients and 1 patient with a lymphnode metastasis of an unknown SCC).

Data collection

A standard questionnaire on the following data were filled in by two trained interviewers: year of birth, smoking habits and the occurrence of cancer in parents and siblings (brothers and sisters) of the patients and their spouses. Only first-degree relatives were included in this study to reduce the possibility of recall-bias. Previous studies on lung cancer have shown that the time-consuming verification of cancer histories by way of death records does not influence the study reliability significantly.[19] Because of this observation cancer history data by a hetero-anamnestic questionnaire have been collected. Smoking was recorded as being positive or negative since it was impossible to count pack-years accurately by hetero-anamnestic questioning. The recording of alcohol consumption of the first-degree relatives by hetero-anamnestic information was assumed to be unreliable and was therefore not included in the questionnaire. Nevertheless, by using the first relatives of the patients' spouses as reference-population in this study, possible bias by differences in alcohol intake was expected to be minimal, as these relatives are members of a similar socio-economic group as the relatives of the patients.

Data analysis

Because of the strong relationship between cancer of the head and neck and cancer of the esophagus and lungs, all sites of cancer of the RUDT were included in our analysis. This is based on the existence of similar risk factors such as smoking and alcohol consumption and on the high rate of occurrence of second primary tumors within the RUDT in head and neck cancer patients, which can be explained by the theory of field cancerization.[20]

Statistical analysis

To compare the occurrence rate of cancer in the parents of patients and in the parents of the spouses, cancer frequencies were established at several sites of the RUDT.

Table 1. Characteristics of first degree relatives of patients and their spouses.

First degree relatives of:		Patients	Spouses
Parents:	Number	207	209
	Year of birth (Median)	1900	1902
	Smokers (%)	53	51
Siblings:	Number	410	409
	Year of birth (Median)	1930	1934
	Smokers (%)	65	62
Parents & siblings	Number	617	618

The differences were tested for statistical significance, using Fisher's exact test.To compare the occurrence rate of cancer in siblings or all first-degree relatives (siblings and parents), however, data were divided into 105 strata, each consisting of either the siblings or all first degree relatives of a matched patient-reference pair. The reason why stratified analyses were performed when dealing with siblings is that siblings are related to each other. An unstratified analysis would ignore this. Because of the small strata, differences were tested for statistical significance using the stratified version of Fisher's exact test. Null hypotheses of no association were tested for either one- or two-sided alternatives. When considering RUDT tumors, the null hypothesis was tested for the one-sided alternative that these tumors are more frequent among relatives of patients. In contrast, when considering other tumors the null hypothesis was tested for the two-sided alternative that such tumors are more frequent in either the relatives of patients or in relatives of their spouses. As a measure for strength of association the relative risk (RR) is used. For stratified data the Mantel-Haenszel (M-H) estimator is used.[21] If possible, a two-sided confidence interval (CI) is reported, either a 90% CI (i.e. when the null hypothesis is tested for a one-sided alternative) or a 95% CI (i.e. when the null hypothesis is tested for a two-sided alternative).

RESULTS

The characteristics of the patients' relatives and the spouses' relatives are listed in Table 1. There were only minor differences in age and smoking between the patients' relatives and the spouses' relatives. Therefore these factors were not considered to be important confounders. 31 cases of cancer of the RUDT were reported in the group of 617 first-degree relatives of the registered patients versus 10 cases in the 618 first degree relatives of the reference group (M-H RR= 3.5, p= 0.0002) (Table 2).

Table 2. Cancer in the respiratory and upper digestive tract (RUDT) in first-degree relatives of patients with SCC of the head and neck vs. cancer in the RUDT in first-degree of the patients' spouses.

	Patient relatives N (%)	Spouse relatives N (%)	Relative Risk (90% CI)	p-value
Cancer of the RUDT:				
Parents:	15 (7.0)	8 (4.0)	1.9 (0.9-3.8)	p = 0.09
Siblings:	16 (3.9)	2 (0.5)	14.6 (3.1-69.1)	p = 0.0001
Parents and siblings:	31 (5.0)	10 (1.6)	3.5 (1.9-6.4)	p = 0.0002
Cancer of the head and neck:				
Parents:	4 (1.9)	1 (0.5)	4.0 (0.6-25.1)	p = 0.18
Siblings:	4 (1.0)	1 (0.2)	2.8 (0.2-33.5)	p = 0.43
Parents and siblings:	8 (1.3)	2 (0.3)	3.4 (0.8-13.7)	p = 0.12

CI: Confidence interval

The significantly increased frequency of SCC was observed in siblings (16 versus 2, M-H RR= 14.6, p= 0.0001) but not in parents (15 versus 8, M-H RR= 1.9, p= 0.09). When regarding head and neck cancer alone, differences in incidence failed to reach significance (8 versus 2, M-H RR= 3.4, p= 0.12). No significant difference was observed for the occurrence of cancers outside the RUDT (not shown).

It was found that a positive family history of SCC of the RUDT was less common in laryngeal cancer patients than in patients with oral and pharyngeal cancer (3.9% versus 6.5% of all first degree relatives) (Table 3). The majority of the relatives of laryngeal cancer patients had lung cancer (77%), while the majority of the positive relatives of oral and pharyngeal cancer patients had cancer of the upper digestive tract (56%).

DISCUSSION

Cancer of the head and neck is frequently described as being a neoplasm that is solely determined by its environment. In an earlier study we found that genetic factors such as HLA antigens and immunoglobulin allotypes play a role in the etiology of head and neck SCC.[22] We believe to have found additional evidence that genetic factors play a major role in the etiology of head and neck SCC.

Table 3. Pattern of heredity in laryngeal versus oral and pharyngeal cancer patients.

	Laryngeal cancer patients	Oral and pharyngeal cancer patients
Probands with:		
RUDT cancer:	N = 13 (out of 335 probands) = 3.9%	N = 18 (out of 277 probands) = 6.5%
Lung cancer:	N = 10 (77%)	N = 8 (44%)
UDT cancer:	N = 3 (23%)	N = 10 (56%)

RUDT: Respiratory and upper digestive tract.
UDT: Upper digestive tract.

This is probably the first time that a direct relationship between a positive family history for cancer of the RUDT and the occurrence of SCC in the head and neck is described.

Previous studies gave RRs for head and neck cancer due to heavy alcohol consumption that ranged from 2.2 to 11.6 and RRs of heavy smoking that ranged from 2.4 to 17.6.[23-25] The M-H RRs in our study varied from 3.5 for all first relatives to 14.6 for siblings alone, which means that genetic factors as well play a major role in the process of carcinogenesis of head and neck SCC. By means of using hetero-anamnestic questionnaires it is impossible to record duration and intensity of smoking and amount of alcohol intake accurately. However, the percentages of smokers were of equal magnitude among the patients' relatives as well as among the spouses' relatives. Taking into account that most of the positive family members had lung cancer (63%) in which alcohol consumption plays just a minor role, a possible bias by differences in alcohol intake should have only a minor effect on our results. No significant excess of various other types of tumors was observed among first degree relatives of the patients. Besides this observation, the choice for the reference-population makes large bias unlikely, because this group belongs to the same socio-economic class as the patients' relatives. Particularly the RR of 14.6 for siblings is of an unexpected magnitude.

