The relationship between histological differentiation and disease recurrence of primary oral squamous cell carcinoma

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INTRODUCTION

Oral squamous cell carcinoma is the sixth most common cancer in world.[1] In India, the National Cancer Registry Programme of the Indian Council of Medical Research reported that up to 80,000 of new oral cancer occur annually.[2] The common risk factors for oral squamous

Abstract

Background: Although advance techniques were available for diagnosis and prognosis of oral cancer, histopathology was used as major method in clinical routine. Of all oral subsites, buccal mucosa squamous cell carcinoma is aggressive in nature with poor survival. Therefore, the aim of the present study was to evaluate the relation of tumor histopathological grade with disease recurrence of buccal squamous cell mucosa carcinoma.

Materials and Methods: A retrospective study was carried out in regional cancer research institute, Tamil Nadu. Demographic, histopathological and participant’s follow-up details were collected from medical records.

Results: Of 198 participants, high frequently encountered with well-differentiated squamous cell carcinoma (n = 98, 49.5%). The clinical characteristics of lymphovascular invasion (P = 0.031), perineural invasion (P = 0.019), tumor stage (P = 0.004), tumor depth (P = 0.048), lymph node (P = 0.02) and metastasis (P = 0.043) had significant association with histopathological grade. In addition, the treatment strategies (P = 0.014) also showed significance at P < 0.05. Further, multivariate revealed cell differentiation (P = 0.048), tumor size (P = 0.037) and depth (P = 0.021) as independent hazard risk of the development of disease using recurrence-free survival of participants at P < 0.05. Of 198 participants, 24 (12.1%) recurrences reported during 34-month follow-up period and the overall estimated recurrence-free survival was 52%. The high frequency of recurrence, 12 (50%), was identified with moderately differentiated tumor cells. However, poorly differentiated tumor showed significantly lower survival (28%) than moderate (54%) and well differentiated (81%) by Kaplan–Meier analysis using log-rank test (P = 0.004, P < 0.05).

Conclusions: The present study concludes high frequency of recurrence observed in moderately differentiated and also revealed lower survival in poorly differentiated tumor. Hence, further treatment plans should focus on moderate and poorly differentiated tumors to improve survival outcome.

Keywords: Aggressiveness, histopathology, prognosis, survival, tumor grade

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cell carcinoma were tobacco habits of chewing with pan, smoking and alcohol.\cite{3} In India, the buccal mucosa squamous cell carcinoma was the common oral subsite which was different disease biologically as compared to rest of oral subsites which was an aggressive in nature and requires multimodality treatment.\cite{4}

Clinical and histopathological methods were the gold standard methods for diagnosis of cancer which had been implicated in prognosis, recurrence and survival of cancer.\cite{5} Morphological assessment of tumors were classified based on the cancer cell differentiation into well, moderately, poorly differentiated carcinomas and anaplastic tumor.\cite{6} Well-differentiated, low-grade oral squamous cell carcinoma usually metastasizes to regional lymph nodes after invading connective tissue, muscle or bone. On the other hand, poorly differentiated, high-grade oral cancer was biologically more aggressive and tends to metastasize to regional lymph nodes early in the course of the disease.\cite{7}

Although the survival remains 50% past several decades, cell differentiation was used as one of the major diagnosis and prognosis factors for oral squamous cell carcinoma.\cite{8} Therefore, there is a need to understand the role of cellular differentiation with disease recurrence. Hence, the study was carried to analyze the relation between cellular differentiation and disease recurrence of buccal mucosa squamous cell carcinoma.

**MATERIALS AND METHODS**

A retrospective study was carried out between 2013 and 2015 in Regional Cancer Centre, Arignar Anna Memorial Cancer Hospital and Research Centre, Kanchipuram, Tamil Nadu. The Institutional and Directorate of Medical Education, Tamil Nadu, ethical clearance was obtained to conduct the study (Ref No. 24984/2013).

The primary buccal mucosa squamous cell carcinoma participants were included in the study. Precancerous and recurrent malignancies were excluded from the study.

**Data collection**

The study included 198 buccal mucosa squamous cell carcinoma participants. The standardized questionnaire was used to retrieve demographic and histopathological variables and their follow-up details from hospital registries.

