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Histological Differentiation of Oral Squamous Cell Cancer in Relation to Tobacco Smoking

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The aim of this study was to assess the potential effect of tobacco and alcohol consumption on the histological differentiation of oral squamous cell carcinomas in 161 consecutive patients. The patients were included in a prospective study to secure valid data on tobacco and alcohol consumption. The histopathological grading system included eight morphological qualities describing both the tumour cell population and the interaction between tumour and host. A mean histological score was calculated as the arithmetic mean of the scored individual morphological parameters. Tobacco consumption, as opposed to alcohol consumption, was shown to be significantly correlated with the mean histological score ($P=0.0009$), and with the four morphological qualities describing the tumour cell population: pattern ($P=0.0044$), cytoplasmic differentiation ($P=0.0008$), nuclear differentiation ($P=0.0054$) and mitosis ($P=0.0001$). Thus, tobacco consumption seems to cause the tumour cells of oral squamous cell carcinomas to undergo a more pronounced dedifferentiation which makes them more aggressive. This effect is enhanced with increasing exposure to tobacco smoke.

Keywords: histological grading, tobacco smoking, oral cancer

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INTRODUCTION

CARCINOGENESIS IS considered to be a multistep process where several initiating and promoting processes interact and a number of aberrant genetic events are involved in the malignant transformation. Several studies have sought to identify common pathways and markers in this multistep progression to malignancy. They have been motivated mainly by the investigators' wish to become able to intervene prophylactically, but also by the prospect of selecting patients for optimal therapy which could improve the course of this disease.

Epidemiological studies [1, 2] have shown a strong association between the development of oral cancer and tobacco and alcohol consumption.

The literature reports excess expression of the p53 protein in head and neck tumours [3], an association between excess expression of p53 and smoking history [4] and an association between the p53 level and the degree of tumour differentiation [5]. In recent years, head and neck cancers have been evaluated histopathologically and their biological aggressiveness classified in terms of their differentiation according to a method originally devised by Broders [6]. Such multifactorial grading systems, which include a number of morphological parameters and imply an evaluation of both tumour cell population and tumour/host interaction are often employed to supplement TNM suggestions for treatment strategy and prediction of disease course. The prognostic value of different malignancy grading systems varies [7]. Holm *et al.* [8] have shown a positive correlation between tumour size and histological grading in patients with cancer of the tongue. Others [9] have demonstrated a higher frequency of lymph node metastasis in cancer of the lip with increasing histological grade, according to the modified system of Jacobsson [10].

We have used the same histological grading system for all head and neck cancers for a number of years and have partly relied on this grading when designing our therapeutic strategies.

The purpose of this study was to examine the association between precisely monitored tobacco and alcohol exposure and the degree of histological differentiation of intra-oral squamous cell carcinoma, in well-described consecutive material of 161 patients.

PATIENTS AND METHODS

Clinico-epidemiologic and histopathological data were assessed by independent observers.

The study population included 161 patients treated for intra-oral squamous cell carcinoma at the Aarhus University Hospital during the period 1 January 1986 to 1 November 1990. This period saw the treatment of a total of 162 patients at

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Table 1. Histological grading of 152 oral squamous cell carcinomas. Each of the eight parameters graded from 1 to 4

Grading	1 Pattern	2 Cytoplasmic differentiation	3 Nuclear differentiation	4 Mitosis	5 Mode of invasion	6 Depth	7 Vascular invasion	8 Cellular response
1	27	26	19	49	14	7	50	29
2	65	95	63	57	62	30	51	75
3	58	30	68	42	70	34	10	42
4	1	1	2	4	5	42	—	3
Total	151	152	152	152	151	113	111	149

Table 2. Correlation between tobacco consumption and morphological histopathological parameters in patients with oral squamous cell carcinoma