Improvements in local and regional cure rates of head and neck cancer patients have not resulted in a proportional increase in survival rates. The main reason for this is that as fewer patients die from an uncontrolled disease in the head and neck, more patients are exposed to the risk of second primary tumors, and to a lesser degree, distant metastases. Second primary tumors occur in 10-40% of all patients with head and neck cancer and the majority of these second primary tumors occur in the RUDT.[26,27]

A number of risk factors of head and neck cancer play a role in the etiology of lung cancer and cancer of the esophagus as well, and therefore it is not surprising that patients with head and neck cancer tend to develop multiple cancers at these sites as well. The incidence and localization of the second primary is related to the localization of the so-called index tumor. In case of index tumors of the oral cavity and the pharynx, the second tumor is relatively frequent located in the upper digestive tract as well, [28,29] while in case of laryngeal cancer the second primary frequently appears in the respiratory tract.[30,31] Moreover, second primary tumors occur less frequently in laryngeal cancer patients than in oral and pharyngeal cancer patients.[26,28,31] Analogous to this pattern, a positive family history for cancer of the RUDT was less common in laryngeal cancer patients than in patients with oral and pharyngeal cancer. Moreover, it was found that of the probands with cancer of the RUDT the majority of the relatives of laryngeal cancer patients had lung cancer, while the majority of the relatives of oral and pharyngeal cancer patients had cancer of the upper digestive tract. This pattern is surprisingly consistent with the pattern of incidence and preferential localization of second primary tumors in head and neck cancer patients. This suggests that different genetic factors play a role in the carcinogenesis of lung and laryngeal SCC when compared to that of oral and pharyngeal SCC. These genetic components probably become active only in those individuals who smoke or drink or those who are exposed to other environmental factors. This predisposition could result from inherited mutations that are not directly on the pathway of carcinogenesis, but either increase the probability of a mutation in the direct pathway or magnify its effect.[32] In this respect, genetically determined metabolism of procarcinogenic components of cigarette smoke by specific enzyme systems in various tissues of the body may be of considerable importance. In other words, quantitative and qualitative differences in cytochrome p450 enzymes [33] in lung and larynx tissue versus pharynx and oral cavity tissue and genetically determined differences of these enzymes between individuals could be an explanation of our observations.

CONCLUSIONS

The current findings suggest that in addition to smoking and alcohol consumption, genetic predisposition is an important, if not mandatory condition for the development of head and neck SCC. To exclude malignant disease comprehensive diagnostics should be utilized. These should rather be used for patients with pathology in the head and neck and who have a positive family history for cancer of the RUDT, than for other patients.

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Chapter 8

SECOND RESPIRATORY AND UPPER DIGESTIVE TRACT CANCER FOLLOWING ORAL SQUAMOUS CELL CARCINOMA

This chapter is based on the following publication:
Jovanovic A, van der Tol IGH, Kostense PJ, Schulten EAJM, de Vries N, Snow GB, van der Waal I. Second respiratory and upper digestive tract cancer following oral squamous cell carcinoma. Oral Oncology, Eur J Cancer, in press.

INTRODUCTION

To reduce the mortality of patients with oral squamous cell carcinoma (SCC), emphasis is put on prevention, early detection and improvement in treatment. Although the cure rates of primary oral tumors have improved, the overall survival rates have hardly increased. This lack of progress is to a large extent due to the occurrence of second primary tumors (SPTs).[1-3] In the literature, attention has been paid to second primary tumors among patients suffering from oral SCC. It has been stated that the most precancerous condition in oral cancer is oral cancer itself.[4]
The percentages of SPTs after oral SCC vary from 10 to 27%.[2,3,5-8] Most likely, due to risk factors such as the use of tobacco and alcohol, a great majority of the SPTs occur in the respiratory and upper digestive tract (RUDT).[7]
The aim of this study was to investigate whether the incidence of SPTs in the RUDT following oral SCC is related to 1) the anatomical site of the index tumor, and 2) the use of tobacco and alcohol.

MATERIAL AND METHODS

Population of study

The population of study consisted of 740 consecutive, previously untreated patients with SCC of the lip and the oral cavity. The SCC was diagnosed at the Free University Hospital in Amsterdam, the Netherlands in the period from January 1, 1971 to January 1, 1991. Age, gender, and the use of tobacco and alcohol were registered at the time of the diagnosis of the index tumor. Concerning the use of tobacco a division of non-smokers, moderate smokers (1 to 20 cigarettes per day) and heavy smokers (more than 20 cigarettes per day) was made. One cigar and one pipeful were assumed to be equal to 4 and 2 cigarette equivalents, respectively. None of the patients had a history of using smokeless tobacco. Intake of alcoholic beverages was expressed in units of alcohol per day, assuming that the amount of alcohol in a consumption of hard liquor, wine and beer is equal (approximately 10 g of alcohol per unit).[9] Patients were divided into non- or incidental drinkers, moderate drinkers (one to four units per day) and heavy drinkers (more than four units per day).
The oral (sub)sites of the index tumor were specified according to the International Union Against Cancer (UICC).[10] Patients with a history of another malignancy, a total of 13 cases, were excluded from this study. The remaining 727 cases were included in the analysis. The population of study comprised 464 male and 263 female patients. The median age was 62 and 69 years respectively. Details of the patients' data have been described elsewhere.[11] All additional tumors following SCC of the lip and the oral cavity located in- and outside the RUDT are presented in Figures 1A and 1B respectively.

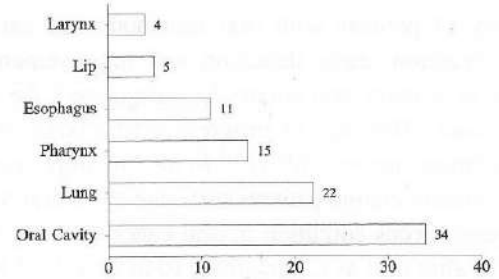

Figure 1A. All additional tumors located in the respiratory and upper digestive tract (n=91).

For the analysis of this study, only tumors in the RUDT were regarded as an eligible SPT. As the study was focussed on the incidence of additional tumors in patients related to the site of the index tumor or tobacco and alcohol habits, the inclusion of third and fourth primary tumors would introduce a bias.[11] Therefore, additional tumors following the SPT were excluded from this analysis.The RUDT is specified as the lip, oral cavity, pharynx, larynx, esophagus and lung and is defined according to the classification of the UICC.[10]

The number of years at risk of SPTs was calculated until the date of the diagnosis of the SPT, until January 1, 1992, the patient's death or the date of the last follow-up visit, whichever occurred first. The follow-up has been described in person-years of follow-up. The incidence rate of SPTs is defined as the total number of SPTs that developed, divided by person-years of follow-up, and expressed per 1,000 person-years of follow-up.[12]

The SPTs were identified by the use of the criteria provided by Warren and Gates.[13] These criteria require that both tumors are histologically malignant, that they are separated by normal healthy mucosa and that one tumor is not a metastasis of the other. The cell types, the degree of differentiation and the presence of regional spread were also taken into account to distinguish between a metastasis and a SPT. Any subsequent SCC at the same site or direct vicinity (<2 cm) of the index tumor, regardless of the time since the diagnosis and treatment of the index tumor, was considered to be a recurrence and therefore subsequently excluded.

Statistical analysis

The product-limit method of Kaplan-Meier was used to assess the incidence rate of SPTs over a period of time. Subgroups were compared by means of the log-rank test (Mantel- Haentzel test for censored survival times). Stratification was applied whenever appropriate to eliminate possible confounding.