Histopathological scoring was prepared according to the World Health Organization (WHO) classification by the pathologist from paraffin sections of pretreatment biopsy specimens and was blindly and retrospectively re-examined by an expert oral pathologist.\cite{9} This classification has been applied in clinical routine for analysis of biopsy and/or surgical specimens.

**Survival outcomes**

The outcome measure was set as disease-free survival (DFS). This was calculated as the time from the first date of treatment to the date of recurrence.\cite{10}

**Statistical analysis**

The results were expressed as numbers and percentages. Descriptive statistics were calculated using Chi-square analysis or Fisher’s exact test. Survival rate was calculated using the Kaplan–Meier method and log-rank test used to compare the survival analysis. All the statistics considered at $P < 0.05$ level significance.

**RESULTS**

The study was conducted with 198 buccal mucosa squamous cell carcinoma participants who comprised 125 (63.1%) male and 73 (36.9%) female participants in 1.7:1 ratio. The mean age of all participants was 54.16 ± 17.25 years (range, 21–88 years). Grade of the tumors (cell differentiation) rendered after analysis of the hematoxylin- and eosin-stained slide scoring. Histopathological scoring comprised degree of keratinization, cellular and nuclear pleomorphism and frequency of mitosis. Table 1 shows scoring according to the WHO grading system as four categories: grade I (well differentiated), Grade II (moderately differentiated), Grade III (poorly differentiated) and Grade IV (undifferentiated).

In the present study, all the participants (100%) had squamous cell carcinoma and none of the participants identified with verrucous carcinoma and basaloid squamous cell carcinoma. Grading was based on the degree of resemblance of the invading carcinoma to the normal epithelium and its ability to form keratinizing islands [Figure 1]. The present study

| Characteristics | I | II | III | IV |
|-----------------|---|----|-----|----|
| Degree of keratinization | >50% cells keratinized | 20%–50% cells keratinized | 5%–20% of cells keratinized | 0%–5% of cells keratinized |
| Nuclear pleomorphism | Little nuclear pleomorphism | Moderately abundant nuclear pleomorphism | Abundant nuclear pleomorphism | Extreme nuclear pleomorphism |
| Number of mitosis/HPF | 0-1 | 2-3 | 3-4 | >5 |

I: Well differentiated, II: Moderately differentiated, III: Poorly differentiated, IV: Undifferentiated, HPF: High-power field
revealed that 98 (49.5%) participants had well-differentiated buccal mucosa carcinoma, 68 (34.3%) had moderately differentiated and 32 (16.2%) had poorly differentiated tumors, and also, the study revealed that none of the participants had undifferentiated tumor [Figure 2].

Table 2 shows association between participants’ demographic and clinicopathological characteristics and tumor grade of buccal mucosa squamous cell carcinoma. The clinicopathological characteristics such as lymphovascular invasion ($P = 0.031$), perineural invasion ($P = 0.091$), tumor stage ($P = 0.004$), tumor depth ($P = 0.048$), lymph node status ($P = 0.02$) and metastasis ($P = 0.032$) had significant association whereas gender, age, tumor size and extracapsular invasion were not associated. In addition, the study also revealed significant association with treatment strategies ($P = 0.014$) by Chi-square analysis at $P < 0.05$. Hence, the study proved that biological behavior of cell differentiation inversely related to clinicopathological characteristics.

Table 3 shows the Cox proportional hazard risk analysis of buccal mucosa squamous cell carcinoma. The significant clinical characteristics, i.e. cell differentiation ($P = 0.000$), lymphovascular ($P = 0.000$), perineural invasion ($P = 0.000$), tumor size ($P = 0.049$) and tumor depth ($P = 0.025$) by univariate analysis were validated by multivariate risk analysis at $P < 0.05$. Thus, cell differentiation ($P = 0.048$), tumor size ($P = 0.037$) and depth ($P = 0.021$) were identified as an independent risk factors for disease development at $P < 0.05$.

All the participants were followed up to 34 months; the median of survival was 24 months (range, 3–34 months). Of 198 participants, 24 (12.1%) participants had recurrence at the last follow-up. An estimated DFS rate of buccal mucosa squamous cell carcinoma was 52% by Kaplan–Meier survival analysis [Figure 3]. Table 4 shows the description of cell differentiation and their survival outcome; most of participants, 12 (50%), were identified recurrence in moderately differentiated buccal mucosa squamous cell carcinoma. However, poorly differentiated participants had lower survival (28%) due to aggressive in nature compared to moderate (54%) and well-differentiated buccal mucosa squamous cell carcinoma (81%) using log-rank test ($P = 0.004$, $P < 0.05$) [Figure 4]. Fortunately, the present study consisted nearly 50% of well-differentiated tumor and showed good prognosis of buccal mucosa squamous cell carcinoma.