	N	Tobacco consumption Spearman's rank correlation coefficient	*P*-value
Histological score	144	0.2734	0.0009
Pattern	143	0.2371	0.0044
Cytoplasmic differentiation	144	0.2752	0.0008
Nuclear differentiation	144	0.2309	0.0054
Mitosis	144	0.3304	0.0001
Mode of invasion	143	0.0634	0.4518
Depth	105	0.1656	0.0913
Vascular invasion	104	0.1816	0.0650
Cellular response	141	0.1513	0.0733

the hospital, which has a catchment area of approximately 1.4 million inhabitants. All 161 patients were allocated to a prospectively designed combined clinico-experimental and epidemiological study. The clinical examination involved a meticulous examination of the oral cavity and TNM classification of the tumour. Furthermore, the patients' tobacco and alcohol consumption was assessed. The patients were asked to fill in a questionnaire on their daily consumption of cigarettes, cheroots, cigars and pipe tobacco. Tobacco exposure was expressed as gram tobacco per day (cigarette equivalents: 1 cigarette was set to the equivalent of 1 g of tobacco, 1 cheroot 1.2 g, 1 cigar 8.0 g and 1 pack of pipe tobacco 50 g). We also assessed the patients' daily alcohol exposure. Alcohol consumption was assessed as the numbers of drinks per day: 1 beer was set to the equivalent of 1 drink, 2 cl of hard liquor 1 drink, and 1 bottle of wine 6 drinks. In 8 patients information about the consumption of tobacco was not available.

Histopathology

The histological grading was based on pretreatment biopsy material. All biopsies were graded by the same pathologist who used the grading system described by Jacobsson *et al.* [10], as modified by Lund *et al.* [11]. Eight individual morphological parameters were assessed: (1) growth pattern, (2) cytoplasmic differentiation, (3) nuclear differentiation, (4) mitosis, (5) mode of invasion, (6) stage of invasion (depth), (7) vascular invasion and (8) cellular response. Each parameter was given a score value in the range 1–4 and ultimately a mean histological score was calculated as the arithmetic mean of the scored individual morphological parameters. This system both describes the tumour cell population (parameters 1–4) and the interaction between tumour and host organism (parameters 5–8). Histopathological grading was performed on a total of 152 patients. We were unable to measure all eight parameters in all the patients. The distribution of the individual parameters is shown in Table 1.

Statistics

Spearman's rank correlation coefficient was used to test for a statistically significant association between tobacco and alcohol consumption on the one hand and histological characteristics on the other. The Kruskall–Wallis test with correction for multiple comparisons was used to test differences in the distribution of tobacco consumption between any two anatomical localisations within the oral cavity. Statistical significance was claimed for a *P*-value less than 0.05.

RESULTS

Table 2 shows Spearman's rank correlation coefficients for the association between tobacco consumption and histological score including the values for the eight individual morphological parameters. There was a significant correlation between increasing tobacco consumption and increasing grades of the four morphological parameters describing the tumour cell population. We also note a significant correlation between the histological mean score and tobacco consumption.

The relationship between tobacco consumption and the morphological parameters mitosis and cytoplasmic differentiation is depicted in Fig. 1.

One hypothesis could be that smokers may develop cancers in certain anatomical localisations within the oral cavity and that differences in the histopathological characteristics of tumours in these sites could account for the observed

association. This was tested by a Kruskall–Wallis test. Five groups were defined: buccal mucosa, upper alveolus and hard palate, lower alveolus and retromolar trigone, tongue and floor of mouth. When comparing these, the Kruskall–Wallis test statistic was 5.89 indicating that there was no statistically significant difference in the distribution of tobacco consumption between the five groups ($P=0.21$). A pairwise comparison between the five groups showed no statistically significant difference between any two of them.

The correlation between the histological mean score and alcohol consumption was not significant ($P=0.065$). Only the association between the morphological mitotic activity score and alcohol consumption came out as statistically significant ($P=0.004$). This is in spite of the association between tobacco and alcohol exposure in this study population as demonstrated in an earlier study [12].