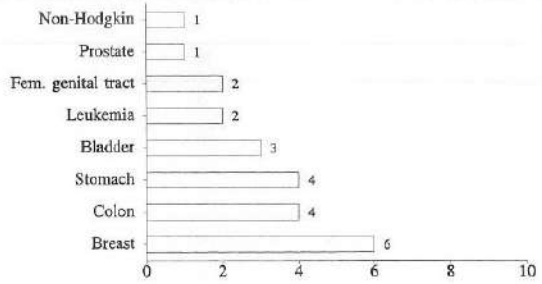

Figure 1B. All additional tumors located outside the respiratory and upper digestive tract (n=23).

RESULTS

The mean follow-up of the 727 patients was 3,63 years, being a total of 2,638 person-years at risk. The incidence rate and localization of the SPTs are shown in Table 1. For all SPTs in the RUDT an incidence rate of 28.0 per 1,000 person-years of follow-up was found.

In Figure 2 the estimate of patients free of SPTs in the RUDT is presented. It appeared that during the entire follow-up period, patients were continuously at risk for a SPT at a steady rate of approximately 2.8 % per year. The Kaplan-Meier estimate for patients free of a SPT was not significantly different for men and women.

In Table 2 an overview is given of the incidence rates of SPTs by the anatomical site of the index tumor. Of the major index (sub)sites, the incidence rates varied from 5.7 for the cheek mucosa to 43.9 for the lower alveolar process. In Figure 3 the incidence of SPTs is related to index tumors of the lower part of the oral cavity (i.e. floor of mouth, retromolar area and lower alveolar ridge) versus the remaining (sub)sites of the oral cavity (i.e. cheek mucosa, tongue, upper alveolar process and hard palate). It appeared that SPTs occurred significantly more often after SCC in the lower part of the oral cavity than after the rest of the group ($p=0.01$). However, after controlling for the use of tobacco and/or alcohol, this difference was not longer statistically significant. In Figure 4 is shown, that a significant trend is observed between the use of tobacco and the incidence of SPTs ($p= 0.045$). After stratification for the use of alcohol, however, no significance was seen. The trend for alcohol drinking appeared to be not significant ($p= 0.07$).

Table 1. Incidence rate and localization of second primary tumors in the respiratory and upper digestive tract (RUDT) in 727 cases of squamous cell carcinoma of the lip and the oral cavity.

Sites in RUDT	N	Incidence*
Lip	3	1.1
Oral cavity	26	9.9
Pharynx	13	4.9
Larynx	5	1.9
Lung	19	7.2
Esophagus	8	3.0
Total	74	28.0

* Incidence rate per 1,000 person-years of follow-up.

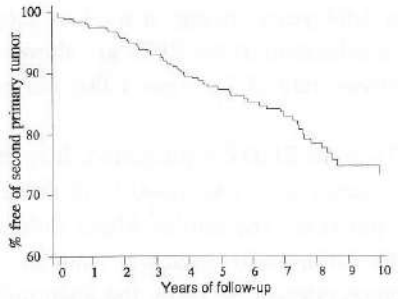

Figure 2. Patients free of second primary tumors in the RUDT.

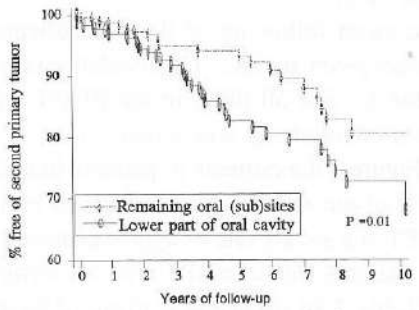

Figure 3. Patients free of second primary tumors in the RUDT. Index tumors in lower part of oral cavity vs. index tumors in remaining (sub)sites of oral cavity.

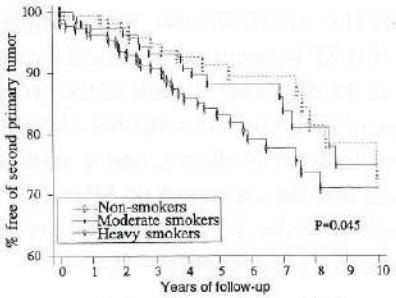

Figure 4. Patients free of second primary tumors in the RUDT related to the use of tobacco.

Table 2. Incidence rate of second primary tumors (SPTs) by gender and (sub)site of the index tumor.

Localization of the index tumor	N SPT / N index tumor		Incidence*		Incidence* 95% CI	
	Male	Female	Male	Female	Both genders	
Lower alveolar ridge	7/31	3/25	50.3	33.8	43.9	21.0 - 80.8
Retromolar area	10/57	0/20	53.0	0	36.9	17.7 - 67.9
Floor of mouth	16/138	6/59	32.8	29.8	31.9	20.0 - 48.4
Lower lip	7/50	1/6	31.6	36.9	32.2	13.9 - 63.5
Tongue	9/134	10/106	18.8	23.5	21.0	12.7 - 32.9
Cheek mucosa	0/26	1/26	0	12.5	5.7	0.1 - 31.7
Upper lip/commissure	0/2	0/1	0	0	0	0 - 174.1
Upper alveolar ridge	2/10	0/11	92.8	0	53.7	6.0 - 193.7
Hard palate	0/8	0/5	0	0	0	0 - 80.1
Simultaneous tumors of lip and oral cavity	1/8	1/4	86.6	119.7	100.5	11.3 - 362.9
All (sub)sites	52/464	22/263	30.9	23.0	28.0	22.0 - 35.2

* Incidence rate per 1,000 person-years of follow-up.
CI = confidence interval

DISCUSSION

In many studies, SPTs are expressed in percentages of patients. Therefore, the period of follow-up is not included. To include the follow-up, the incidence rate of SPTs is expressed per 1,000 person-years of follow-up.[12] In the present study 28 SPTs per 1,000 person-years of follow-up are seen in the RUDT. This means that 1 SPT develops during one year follow-up of 36 patients (1,000/28).

According to the literature, most cases of SPTs occur in the RUDT[6,14,15] with high occurrence rates in the oral cavity itself.[3,7,14,15] In our study about 40% of the SPTs in the RUDT affected the lip and the oral cavity again (Table 1). Traditionally, this was explained by the susceptibility of the epithelium of the oral cavity - field cancerization [16]- to carcinogens such as tobacco and alcohol. Recently, experimental evidence for this concept has been provided.[17]

Nowadays, it is well accepted that the whole RUDT is susceptible to common exogenic influences.[18] In addition, it has also been reported that the presence of oral leukoplakia among patients with oral SCC does increase the risk of additional SCCs at the site of the leukoplakia.[19] The development of second malignancies, however, remains the most obvious expression of field cancerization.

