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Analysis of histopathological and immunohistochemical differences of oral squamous cell carcinoma in young and old patients in Sri Lanka

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BACKGROUND: Oral squamous cell carcinoma (OSCC) most commonly occurs in the middle-aged and older individuals. The purpose of the present study was to evaluate the histopathological and immunohistochemical differences of the younger (<40 years) and the older (more than 50 years) groups.

METHODS: The histopathological parameters of 112 patients (younger 56 and older 56) were compared according to three grading systems (Broder *JAMA* 1920; 74: 656, Anneroth et al. *Scand J Dent Res* 1987; 95: 229, Bryne et al. *J Pathol* 1992; 166: 375) and as individual histopathological parameters. Further, the expression of p53 and Proliferative Cell Nuclear Antigen (PCNA) index was also compared.

RESULTS: Although there was no significant difference between two groups regarding the three grading systems, a significantly higher number of nuclear aberrations was found in younger group ($P < 0.001$). Interestingly, higher number of mitoses ($P < 0.05$) and lymph node metastasis ($P < 0.05$) were observed in the older group ($P < 0.05$). Furthermore, significantly a higher PCNA index was found in the older group ($P < 0.005$).

CONCLUSIONS: Although tumours of the young patients showed more nuclear aberrations, OSCC of the older patients is proliferative and showed higher metastatic rate.

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Introduction

Cancers of the oral cavity accounted for 274 000 cases in 2002, with almost two-thirds of them occurring in men (1). In countries such as Sri Lanka, India, Pakistan and Bangladesh, oral cancer is the most common cancer. In developed countries, oral cancer is known to be less common. However, in some developed countries a high incidence of oral cancer has been reported in isolated areas (2). Oral squamous cell carcinoma (OSCC) typically occurs in the elderly men during the fifth to eighth decade of life (1). The incidence of oral cancer in younger patients is reported to be approximately 6% of all oral cancers in the United Kingdom (3) and the same was 4.5–5.5% for the Sri Lankan population (4). In the present study, we selected two groups based on the age of the patient as younger (< 40 years) and older (> 50 years).

The histopathological grading of tumours has been used for many decades in an attempt to predict the clinical behaviour of squamous cell carcinoma. Very few studies have analysed the pathology of these lesions to confirm whether these lesions are histologically similar (5). Alterations of cell cycle proteins contribute to the biological behaviour of cancers. Studies regarding cell cycle regulators comparing the young and the old are sparsely available in the literature (6). After the p53 tumour suppressor gene plays multiple integral roles in apoptosis, cell cycle control and DNA repair (7). Although there were very few studies in the English literature comparing two groups based on the pathological and molecular difference (5, 6, 8): the sample size

Table 1 Different grading systems for squamous cell carcinoma

Broder's grading system – tumour area only

Grade	*Differentiated (%)*	*Undifferentiated (%)*
I	100–75	0–25
II	75–50	25–50
III	50–25	50–75
IV	25–0	75–100

Anneroth et al.'s grading system (1987) – whole tumour area, including connective tissue stroma

	Score 1	*Score 2*	*Score 3*	*Score 4*
Degree of keratinization	Highly keratinized	Moderately keratinized	Minimal keratinization	No keratinization
Presence of nuclear aberrations	Few	Moderate	Abundant with anaplastic nuclei	Abundant with many anaplastic nuclei
Number of mitoses (mean value of 4 high power field)	Few (0–2) cells	Moderate (3–4) cells	Numerous (5–6) cells	Extremely numerous (more than 6) cells
Pattern of invasion	Large islands with a pushing border	Small islands	Thin strands (< 5 cell thickness)	Individual tumour cells
Inflammatory response	Dense	Moderate	Light	None

Bryne et al.'s grading system (1992) – invasive front area, including connective tissue stroma

Degree of keratinization	Highly keratinized	Moderately keratinized	Minimal keratinization	No keratinization
Presence of nuclear aberrations	Few	Moderate	Abundant with anaplastic nuclei	Abundant with many anaplastic nuclei
Pattern of invasion	Large islands	Small islands with a pushing border	Thin strands (< 5 cell thickness)	Individual tumour cells
Inflammatory response	Dense	Moderate	Light	None

was smaller. Therefore, it is worth to compare the tumour behaviour of above two age groups, histopathologically and immunohistochemically in a large cohort of patients.