**DISCUSSION**

Oral cancers were had geographical variation with respect to the age, gender and stage of diagnosis. The present study showed a male predominance in the occurrence of buccal mucosa squamous cell carcinoma in this region with the proportion of 1.7:1 (male: female). Similarly, one of the studies from Kerala reported with 2.2:1. This high proportion of oral cancers in males may be attributed to the increased risk habits.

The previous literature reported with high frequency of well-differentiated oral squamous cell carcinoma. In contrary, there were some studies reported with majority of...
However, the present study revealed with high frequency of well differentiated, nearly half of patients (49.5%) at diagnosis and followed moderately and poorly differentiated buccal squamous cell carcinoma.

Histopathology grading system was very effective for assessing the fundamental characteristics of carcinoma such as local extent, regional dissemination and distant metastasis. Fang et al. reported the histological differentiation with clinical manifestation of oral squamous cell carcinoma. He did not find any significant difference with age, sex, tumor, node, metastasis stage, bone invasion, depth of invasion and history of carcinogen exposure whereas nodal metastasis, extracapsular spread and perineural invasion had highly significant difference among grade of tumor. Similarly, Chen et al. revealed from their study that tumor, nodal stage and surgical margin had strong significant difference with cellular differentiation of oral squamous cell carcinoma. Thus, our results were in accordance with previous finding clinicopathological characteristics of lymphovascular, perineural invasion,
tumor stage, tumor depth, lymph node and metastasis association with cellular differentiation. In addition, an interesting finding in our study was that the grade of buccal mucosa squamous cell carcinoma was highly associated with treatment strategies.

The 5-year survival rate of buccal mucosa squamous cell carcinoma participants had been reported approximately 50%, which is not satisfactory despite new treatment modalities.\textsuperscript{[3]} Fang \textit{et al.} reported that cell differentiation was the most significant factor affecting prognosis and survival of oral squamous cell carcinoma.\textsuperscript{[14]} Nance \textit{et al.} conducted study in head and neck carcinoma using Brandwein histological grading system and identified most of recurrence in moderately differentiated (intermediate) squamous cell carcinoma.\textsuperscript{[16]} The present study also found with similar results, 50% recurrence identified in moderately differentiated squamous cell carcinoma using the WHO grading system. Hence, treatment strategies should focus on moderately differentiated squamous cell carcinoma to improve survival.

A recent study from Rikardsen \textit{et al.} reported the survival difference among cellular differentiation. They concluded that patients with well-differentiated tumors live longer than patients with low-differentiated oral squamous cell carcinoma.\textsuperscript{[17]} Similarly, another report from Cho \textit{et al.} proved the significant survival difference and well differentiated had better survival of buccal mucosa squamous cell carcinoma \((P = 0.003, P < 0.05)\).\textsuperscript{[18]} In accordance to the previous reports, the present study conducted with specific oral subsite of buccal mucosa squamous cell carcinoma and revealed survival difference among the cellular differentiation. Due to study limitation, the present study showed with 34-month follow-up survival of buccal mucosa carcinoma. The study showed 52% of DFS of buccal mucosa squamous cell carcinoma, and also, the present study supports previous reports; the study revealed that poorly differentiated had lower survival compared to moderate and well differentiated squamous cell carcinoma.

**CONCLUSIONS**

The present study concludes with high frequency of recurrence (50%) in moderately differentiated buccal squamous cell carcinoma which affects survival outcome of participants. However, poorly differentiated was revealed lower survival because of aggressive nature than
moderate and well-differentiated tumor cells. Further, cellular differentiation (histopathological tumor grade) had significant relation with clinical characteristics of buccal mucosa carcinoma. Thus, the study reports that cell differentiation is an important prognostic factor for buccal mucosa squamous cellular carcinoma and further treatment plans should focus on moderately and poorly differentiated tumors to improve survival outcome and to reduce disease recurrence rate.

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Conflicts of interest
There are no conflicts of interest.

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