This study confirms previous studies which report on a virtually constant risk of a SPT in the RUDT in the course of time.[3,20,21] In our study the patients were at a continuous risk of a SPT of approximately 2.8 % per year for at least 10 years. No significant difference was seen between male and female patients. In the literature, a preference for female as well as for male patients is reported.[3,22]

There is evidence that smokers and alcohol drinkers are more at risk of developing SPTs following oral cancer than non-smokers and non-drinkers, respectively.[22-25] However, it is not entirely clear whether the risk decreases if the use of tobacco and alcohol is discontinued after diagnosing the first neoplasm.[26] When changes in life-style do not result in a decrease of risk of a SPT, the entire mucosa may be primed for neoplasia before the first clinical cancer has actually occurred. On the other hand, it has been reported that among non-smokers the frequency of SPTs is similar to that among smokers.[27,28]

According to a previous study of our group, patients with index tumors in the lower part of the oral cavity (i.e. floor of mouth, lower alveolar process and retromolar area) had a significantly higher incidence rate of SPTs than patients with index tumors in the remaining (sub)sites of the oral cavity (i.e. cheek mucosa, tongue, upper alveolar process and hard palate).[7] After stratification for the use of tobacco and/or alcohol no significance was observed. This supports the theory that SCC of the lower part of the oral cavity is more related to the use of tobacco and alcohol than the rest of the oral cavity.[29,30] This explains the higher incidence rate of SPTs in the RUDT that is seen in the index tumors of these sites.

Furthermore, a significant trend in smoking could be seen for the development of SPTs. However, after the stratification for the use of alcohol no significance was observed. The trend for alcohol drinking appeared to be not significant ($p = 0.07$, one-sided). No reliable information could be retrieved from our data on whether the use of tobacco and alcohol was continued after diagnosis of the index tumor.

The development of SCC of the oral cavity is, to a large extent, related to exogenous carcinogens in cigarette smoke and to alcohol abuse. Occupational and dietary factors play a role as well,[31,32] while the role of viral infections with the human papilloma virus and herpes simplex remains to be elucidated.[33] The fact that only a minority of heavy smokers and drinkers develop cancer of the oral cavity points to a - possibly endogenous - individual susceptibility to these carcinogens. Several of such endogenous factors that may play a role in the etiology of oral cancer have been reported. These factors include HLA antigens, immunoglobulin allotypes,[34,35] and an increased occurrence of cancers in the RUDT in first degree relatives of oral cancer patients (data not shown). With regard to the endogenous factors, the mutagen sensitivity, presumably the result of a DNA repair deficiency, might be associated with an increased risk of developing malignancies.[36] Schantz et al.[37] reported that patients with a decreased DNA repair capacity, after controlling for the use of tobacco and alcohol, indeed experienced over four times the risk of developing a SPT compared to patients with a normal DNA repair capacity.

CONCLUSIONS

Patients with an index tumor in the lower part of the mouth that is more related to the use of tobacco and/or alcohol seem to be more at risk of developing a SPT than patients with an index tumor in the other (sub)sites of the mouth. Apart from factors such as the use of tobacco and alcohol, other factors such as occupation, nutrition, viral infection, genetic and as yet unknown factors may play a (major) role in the risk of developing SPTs as well.

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Chapter 9

RISK OF MULTIPLE PRIMARY TUMORS FOLLOWING ORAL SQUAMOUS CELL CARCINOMA

This chapter is based on the following publication:
Jovanovic A, van der Tol IGH, Kostense PJ, Schulten EAJM, de Vries N, Snow GB, van der Waal I. Risk of multiple primary tumors following oral squamous cell carcinoma. Int J Cancer, in press.

INTRODUCTION

In the literature the percentage of multiple primary tumors (MPTs) following oral squamous cell carcinoma (SCC) varies from 10 to 27 %.[1-3] It is important to investigate whether these additional tumors occur more frequently than expected in the general population. The person-year approach, proposed by Schoenberg and Myers,[4] applied to data derived from a well-defined population makes it possible to compare the observed and the expected number of additional primary cancers. The aim of this study was to calculate the risk of MPTs following SCC of the lip and the oral cavity.

MATERIAL AND METHODS

Population of study

The data were gathered from 740 consecutive, previously untreated patients suffering from a primary SCC of the lip and the oral cavity, diagnosed in the period between January 1, 1971 and January 1, 1991, at the Free University Hospital, Amsterdam, the Netherlands. The oral (sub)sites of the tumor were specified according to the classification of the International Union Against Cancer (UICC).[5] MPTs were identified according to the criteria provided by Warren and Gates.[6] Briefly, each tumor must be 1) malignant 2) distinct, and 3) no metastasis of the other. Any subsequent SCC at the same site or direct vicinity of the index tumor (<2 cm), regardless of time since the diagnosis and treatment of the index tumor, was considered to be a recurrence and therefore excluded from the analysis.

Patients with a history of another malignant tumor, a total of 13 cases, were excluded. The remaining 727 cases consisted of 464 male and 263 female patients. The median age was 62 and 69 years respectively. Details of the patients' data have been described elsewhere.[7] The number of years at risk of MPTs was calculated until January 1, 1992, the patient's death or the date of the last follow-up visit, whichever occurred first. Concerning the treatment modalities, 568 patients were treated for their primary tumor by surgery alone or in combination with radiotherapy and/or chemotherapy. 227 patients received radiotherapy alone or in combination with other treatment modalities. For the purpose of this study, MPTs were divided into tumors located in- and outside the respiratory and upper digestive tract (RUDT). The RUDT is specified as lip, oral cavity, pharynx, larynx, esophagus and lung. Definitions of these sites are in accordance with those of the UICC.[5] Skin tumors, carcinoma in situ or carcinomas found at the autopsy were not regarded as MPTs. Cases of MPTs were divided into synchronous (i.e. diagnosed within six months) and metachronous tumors (i.e. diagnosed after six months). To prevent an overestimation of risks by counting tumors of which the onset preceded the follow-up period, synchronous tumors were excluded for the risk analysis.

Statistical analysis

Identifying each patient's risk of developing MPTs, the person-years approach proposed by Schoenberg and Myers [4] was used, applying age-, gender-, and site-specific cancer incidence rates from the general population to the appropriate persons-year of the follow-up of the patients with SCC of the lip and the oral cavity. In order to express these risks the observed (O) numbers of MPTs are compared to the numbers of expected (E) tumors in the general population. The population-based cancer registry data of the period 1978-1987 on the population of southeastern Netherlands, were used for this purpose.[8]

Due to non-specified data the incidence rates of the oral cavity and pharynx were assembled.A comparison of the observed and expected numbers was performed by calculating the O/E ratio and expressed in standardized incidence ratio's (SIR). Statistical significance was tested on the assumption of a Poisson distribution and the 95% confidence intervals were calculated.

Table 1. Incidence rate and onset of 114 multiple primary tumors (MPTs) in -and outside the respiratory and upper digestive tract (RUDT) in 96 patients.

	Total	Synchronous	Metachronous	Incidence*
MPT in the RUDT	91	12	79	31.8
MPT outside the RUDT	23	4	19	8.0
Total MPT	114	16	98	39.8

* Incidence rate per 1,000 person-years of follow-up

RESULTS

Ninety-six of the 727 analyzed patients developed a total number of 114 MPTs during 2860.2 person-year of follow-up. 80 % of the MPTs occurred in the RUDT, 13% of which was synchronous (Table 1). The distribution of the metachronous MPTs in the RUDT by (sub)site of the index tumor and gender is presented in Table 2. MPTs among female patients were mainly located in the oral cavity (68%), whereas among male patients the MPTs were spread over the entire RUDT. MPTs of the esophagus were mainly seen among patients with an index SCC in the lower part of the mouth, i.e. the lower alveolar ridge, floor of mouth and retromolar area.

Table 2. Distribution of metachronous multiple primary tumors (MPTs) in the respiratory and upper digestive tract (RUDT) following initial SCC of the lip and the oral cavity for males (M) and females (F).