Methods

Archived paraffin-embedded tissue specimens from 112 previously untreated patients with OSCC were obtained from the Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka. The sample comprised two groups from both the genders based on the age of the patient. The sample was divided into two, as young (< 40 years) and old (> 50 years). The older group was randomly selected during the same time period that the young cancers were received.

The sections from all cases were stained with haematoxylin and eosin (H & E). A representative section containing the full thickness of the tumour (including invasive front), together with other sections were used for histopathological gradings. Broder's (9), Anneroth et al.'s (10) and Bryne et al.'s (11) classification systems (Tables 1 and 2) were used to assess the histopathological parameters. Details of the lymph node metastasis were gathered from the patients' records.

Immunohistochemical analysis

The tumour tissues (36 cases were selected randomly from each group) were cut into 4 μM thick sections. For immunohistochemical examination of p53 and Proliferative Cell Nuclear Antigen (PCNA), the EnVision + system (Dako, Glostrup, Denmark) was used. The tissue sections were deparaffinized and rehydrated in a graded series of alcohols. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol for 30 min. The sections were microwaved three times for 5 min each in citrate phosphate buffer (pH 6.0) for antigen retrieval. The sections were then incubated with protein block serum-free medium for 10 min to block non-specific binding. Monoclonal antibodies of p53 (Novocastra Lab., Newcastle Upon Tyne, UK) and PCNA (Dako, Carpinteria, USA) were applied as primary antibodies and incubated at 4°C overnight. After washing with PBS, peroxidase-conjugated secondary antibody was applied to the sections, which were then incubated for 1 h at room temperature. Peroxidase was visualized with diaminobenzidine (DAB). Sections were counterstained with Mayer's haematoxylin, dehydrated and mounted. Expression of p53 protein was assessed as (+) where more than 30% of the tumour cells were positive and (–) if it is < 30% or completely negative.

Table 2 Scoring system of both Anneroth et al.'s and Bryne et al.'s grading systems

	Score	*Grade*
Anneroth et al.	5–10	I (well differentiated)
	11–15	II (moderately differentiated)
	16–20	III (poorly differentiated)
Bryne et al.	4–8	I (well differentiated)
	9–12	II (moderately differentiated)
	13–16	III (poorly differentiated)

PCNA index

Three PCNA-positive areas were selected, and the selected fields were microphotographed. The positive cells and the total number of cells were counted (using a ADOBE PHOTOSHOP software), and the index was taken as the number of positive cells per total number of the cells. The average was taken from three sites of each tumour.

Statistical analysis

The correlation between the young and the old groups was tested by the chi-squared test. PCNA index was analysed by *T*-test. A *P*-value of < 0.05 was considered as significant.

Results

We previously published the clinical data on these two age groups (4). Although the prognosis-based recent grading system is Bryne et al.'s (11), we used all the three grading systems to find out whether there is any difference in the two age groups. A statistically significant difference was not found between the two age groups according to Broder's, Anneroth et al.'s and Bryne et al.'s grading systems (Table 3; 9–11).

Some authors described that individual parameters have a prognostic value, hence we examined the individual histopathological parameters (12) in the whole tumour area, according to the method of Anneroth et al. (Table 1; 10). Anaplasia or loss of differentiation is a characteristic feature of cancer cells, and it consists of morphological and functional changes. Interestingly, a significantly higher number of nuclear aberrations was found in the younger patients compared with the older patients ($P < 0.001$). Some authors have suggested that cancer in the young adults tends to be more frequently anaplastic resulting in a more aggressive behaviour and poor prognosis (13, 14). We found that well, moderately and poorly differentiated carcinomas are more or less equally distributed within the two age groups. The differentiation was graded according to Anneroth et al.'s criteria (Table 2; 10).

