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Histological characteristics of early-stage oral tongue cancer in young versus older patients: a multicenter matched-pair analysis

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Introduction

The mean age of diagnosis of new oral squamous cell carcinoma (OSCC) cases is around 60 years with less than 5% of all patients aged 45 years or younger (Taybos, 2003). Oral tongue squamous cell carcinoma (OTSCC) is the most common, and presents with the worst prognosis (Ng et al., 2017). Several risk factors have been associated with OTSCC, many of which appear to be more related to older age (Farquhar et al., 2018). Young OTSCC patients lack these risk factors or when present, they have much shorter exposure time to the risk factors, suggesting different genomic and molecular changes than seen in older OTSCC patients (Llewellyn et al., 2004).

Globally the increased incidence of OTSCC in the past four decades is partly attributable to increased cases among younger patients, with some countries experiencing up to four fold increase (Muller et al., 2008, Ng et al., 2017). Suggestions for this increase included oncogenic virus infection, exposure to yet unidentified mutagens, alterations in diet or oral microbiome and inherited genetic defects predisposing to OTSCC (Gu et al., 2019). However, conflicting reports about clinical behavior and prognosis of young OTSCC patients compared with older patients abound, as reviewed by different groups (Campbell et al., 2018, Paderno et al., 2018).

Previous studies have focused mainly on the clinical outcome and molecular characteristics, but not on the histopathological characteristics to explain the possible differences and similarities of OTSCC between young and older patients. The purpose of this study was therefore to compare several OTSCC epithelial and stromal histological features with independent prognostic potential like tumour budding (TB), depth of invasion (DOI) and worst pattern of invasion (WPOI) (Almangush et al., 2015, Almangush et al., 2014), tumour-stroma ratio (TSR) (Almangush et al., 2018) and stromal tumour-infiltrating lymphocytes (TIL) (Heikkinen et al., 2019), in addition to routine parameters like

tumour grade and perineural invasion (PNI) in patients with early-stage OTSCC between patients ≤ 45 years and those >60 years.

Methods

A previously studied cohort of 311 patients treated for early-stage (cT1-2N0M0) OTSCC from 1979 to 2009 at six university hospitals in Finland and Brazil was used (Almangush et al., 2018). Only 42 patients were aged ≤ 45 years. These cases were extracted and matched according to the clinical tumour stage (stage I or II), gender and centers of management with patients >60 -year-old. Patients aged 46-60 years were excluded because their cancer-related events rate were much more similar to the young patients than the elderly group.

The histopathological features extracted for comparison included those often included in pathology reports such as WHO tumor grade (Kademani et al., 2005), DOI (Brierley et al., 2017), PNI (Brandwein-Gensler et al., 2010), and WPOI (Brandwein-Gensler et al., 2010), in addition to TB (Almangush et al., 2014), TSR (Almangush et al., 2018), TILs (Heikkinen et al., 2019) and histologic risk score (HRS) (Brandwein-Gensler et al., 2010) (Table 1). These histopathologic features were scored as illustrated in Figure 1. The procedures for evaluating the parameters studied are outlined in the published studies (Tam et al., 2019, Almangush et al., 2015, Almangush et al., 2018, Heikkinen et al., 2019, Brandwein-Gensler et al., 2010). Cross-tabulation analysis of the differences in histopathologic characteristics and other variables between paired patients groups were done. Statistical significance was evaluated using McNemar (2x2 tables) or McNemar-Bowker (3x3 tables) tests as the compared groups were not considered independent, since the subjects were chosen based on similar characteristics. IBM SPSS software version 23 was used for statistical analysis.

Results

With minor differences, the WHO grade, depth of invasion and perineural invasion bear close resemblance in both groups (Table 1). Regarding other histological characteristics, the young group had more high-intensity TB (43% vs. 24%) and high HRS (76% vs. 64%) when compared with the

older patients. Similarly, higher WPOI (86% vs. 69%) and TSR (36% vs 31%) were noted more in young patients compared with older patients. Low TIL was observed in 14% of tumours in the young group compared with 17% in the older group. No statistically significant difference was observed between the two groups in all the parameters evaluated ($P>0.05$) (Table 1).