	Lower lip		Tongue		Lower alv. ridge		Floor of mouth		Cheek mucosa		Retromolar area		Other sites		All index SCC	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
	50	6	134	106	31	25	138	59	26	26	57	20	28	21	464	263
Lip	2	-	-	-	1	-	-	-	-	-	-	-	1	-	4	-
Oral cavity	2	1	3	6	5	3	3	5	-	1	2	-	2	1	17	17
Pharynx	-	-	2	3	-	-	3	-	-	-	2	-	1	-	8	3
Larynx	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2	0
Esophagus	-	-	-	-	1	1	3	2	-	-	1	-	1	-	6	3
Lung	4	-	5	1	2	-	3	1	-	-	3	-	-	-	17	2
Total RUDT	8	1	10	10	9	4	12	8	-	1	10	-	5	1	54	25

Other sites = Upper lip, commissure, upper alveolar ridge, hard palate, simultaneous MPTs in the lip and the oral cavity.

Table 3. Observed (O) and expected (E) number of multiple primary tumors (MPTs) in the respiratory and upper-digestive tract (RUDT) following SCC of the lip and the oral cavity for males (M) and females (F).

Localization of additional tumors	Observed		Expected		O/E ratio			
	M	F	M	F	M	95% CI	F	95% CI
Lip	4	0	0.2	0.01	24.3	6.6 - 62.2	0	0 - 488.7
Oral cavity/pharynx	25	20	0.3	0.1	74.7	48.3 - 110.2	190.4	116.3 - 294.1
Larynx	2	0	0.5	0.02	3.8	0.5 - 13.9	0	0 - 197.6
Esophagus	6	3	0.2	0.07	24.6	9.0 - 53.6	45.3	9.3 - 132.3
Lung	17	2	8.4	0.3	2.0	1.2 - 3.3	6.9	0.8 - 25.1
Total RUDT	54	25	9.7	0.5	5.6	4.2 - 7.3	51.5	33.3 - 76.0

O/E ratio : Standardized Incidence Ratio (SIR)
CI : confidence interval

In Table 3 the calculated risks (SIRs) of MPTs in the RUDT are presented. Patients with a primary SCC of the lip and the oral cavity run a significantly higher risk (compared to the general population) of developing an additional cancer in the oral cavity and/or pharynx, being 74.7 for males and 190.4 for females, respectively. Furthermore, the SIR for developing an additional esophageal tumor was 24.6 and

45.3, respectively. The SIRs for cancer of the lung and larynx were less elevated. The risks of an additional tumor outside the RUDT in patients with a history of SCC of the lip and the oral cavity are presented in Table 4. Considering MPTs both in- and outside the RUDT, the SIR of male- and female patients was 2.4 (95% CI: 1.9-3.1) and 3.6 (95% CI: 2.5-5.0), respectively.

Table 4. Observed (O) and expected (E) number of multiple primary tumors (MPTs) outside the respiratory and upper digestive tract (RUDT) following SCC of the lip and the oral cavity for males (M) and females (F).

Localization of	Observed		Expected		O/E ratio			
additional tumors	M	F	M	F	M	95% CI	F	95% CI
Stomach	0	2	1.9	0.8	0	0 - 2.0	2.3	0.3 - 8.7
Colon	3	1	2.0	1.4	1.5	0.3 - 4.4	0.7	0 - 4.1
Bladder	3	0	1.6	0.3	1.9	0.4 - 5.5	0	0 - 12.4
Non-Hodgkin lymphoma	0	1	0.6	0.4	0	0 - 60.8	2.4	0.1 - 13.2
Leukemia	1	0	0.5	0.2	1.9	0.1 - 10.5	0	0 - 18.0
Prostate	0	-	3.2	-	0	0 - 1.1	-	
Breast	-	6	-	2.8	-		2.2	0.8 - 4.7
Female genital tract	-	2	-	1.2	-		1.6	0.2 - 5.9

O/E ratio : Standardized Incidence Ratio (SIR)
CI : 95% confidence interval

DISCUSSION

According to the literature, the vast majority of MPTs after oral SCC occurs in the RUDT, the oral cavity itself being most frequently affected.[2,9] Among female patients MPTs occurred mainly in the oral cavity, whereas among male patients the MPTs occurred in all sites of the RUDT. However, with these findings one cannot judge in a reliable way whether these additional tumors occur more often than expected among the general population. The person-years approach defined by Schoenberg and Myers,[4] applied to data derived from the general population, meets the demand to judge whether a changed or an unchanged risk of developing MPTs at different sites of the body is present.

Including the additional malignancies diagnosed synchronously with the index tumor may lead to an overestimation of risks by counting the tumors of which the onset preceded the follow-up period used for the computation of the expected cases. To overcome this potential bias, the present study only analyses metachronous, as reported by Franco.[10] Several studies on the risk of MPTs after oral cancer have been published.[9,11-13] All these reports state that patients with an oral index tumor have, compared to the general population, a significantly higher risk of an additional tumor in the oral cavity and pharynx (SIRs ranging from 22 to 79) as well as in the esophagus (SIRs ranging from 6.5 to 52). Regarding MPTs in the larynx and lung, however, the reports in the literature are contradictory. In this respect, both elevated and non-elevated risks have been reported.[9,13,14] Although patients in the present study seem to have an elevated risk of developing cancer of the lung and larynx, the risks are less elevated than for cancer of the upper digestive tract (i.e. oral cavity, pharynx, esophagus).

The fact that the risk of MPTs in the RUDT is higher in both genders, but more distinct in female patients, has also been reported by other authors.[3,11] This can be explained by the fact that females are, in general, less affected by cancer in the RUDT, and, therefore, the relative risk might be higher than among male patients.

80 to 90 percent of the etiological factors of oral SCC can be contributed to the use of tobacco and alcohol.[15] However, there is a less clear pattern concerning the key factors in developing additional cancers after oral SCC. The concept of field cancerization used to imply a local epithelial susceptibility to cancer.[16] Nowadays it also implies a regional epithelial susceptibility to cancer,[17] and is most probably related to environmental carcinogens. This explains the high percentage of additional tumors in the RUDT. Although all these epithelial tumors in the RUDT are mainly related to the use of tobacco, the risks of MPTs in the larynx and lung are lower than for cancer of the upper digestive tract. These differences in SIRs may be explained by the carcinogenic effect of alcohol, which seems to be more related to cancer of the upper digestive tract compared to cancer of the lung and larynx. On the other hand, these results support the theory that different genetic factors may play a role in the carcinogesis of lung and laryngeal SCC when compared to that of oral and pharyngeal SCC.[18]

In general, it is thought that smoking and/or alcohol drinking increases the risk of oral cancer three to fifteen times.[19,20] However, the increase in the risk of MPTs in the oral cavity, pharynx and esophagus, by 25 times or more, is higher than one would expect to result from the use of tobacco and alcohol alone. This indicates that these patients may be more vulnerable to cancer because of host factors [21] or other, as yet unknown, factors.

Our analysis establishes no higher risks of developing cancer outside the RUDT. This is in accordance with the literature.[9,14] Apart from the urogenital tract perhaps, no higher risk would be expected, as no genotoxic effects of environmental chemical carcinogens appear to be active on internal organs. In our study the SIR of bladder cancer among male patients was 1.9.

As oral SCC is often accompanied by oral leukoplakia,[22] the latter lesion also may play a role in the occurrence of additional primary tumors. Shibuya et al. [23] noted that the incidence of multiple oral carcinomas in patients with tongue carcinoma together with leukoplakia was five times higher than among patients without leukoplakia.