In the present study, we found a significantly increased number of mitoses in the older group ($P < 0.05$, Table 3). The PCNA index was also significantly higher in the same group ($P < 0.005$, Fig. 1). Therefore, we can postulate that the OSCC of the older group is more proliferative, compared with the younger patients. To support the above statement further, a significantly higher number of metastasis was found in the older group ($P < 0.05$, Fig. 2).

A significant difference was not found with regard to the pattern of invasion, keratinization and inflammatory response. Positive p53 staining was 52.7% (19 of 36) and 55.5% (20 of 36) in the young and the old patients, respectively (Table 4, Fig. 3). The above results were not significant statistically.

Discussion

There are reasons to believe that the aetiology and pathogenesis of the OSCC may be different in younger

Table 3 Distribution of histopathological parameters within two age groups

	Number (%) in each group		
Factor	*Young (< 40 years)*	*Old (> 50 years)*	*P-value*
Broder's grade			
I	17 (30.35)	22 (39.28)	ns
II	19 (33.92)	19 (33.92)	
III	19 (33.92)	12 (21.42)	
IV	1 (1.78)	3 (5.35)	
Anneroth et al.'s grade			
I	25 (44.6)	26 (46.4)	ns
II	26 (46.4)	23 (41.7)	
III	5 (8.9)	7 (12.5)	
Bryne et al.'s grade			
I	25 (44.6)	24 (42.8)	ns
II	23 (41.0)	26 (46.4)	
III	8 (14.2)	6 (10.7)	
Pattern of invasion			
I	6 (10.7)	7 (12.5)	
II	13 (23.2)	15 (26.7)	
III	25 (44.6)	21 (37.5)	
IV	12 (21.4)	13 (23.2)	
Degree of keratinization			
High	14 (25)	12 (21.4)	ns
Moderate	26 (46.4)	34 (60.7)	
Light	11 (19.6)	6 (10.7)	
None	5 (8.9)	4 (7.1)	
Nuclear aberrations			
Few	12 (21.4)	25 (44.6)	< 0 001[a]
Moderate	27 (48.2)	25 (44.6)	
Abundant	12 (21.4)	2 (3.5)	
Abundant + anaplastic	5 (8.9)	4 (7.1)	
Number of mitoses			
Few (0–2)	21 (37.5)	20 (35.7)	< 0.05[a]
Moderate (3–4)	23 (41.7)	18 (32.1)	
Numerous (5–6)	11 (19.6)	12 (21.4)	
Ex numerous (> 6)	1 (1.7)	6 (10.7)	
Host immune response			
Dense	13 (23.1)	10 (17.8)	ns
Moderate	22 (39.2)	22 (39.2)	
Light	14 (25.0)	22 (39.2)	
None	7 (12.5)	2 (3.5)	

[a]Chi-squared test.
ns, not significant.

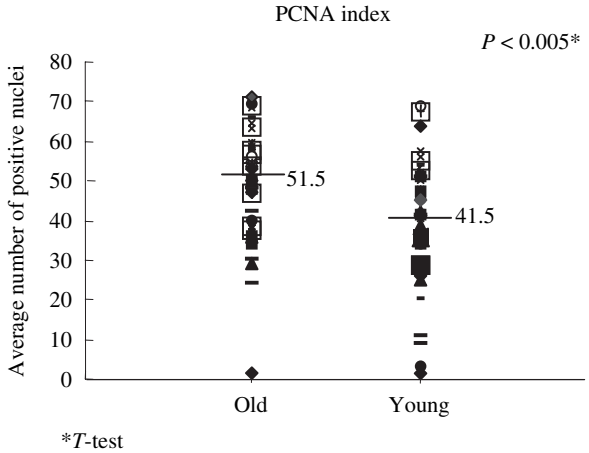

Figure 1 Proliferative Cell Nuclear Antigen index (PCNA) shows the average number of positive nuclei for PCNA per total number of nuclei. Older patients had higher proliferative index compared with younger patients.