Discussion

Some workers reviewed the several conflicting reports regarding the prognosis of OTSCC in young patients in comparison to older patients among American patients (Campbell et al., 2018). They adduced several reasons for this incongruity, including lack of statistical power in studies, absence of a control group of older patients, and use of different survival end points thereby making comparison between studies difficult. In general, the more recent studies, with few exceptions, have reported better survival outcome for younger patients but the controversy still lingers (Campbell et al., 2018, Paderno et al., 2018).

We have earlier established that some histopathological characteristics are independently associated with prognosis of early-stage OTSCC patients (Almangush et al., 2014, Almangush et al., 2015, Almangush et al., 2018, Heikkinen et al., 2019). A previous study which compared stromal myofibroblast quantity in matched young and older OTSCC patients found no significant differences between both groups (Fonseca et al., 2014). Increased stromal myofibroblasts has been associated with poor prognosis in OTSCC (Bello et al., 2011). In a previous study, we suggested that stromal myofibroblasts are probably more influential in late-stage OTSCC than early stages (Almangush et al., 2014).

In this present study, no significant differences in the histopathological characteristics of early-stage OTSCC was seen between clinically matched young and elderly groups. Based on this, it is therefore not possible to suggest or conclude that the management protocol for early-stage OTSCC should be different between the two groups. This lack of difference in the histopathological features turns the attention to genomic and molecular differences. Some studies have reported lack of differences in the genomic features between young and older OTSCC patients based on whole exome-

sequencing and copy number analysis (Pickering et al., 2014). However, diverse copy number variations seemed to be related to worse prognostic phenotype within young patients (Gu et al., 2019). DNA ploidy studies have suggested increased genomic instability in young OTSCC patients with increased aneuploidy, tetraploidy and mean higher DNA index noted (Santos-Silva et al., 2011). A recent review on molecular testing concluded that OTSCC is essentially similar in young and older patients (Campbell et al., 2018). Genetic studies are usually limited to a small number of patients, and more needs to be done to establish the true nature of OTSCC in young patients, particularly early-stage disease.

In summary, this study did not find any significant differences in the histopathologic features of early-stage OTSCC between the young (≤ 45 years) and the elderly patients (>60 years) in our multi-institutional series. This invites further validation studies in larger cohorts of early-stage OTSCC. More refined genomic, metagenomics and molecular investigations in these patient groups are also warranted to elucidate whether their treatment protocols should be developed differently.

Compliance with Ethical Standards

This study was approved by the Finnish National Supervisory Authority for Welfare and Health (VALVIRA), the Brazilian Human Research Ethics Committee, and the Ethical Committee of University Hospital of Helsinki, Oulu, Kuopio, Turku, and Tampere in Finland and the A.C. Camargo Cancer Center, São Paulo, Brazil.

Conflict of Interest

None declared

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Author Contributions

IOB, AA contributed to conception, study design and data interpretation. **IH, CJ, RDC, LPK, AAM, IL, TS** contributed to data collection and assembly and interpretation. **PN** did the statistical analysis. **IL, TS** provided logistical support and work integrity. All the authors contributed to the drafting of the article, provided critical revision for important intellectual content, and approved the final version.

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Table 1: Frequency and percentage distributions of various tumor-related epithelial and stromal histopathologic characteristics among young (≤ 45 years) and old patients (> 60 years).