The occurrence of MPTs after oral cancer leads to philosophical thoughts as to how to fight this problem. Theoretically, three approaches would seem possible: a) a better identification of risk factors, b) an early detection and c) (chemo)prevention. Several etiological factors - both for oral cancer and for a subsequent development of second tumors - have been identified, such as smoking, alcohol consumption, genetic factors, mutagen sensitivity, dietary factors, occupation and viral infections.[21,24,25] In view of developing MPTs, all patients should be advised to quit smoking and alcohol drinking as well as to include consumption of fruit and vegetables in their diet.

The incidence of oral cancer in general is too rare to screen in the general population. However, among patients with premalignant lesions of the oral cavity and among patients who have been treated for oral cancer such programs seem rational. On the other hand, several studies have shown that the value of routine follow-up visits with the aim to find MPTs in an early stage are of rather limited value.[26,27] Serial panendoscopy during follow-up is clearly not feasible because of cost-effectiveness and the extra burden for patients and personnel. Due to the relative low risk of MPTs in the lung, the value of routine chest film among oral cancer patients seems to be disputable as well.

At present, chemoprevention seems to be a promising tool to decrease the incidence of MPTs. Positive results have been reported, while several chemoprevention studies of early stage oral cancer patients are ongoing.[28,29] The ideal chemopreventive agent (or combination of agents) with optimal effectiveness, with minimal side-effects and toxicity, dose and length of administration remains to be established. The ideal chemopreventive agent (or combination of agents) with optimal effectiveness, with minimal side-effects and toxicity, dose and length of administration remains to be established.

CONCLUSIONS

Patients with a primary SCC of the lip and the oral cavity have, compared to the general population, a significantly higher risk of developing an additional cancer in the RUDT. The risk of a MPT in the oral cavity, pharynx and esophageal appeared to be higher than the risk of a MPT in lung and larynx. No elevated risks were established in organs outside the RUDT.

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Chapter 10

SUMMARY AND CONCLUSIONS

In this thesis the epidemiology of squamous cell carcinoma (SCC) of the mucosa of the lip and the oral cavity in a Dutch population is described. A general introduction on the study and an outline of the aims of the study are presented in **chapter 1**. Furthermore, a summary of the literature on the epidemiology and etiology of SCC of the lip and the oral cavity is given.

The incidence rates of cancer of the lip and the oral cavity vary worldwide with geographic latitude, race and life-style. The highest incidence rates for lip cancer are reported in Southern Australia, whereas the highest incidence rates for oral cancer are reported in parts of France and India. In the Netherlands, 1 to 2 % of all cancers are located in the lip and the oral cavity. Annually about 550 cases of SCC of the lip and the oral cavity are registered in the Netherlands.
Both the use of tobacco and alcohol have been accepted as independent risk factors for cancer of the lip and the oral cavity. Besides smoking cigarettes, a variety of other forms of tobacco use is associated with SCC of the lip and the oral cavity, such as smoking cigar or pipe, reverse smoking (smoking with the burning end inside the mouth) and the use of smokeless tobacco. Furthermore, factors such as occupation, nutrition, the dental status, host factors and viral infections are related to the etiology of SCC of the lip and the oral cavity.

In **chapter 2** the referral pattern of 140 patients with oral mucosal lesions, who had been referred to the Department of Oral & Maxillofacial Surgery at the Free University Hospital, is described. Patients with oral mucosal lesions consulted the dentist equally often as the general practitioner (GP). In case of symptoms due to a SCC, the GP was consulted significantly more often than the dentist. GPs were more likely to refer patients with oral mucosal lesions to medical specialists, whereas dentists only referred to oral and maxillofacial surgeons.

In **chapter 3** the patients' and doctors' delay of 50 patients with oral SCC is discussed. Twenty-six patients consulted the GP as the first source of help. In seven cases the cancer had been initially detected by the dentist during a routine dental examination. The mean patients' and doctors' delay was 103 days and 22 days respectively, the median being 35 and 11 days respectively. The doctors' delay of the dentist and the GP did not differ significantly from each other. Patients under the age of 40 had a relative long doctors' delay, compared to older patients.
The gender, dental status, site and tumor size did not show a significant correlation with the delay. Measures for early detection of oral cancer should place special emphasis on the patients' delay.

In **chapter 4** the epidemiological data of 740 patients with primary SCC of the lip and the oral cavity are reported. The results were similar to other reports in the Western literature. The mean age of the patients was 63 years. Female patients with SCC were seven years older than male patients on average. Most patients under the age of 40 years were men. The male-to-female ratio ranged from 8.3 for the lower lip to 0.9 for the upper alveolar ridge with an overall ratio of 1.8. The tongue and the floor of mouth were the most affected sites. No significant association was found between the age or gender of patients compared to the T classification of the tumor. However, a significant association was seen between the localization and the T classification. The size of the lesions of the lower lip was significantly smaller than the size of the tumors in other sites.

In **chapter 5** the relation of the use of tobacco and alcohol to the anatomical site of the SCC of the lip and the oral cavity is described. For this purpose, a case-case study has been performed. Smoking and drinking were adjusted to each other. Patients with SCC of the retromolar area and floor of mouth had the highest association with smoking and drinking. The lowest association with smoking and drinking was found in the cheek mucosa. It appeared that the lower part of the oral cavity (i.e. floor of mouth, retromolar area and lower alveolar ridge) was more related to the mentioned carcinogens than the other sites in the oral cavity. The possible local and systemic factors responsible for these variations of susceptibility for the use of tobacco and alcohol within the oral cavity are discussed.

In **chapter 6** the relation between the tumor suppressor protein p53 and the use of tobacco and alcohol of 40 patients with SCC of the tongue is described. Thirty percent of all neoplasms showed positive p53 reactivity, suggesting increased levels of p53 protein. No alcohol or tobacco risk factors were evident in 33.3% (4/12) of p53-positive neoplasms, whereas only 7.1% (2/28) of the p53-negative neoplasms showed an absence of these risk factors. Twenty-five percent (3/12) of the p53-positive neoplasms and 71.4% (20/28) of p53-negative neoplasms were found in patients who had been exposed to the use of both alcohol and tobacco. A similar negative association with p53 reactivity was found when either tobacco or alcohol were used in isolation. The results contrast with previous observations in oral carcinomas where a positive link is observed between p53 expression and smoking and drinking. This indicates that the association of the use of alcohol and/or tobacco with p53 expression is not clear yet.

In **chapter 7** possible genetic factors in the etiology of SCC of the head and neck are described. For this purpose, a study has been undertaken in which the occurrence of cancer in first-degree relatives of the patients and the patients' spouses is analyzed. The first-degree family of spouses acted as the reference group, where environmental conditions are considered to be equal. Among first-degree relatives (N= 617) of head and neck cancer patients (N= 105), 31 cases of cancer of the respiratory and upper digestive tract (RUDT) were reported versus 10 of these cases among the first-degree relatives (N= 618) of the patients' spouses (relative risk: 3.5, significant difference: p= 0.0002). This relative risk was even higher in siblings (16 versus 2, relative risk: 14.6, p= 0.0001). These findings suggest that a genetic predisposition is an important risk factor for head and neck SCC. Moreover, it was found that the majority of the tumor positive relatives of laryngeal cancer patients had lung cancer (77%), while the majority of the tumor positive relatives of oral and pharyngeal cancer patients had cancer of the upper digestive tract (56%). This pattern is consistent with that of the preferential localization of second primary tumors in head and neck cancer patients, suggesting that different genetic factors are of importance in the carcinogenesis of lung and laryngeal SCC if compared to oral and pharyngeal SCC.