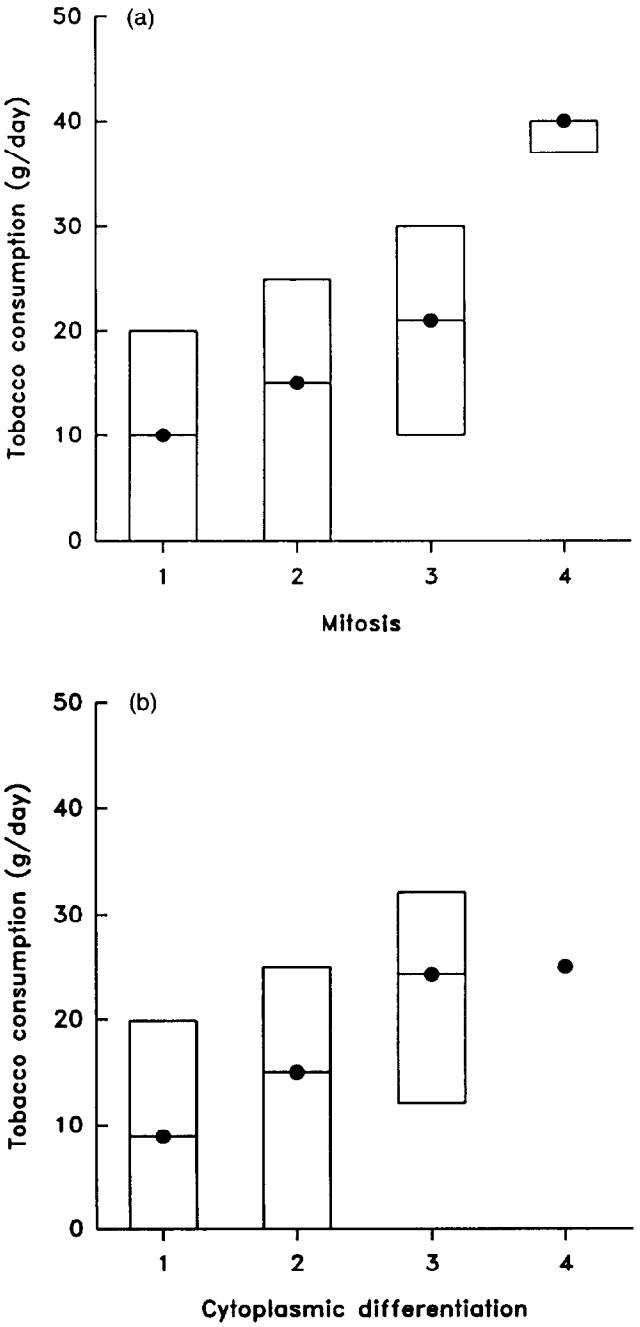

Fig. 1. Tobacco consumption for tumours with various grades of mitosis (a) and cytoplasmic differentiation (b). Filled circles indicate median consumption, top and bottom lines of the boxes indicate the upper and lower quartiles of the distribution.

DISCUSSION

Epidemiological studies have firmly established the aetiological importance of both tobacco and alcohol consumption in intra-oral squamous cell carcinoma. However, it remains unclear at exactly which level of carcinogenesis tobacco exercises its role. Previous studies have shown an association between tobacco smoking and excess expression of the p53 protein, which would seem to indicate that the *p53* gene could be a target for specific mutagens in tobacco smoke [5]. Other studies have established an association between histological differentiation and excess expression of p53 protein [5], and p53 has been found to be associated with the histopathological malignancy grade [3]. Components in tobacco smoke presumably have a differential effect on the cell proliferation that prompts the expansion of preneoplastic and neoplastic squamous epithelial cells during carcinogenesis [13].

We found an association between histological score and sex ($P=0.005$). We have previously demonstrated a highly significant association between tobacco, alcohol and sex, as men are generally more exposed to tobacco and alcohol than women [12].

Remarkably, there was a significant association between tobacco exposure and the morphological parameters describing the tumour cell population, but no such significant association could be demonstrated between tobacco exposure and the parameters describing tumour–host interactions. This indicates that the effect of tobacco is directly reflected in the histopathological tumour characteristics.

The association between alcohol/tobacco consumption and mitotic activity is supported by a study of oral cancer in women [14] in which users of tobacco and alcohol had a significantly higher mitotic activity than non-users. A stratification of the present study population into users and non-users showed a borderline significant difference in the morphological score of mitotic activity ($P=0.056$) [15].

All data on tobacco and alcohol consumption were gathered at the time of diagnosis and all biopsies were graded before treatment, i.e. without any knowledge of the course of the disease. All biopsy specimens were graded by the same pathologist and the pathologist was without any knowledge of the tobacco and alcohol consumption. Any such bias may, therefore, be excluded.

It remains a conjecture whether a genetic link is instrumental in the association between exposure to tobacco smoke and development of oral cancer or if the dedifferentation process prompting carcinomatous development is indeed initiated by elements in tobacco smoke (and promoted by alcohol, among others) and becomes more pronounced the greater the exposure, as suggested in this work. If this is the case it is important to focus on tobacco as an actiologic factor, not least because our results establish that tumours become biologically more aggressive with increasing tobacco exposure which in turn aggravates the prognosis. Further evidence to this effect has been published in a paper which demonstrates that tobacco consumption is an independent prognostic parameter in intra-oral squamous cell carcinoma [12].

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