Variable	Young (≤ 45 yrs) (n=42)	Old (> 60 yrs) (n=42)	<i>P</i> value [*]
Tumor grade			0.948
Well-differentiated	13 (31%)	15 (36%)	
Moderately-differentiated	20 (48%)	18 (43%)	
Poorly-differentiated	9 (21%)	9 (21%)	
Depth of invasion[#]			0.804
Superficial	14 (33%)	16 (38%)	
Deep	28 (67%)	26 (69%)	
Perineural invasion			0.727
Absent	38 (91%)	36 (86%)	
Present	4 (9%)	6 (14%)	
Tumor budding[#]			0.077
Low intensity	24 (57%)	32 (76%)	
High intensity	18 (43%)	10 (24%)	
Histologic risk score^{##}			0.225
Low-risk (0)	1 (2.4%)	1 (2.4%)	
Intermediate-risk (1-2)	9 (21.4%)	14 (33.3%)	
High-risk (≥ 3)	32 (76.2)	27 (64.3%)	
Worst pattern of invasion			0.065
Cohesive	6 (14%)	13 (31%)	
Infiltrative	36 (86%)	29 (69%)	
Tumor - stroma ratio (TSR)			0.180
Low	27 (64%)	33 (79%)	
High	15 (36%)	9 (31%)	
Tumor-infiltrating lymphocytes			> 0.999
Low response	6 (14%)	7 (17%)	

High response

36 (86%)

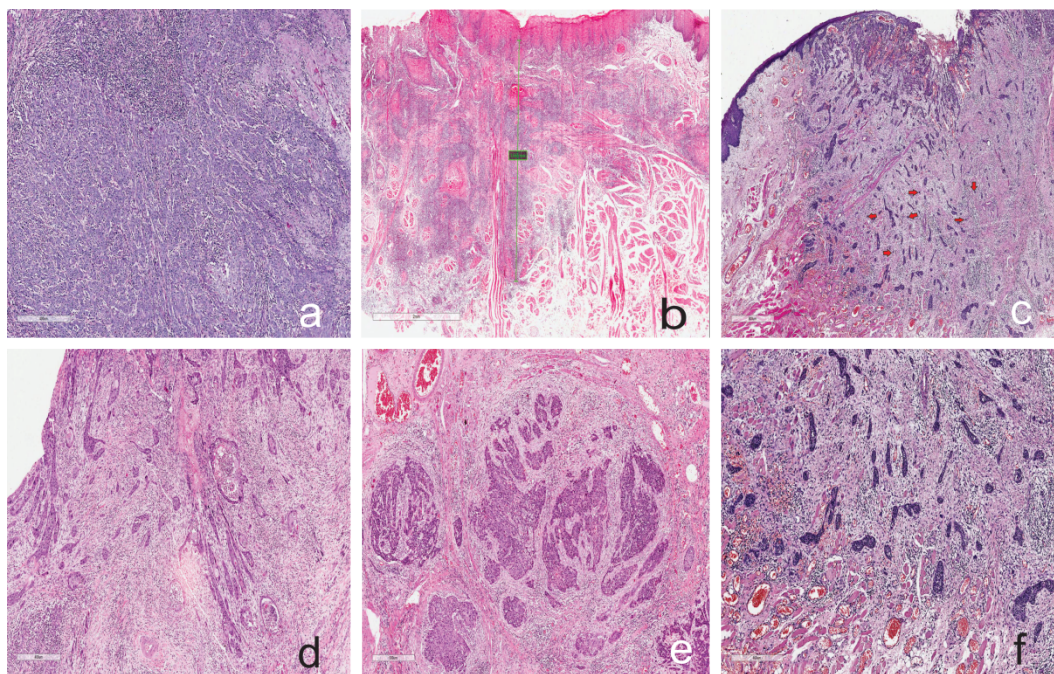
34 (83%)

* *P* value of McNemar test (2x2 table) or McNemar-Bowker (3x3 table) test.

#Depth of invasion was classified as superficial when less than 4mm. High tumor budding was defined as presence of 5 or more tumor buds (see figure 1) at the invasive front of the tumor.

Worst pattern of invasion, TSR and TIL are briefly explained in Figure 1.

Histologic risk score comprises both tumor cell-related and stromal components (WPOI, lymphocytic host response and perineural invasion). Final score used to categorize cases for HRS are in parenthesis.



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