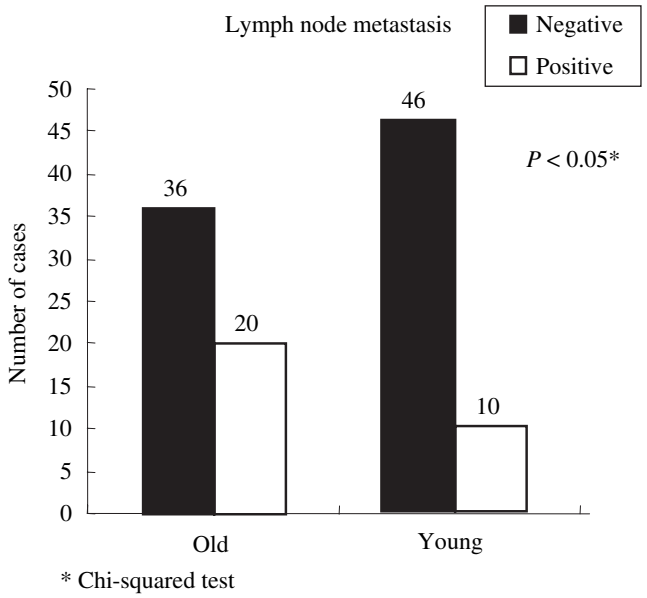

Figure 2 Nodal metastasis of two age groups. Older group shows higher metastatic rate than their younger counterparts and the difference is statistically significant.

Table 4 Immunohistochemical differences of younger and older patients

	Young (< 40 years)	*Old (> 50 years)*	*P-value*
p53			
−	17	16	ns
+	19	20	
Proliferative Cell Nuclear Antigen (average)	41.5	51.5	0.005[a]

[a] *T*-test.

vs. older patients. A shorter duration of exposure to environmental carcinogens and lack of pre-existing lesion in the former suggest the possibility of different molecular mechanisms in two groups. A few studies on the squamous cell carcinomas on malignancy grading with different clinical parameters, such as clinical staging, recurrence and prognosis have been published (4, 5, 10, 15). We retrospectively reviewed the pathology of lesions from young patients with the squamous cell carcinoma of the oral cavity, and compared them with the OSCC from the older patients in order to determine if there were any defining characteristics in the younger population. In this study, we used three histopathological grading systems.

Broder initiated the quantitative grading of cancer in 1920 (9), and his classification system has been in use for many years. However, lack of correlation between Broder's grading system and prognosis has been mentioned. One of the main reasons for the above could be that, the squamous cell carcinoma usually exhibits a heterogeneous cell population (10, 15). In 1987, Anneroth et al. modified the multifactorial grading system developed by Jakobsson et al. (16) in order to obtain a more precise morphological evaluation of the growth potential of the squamous cell carcinoma in the head and neck region. This system included not only an analysis of the cell population of cancer, but also an evaluation of the tumour host relationship. Recently, Bryne et al. introduced a multifactorial grading system only for the deep invasive margins of the squamous cell carcinoma, which proved to be of high prognostic value (11).

In the present study, despite the use of different histopathological grading systems, two groups are more or less equally distributed within each grade in each classification. Further, we found that the differentiation