In **chapter 8** the incidence of second primary tumors (SPTs) in the RUDT following SCC of the lip and the oral cavity is described. Seventy-four of the 727 patients (10%) developed at least one SPT in the RUDT. The incidence of SPTs was expressed per 1,000 person-years of follow-up; about 28 SPTs per 1,000 person-years of follow-up were seen in the RUDT. Patients ran the risk of developing a second primary tumor at a steady rate of approximately 2.8 % per year during at least 10 years. Furthermore, patients with an index tumor in the lower part of the mouth (i.e. floor of mouth, retromolar area and lower alveolar ridge), which seems to be more related to the use of tobacco and/or alcohol, were more at risk of developing SPTs than patients with an index tumor in the other localizations of the oral cavity. Furthermore, an increasing incidence of SPT could be observed in case of an increasing intensity of tobacco use.

Finally, in **chapter 9** the risk of an additional primary tumor after SCC of the lip and the oral cavity is compared to the risk of a malignancy in the general population. The follow-up of 727 patients with SCC of the lip and the oral cavity has been used for this risk analysis. Cancer incidences in the general population have been applied to the appropriate persons-years of follow-up of the patients with SCC of the lip and the oral cavity (observed-expected ratio design). The results indicate that patients with a primary SCC of the lip and the oral cavity have, compared to the general

population, a significantly higher risk of developing an additional cancer of the oral cavity and/or pharynx, with a multiplification factor of 74.7 for males and 190.4 for females respectively. A 24.6 and 45.3 times higher risk of an additional esophageal tumor, in males and females respectively was found. The risk of cancer of the lung and pharynx was less elevated compared to the risk of cancer of the upper digestive tract. No elevated risks were established in organs outside the respiratory and upper digestive tract.

Chapter 11

SAMENVATTING EN CONCLUSIES

In dit proefschrift wordt de epidemiologie van het plaveiselcelcarcinoom (PCC) van de slijmvliezen van de lip en de mondholte in een Nederlandse populatie beschreven. In **hoofdstuk 1** worden een algemene inleiding op het onderzoek en een uiteenzetting van de belangrijkste doelstellingen gegeven. Bovendien wordt aan de hand van de literatuur een overzicht gegeven van de epidemiologie en etiologie van het PCC van de lip en de mondholte.

De incidentie van kanker van de lip en de mondholte varieert wereldwijd en wordt deels bepaald door de geografische ligging, het ras en de gewoonten. De hoogste incidentie voor kanker van de lip komt voor in Zuid-Australië, terwijl kanker van de mondholte het meest voorkomt in delen van Frankrijk en India. In Nederland komt slechts 1 tot 2 % van alle kwaadaardige afwijkingen in de lip en de mondholte voor. Jaarlijks worden ongeveer 550 plaveiselcelcarcinomen van de lip en de mondholte geregistreerd. Tabak en alcohol worden beschouwd als onafhankelijke risicofactoren. Naast het roken van sigaretten worden er verschillende andere vormen van tabaksgebruik geassocieerd met het PCC van de lip en de mondholte, zoals het roken van sigaren of pijp, het roken met het brandende uiteinde van de sigaret/sigaar in de mond ("reverse smoking") en het gebruik van tabak voor oraal gebruik ("smokeless tobacco"). Beroep, voeding, gebitsstatus, endogene factoren en virale infecties worden eveneens als etiologische factoren van het PCC van de lip en de mondholte beschouwd.

In **hoofdstuk 2** wordt het verwijzingspatroon van 140 patiënten met een mondslijmvliesafwijking beschreven. De patiënten met een mondslijmvliesafwijking bleken hiervoor even vaak de tandarts als de huisarts als eerste te consulteren. In geval van klachten gebaseerd op een PCC werd de huisarts significant vaker geraadpleegd. Huisartsen verwezen patiënten met slijmvliesafwijkingen vaker naar een medisch specialist dan naar een tandarts of kaakchirurg. De tandarts verwees eigenlijk altijd naar de kaakchirurg.

In **hoofdstuk 3** wordt van 50 patiënten de periode tussen het optreden van de eerste symptomen en de uiteindelijke diagnose van het PCC van de mondholte -"delay"- beschreven. De meeste patiënten consulteerden de huisarts en niet de tandarts. In 7 gevallen ontdekte de tandarts het PCC tijdens een halfjaarlijkse controle. De diagnose van het PCC werd bij patiënten jonger dan 40 jaar later gesteld dan bij oudere patiënten. Patiënten wachtten gemiddeld drie maanden voordat ze professionele hulp zochten. Met betrekking tot de tijdsduur tussen het eerste consult van de patiënt en de definitieve diagnose, bleek er geen significant verschil te bestaan tussen verwijzing respectievelijk via de tandarts en de huisarts. Geslacht,

gebitsstatus, localisatie en grootte van de tumor hadden geen significante invloed op het "delay". Aanbevolen wordt dat de tandarts de slijmvliezen van de lip en de mondholte regelmatig controleert, zodat (pre)maligne afwijkingen in een vroeg stadium kunnen worden opgespoord.

In **hoofdstuk 4** worden de epidemiologische gegevens van 740 patiënten met een PCC van de lip en de mondholte beschreven. De resultaten komen overeen met die van de Westerse literatuur. De onderzoekspopulatie omvatte 473 mannen en 267 vrouwen met een gemiddelde leeftijd van 63 jaar. Vrouwen met een PCC waren ten tijde van de diagnose gemiddeld 7 jaar ouder dan de mannen. Van de 32 patiënten die jonger dan 40 jaar waren, waren er 30 van het mannelijke geslacht. De man-vrouw-verhouding was 1.8 en varieerde van 8.3 voor de onderlip tot 0.9 voor het slijmvlies van de processus alveolaris superior. De tong en mondbodem waren het meest frequent aangedaan. Er bleek geen significant verschil te bestaan tussen leeftijd of geslacht vergeleken met het T-stadium van de tumor. Daarentegen bleek de localisatie van de tumor wel geassocieerd te zijn met het T-stadium. Tumoren van de lip bleken ten tijde van de diagnose namelijk significant kleiner te zijn dan tumoren in andere localisaties.

In **hoofdstuk 5** wordt de relatie beschreven tussen het gebruik van tabak en alcohol enerzijds en de anatomische localisatie van het PCC van de lip en de mondholte anderzijds. Het bleek dat het optreden van een PCC in de mucosa van het trigonum retromolare en de mondbodem het sterkst geassocieerd werd met het rook- en drinkgedrag, terwijl het PCC van het wangslijmvlies het minst geassocieerd werd met het genoemde gedrag. PCC van het slijmvlies van het onderste gedeelte van de mondholte (mondbodem, trigonum retromolare en onderkaak) werd meer gerelateerd aan het gebruik van tabak en alcohol dan de overige localisaties in de mondholte. De mogelijke locale en systemische effecten die hiervoor verantwoordelijk kunnen zijn, worden beschreven.

In **hoofdstuk 6** wordt het tumor suppressor eiwit p53 gerelateerd aan de rook- en drinkgewoonten van 40 patiënten met een PCC van het tongslijmvlies. Dit eiwit controleert de celvermeerdering en bij afwezigheid en/of mutatie van dit eiwit zou een ongecontroleerde celgroei kunnen ontstaan. In de literatuur wordt de afwezigheid en/of mutatie van het tumor suppressor eiwit p53 geassocieerd met rook- en drinkgewoonten. In het huidige onderzoek werd bij 30% van de tumoren p53 aangetoond. In tegenstelling tot eerdere bevindingen in de literatuur, bleek echter dat de p53 positieve tumoren zowel bij patiënten met als zonder rook- en drink

gewoonten voorkwamen. De mogelijke verklaringen voor deze discrepantie worden in het hoofdstuk beschreven.

In **hoofdstuk 7** worden de mogelijke genetische factoren in de etiologie van het hoofd-hals PCC beschreven. Hiervoor werd zowel aan de patiënt als aan de partner van de patiënt (referentiegroep) gevraagd naar het voorkomen van kanker bij hun broers, zusters en ouders. Onder de broers, zusters en ouders (N= 617) van de 105 patiënten met een hoofd-hals PCC waren er 31 gevallen van kanker van de luchtwegen en de bovenste voedselwegen, versus 10 gevallen in de referentiegroep (N= 409). Er bleek een relatief risico (RR) van 3,5 te bestaan (p= 0.0002). Als alleen naar het voorkomen van kanker bij broers en zusters werd gekeken, was het relatieve risico zelfs 14,6 (p= 0.0001). Een tweede bevinding was dat bij de tumor-positieve familieleden van patiënten met een larynx PCC de meerderheid longkanker had (77%). De tumor-positieve familieleden van de patiënten met een mondholte- en pharynx PCC bleken daarentegen voornamelijk tumoren van de bovenste voedselwegen (56%) te hebben ontwikkeld. Dit komt overeen met het patroon van de localisatie van een tweede primaire tumor bij patiënten met een hoofd-hals PCC en suggereert dat verschillende genetische factoren een rol spelen in de carcinogenese van het PCC van de long en larynx vergeleken met het PCC van de mondholte en pharynx.

In **hoofdstuk 8** wordt de incidentie van tweede primaire tumoren in de luchtwegen en bovenste voedselwegen beschreven na het ontwikkelen van een primair PCC van de lip en de mondholte. Bij nacontrole van 727 patiënten bleken 74 patiënten (10%) minstens één tweede primaire tumor te hebben ontwikkeld. Gedurende 1.000 follow-up jaren traden 28 tweede primaire tumoren op. Patiënten hadden een constant risico van 2,8% per jaar op het ontwikkelen van een tweede primaire tumor in een periode van ten minste 10 jaar.
De incidentie van tweede primaire tumoren bleek geassocieerd te zijn aan de localisatie van de index-tumor. Significant meer tweede primaire tumoren ontwikkelden zich na een index tumor in het slijmvlies van de mondbodem, het trigonum retromolare en de processus alveolaris inferior ten opzichte van de overige localisaties in de mondholte. Tevens bleek het voorkomen van tweede primaire tumoren significant toe te nemen met de intensiteit van het rookgedrag.

Tenslotte wordt in **hoofdstuk 9** voor patiënten met een PCC van de lip en de mondholte het risico beschreven op het optreden van een tweede of volgende tumor, afgezet tegen het risico voor het optreden van een kwaadaardige tumor in de Nederlandse bevolking. Voor de risicoanalyse werd enerzijds de nacontrole van 727

patiënten met een PCC van de lip en de mondholte en anderzijds de kankerincidentiecijfers van een deel van de Nederlandse populatie gebruikt. De resultaten geven aan dat patiënten met een PCC van de lip en de mondholte, vergeleken met de Nederlandse bevolking, een sterk verhoogd risico hebben op het ontwikkelen van een tweede of volgende tumor van de mondholte of de pharynx, namelijk 74,7 voor de man en 190,4 voor de vrouw. Bovendien blijkt een verhoogd risico te bestaan voor het ontwikkelen van een maligniteit van de slokdarm (risico's van respectievelijk 24,6 en 45,3 voor de man en vrouw). De risico's op een maligniteit in de overige organen van de luchtwegen en de bovenste voedselwegen waren minder sterk verhoogd. Tenslotte werd er geen verhoogd risico gezien voor het ontwikkelen van een maligniteit buiten de luchtwegen en de bovenste voedselwegen.

CURRICULUM VITAE

De auteur van dit proefschrift werd op 2 april 1965 geboren te Isny in Duitsland. In 1984 behaalde hij het eindexamen aan het Gymnasium Erasmianum te Rotterdam. In datzelfde jaar werd begonnen met de studie tandheelkunde aan het Academisch Centrum Tandheelkunde Amsterdam (ACTA). Het tandartsexamen werd in 1989 behaald. Op 1 februari 1990 is hij gestart met het promotieonderzoek op de afdeling Mondziekten en Kaakchirurgie en Orale Pathologie, Vrije Universiteit/ ACTA te Amsterdam (hoofd: Prof. dr I. van der Waal).
In 1991 behaalde hij het doctoraalexamen van de studie geneeskunde aan de Universiteit van Amsterdam (UvA). Sinds 1 januari 1992 is hij in opleiding tot specialist in de Mondziekten en Kaakchirurgie (opleider: Prof. dr I. van der Waal).

STELLINGEN

behorend bij het proefschrift

SQUAMOUS CELL CARCINOMA OF THE LIP AND THE ORAL CAVITY

An epidemiological study

Amsterdam, 3 december 1993

A. Jovanovic

1. Binnen de mondholte zijn het plaveiselcelcarcinoom van het trigonum retromolare en de mondbodem het sterkst gerelateerd aan het rook- en drinkgedrag.

   Dit proefschrift

2. De expressie van het tumor suppressor gen p53 en de veronderstelde relatie met het rook- en drinkgedrag staat ter discussie.

   Dit proefschrift

3. Het risico op een tweede primaire tumor in de luchtwegen en bovenste voedselwegen is gedurende minstens tien jaar na een plaveiselcelcarcinoom van de lip en de mondholte constant van grootte.

   Dit proefschrift

4. Patiënten met een plaveiselcelcarcinoom van de lip en de mondholte hebben geen verhoogd risico op een tweede primaire tumor buiten de luchtwegen en bovenste voedselwegen.

   Dit proefschrift

5. De tandarts draagt een grote verantwoordelijkheid bij de vroege diagnostiek van mondkanker.

6. Gezien de toegenomen hoeveelheid soorten osteosynthese-materialen wordt het tijd dat nieuwe typen fracturen in het cranio-faciale gebied worden geclassificeerd.

7. Het gebruik van "smokeless tobacco" in Zweden staat vooralsnog het toetreden van Zweden tot de EEG in de weg.

8. Endocarditis profylaxe sluit het ontwikkelen van een bacteriële endocarditis niet uit.

9. "Pret-echo's" geven de aanstaande ouders een schijnveiligheid en zorgen voor onnodig medicalisering van zwangerschap en geboorte.

10. Het hoopvolle woord wapenstilstand heeft sinds de burgeroorlog in voormalig Joegoslavië veel van zijn kracht verloren.

11. In Nederland wordt het milieubeleid gemaakt en uitgevoerd door adviesbureaus.

12. Degene die snurkt valt vaak als eerste in slaap.