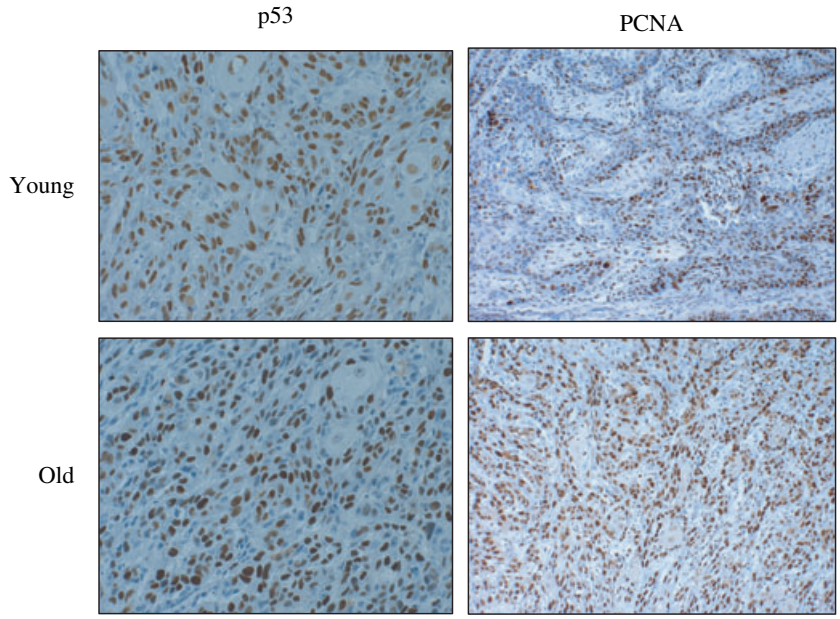

Figure 3 A representative case of p53 protein and Proliferative Cell Nuclear Antigen expression in younger and older groups (×100).

of the tumours (well, moderate, poor) showed no difference between the two groups. In contrast, some authors described that a majority of the tumours of the young adults were well differentiated (5, 17). So far, there are no studies comparing the young and the old OSCC with regard to individual histological parameters in the literature. We analysed the differences of the individual parameters of Anneroth et al.'s grading between the two age groups. It is interesting to note that significantly higher nuclear aberrations were observed in the younger patients. Of the few studies of the squamous cell carcinoma in young patients, some authors suggested that tumours in young patients could behave more aggressively (13, 14). We previously reported that a majority of the older group had betel chewing habits (betel quid ingredients are; piper betel leaf, areca nut, slake lime and tobacco). Areca nuts contain different kinds of alkaloids and are coline (18). Lin et al. demonstrated that areca nut extracts activate mitogen-activated protein kinases (18). In the present study, we found that a significantly higher number of mitoses in the older patients compared with the younger patients and a large majority of patients of the older group were betel chewers.

Generally, cervical lymph node metastasis in OSCC patients indicates a poor prognosis (17, 19). In the present study, a significantly higher rate of lymph node metastasis was found in the older group when compared with the younger group. The metastatic spread of a tumour is not a random process. A common pattern for carcinomas is that the regional lymph nodes are the first sites to develop metastases, either draining via the pre-existing afferent lymphatic vessels and/or via the newly formed lymphatic capillaries (20, 21). Therefore, higher mitotic activity and higher PCNA index may proceed lymphatic invasion and that is in correlation with the higher lymph node metastasis in the older group of the patients. Although there has been no overall consensus about the difference in prognosis between young and old groups, more recent studies indicate that there may be improved survival among the younger individuals (22–24).

The inactivation of the p53 tumour suppressor gene is the most common genetic alteration in all cancer types (25), and is common in the squamous cell carcinoma with approximately 50% of the lesions expressing a mutant form of protein (26, 27). Detectable levels of the p53 protein are often due to the production of a mutant p53 gene or the stabilization of the wild-type protein. Although nearly 50% of the tumours overexpressed the p53 protein, we could not find any significant difference between the two age groups. Supportively, Regezi et al. (6) also reported that the p53 expression was not significant in their comparative study of young and old groups. Jin et al. (8) described that the incidence of LOH at 3, 9 and 17p regions was similar in both the younger and the older groups.

In conclusion, although the OSCC of the younger patients showed more nuclear aberrations, the older patients had more proliferative tumours with higher lymph node metastasis. Further, it is evident that the existing histopathological grading systems show that the two groups are equally distributed among them. Therefore, we suggest, studies of genome-wide analysis are necessary, other than histopathological features, in deciding the prognosis and survival of these two important categories.

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