Tongue Squamous Cell Carcinoma an Immunohistochemical Analysis of Prognostic Markers

HANAN H.M. ALI, M.D.* and WALID SOROUR, MRCS, DOHNS**

The Departments of Pathology, Faculty of Medicine, Cairo University* and Otolaryngology Head & Neck Surgery, Imperial College, Healthcare NHS Trust**

Abstract

Tongue cancer is the most common malignancy diagnosed within the oral cavity. Though oral tongue cancer is generally diagnosed at an early stage, the prognosis is poor due to frequent recurrence. Therefore, it is important to identify factors predictive of recurrence and to treat those patients with a high probability of recurrence with specialized treatment strategies. Between 2004 and 2009, a total of 37 specimens from patients with tongue squamous cell carcinoma were evaluated. Twenty four cases were studied by immunohistochemistry for EGFR, p53, Cyclin D1 and Ki-67 protein expression. All cases expressed EGFR, 70% expressed p53, 75% overexpressed cyclin D1, and 20% had high ki67 proliferation index. Expression of p53 and overexpression of cyclin D1 were correlated with moderately/poorly differentiated tumors. Cases who did not develop tumor recurrence, grade II and stage I tumors were associated with low ki67 proliferation index. Tumor recurrence was associated with stage II-III, and cases originated from tongue base. However tumor recurrence was not associated with factors such as age, sex, tumor grade and any of the studied biomarkers.

Key Words: Tongue cancer — SCC — EGFR — P53 — Cyclin D1 — Ki-67.

Introduction

SQUAMOUS cell carcinoma (SCC) is the most common form of oral malignancy including tongue cancer ill. SCC of the tongue is characterized by an unpredictable course as some patients with early lesions may develop local recurrence and regional metastases despite adequate surgery, and so the identification of prognostic clinicopathological and immunohistochemical markers (IHC) would enable clinicians to target patients who may benefit from a specifically tailored treatment strategy [2]. The molecular markers of interest are those involved in cell cycle regulation of tumor cells and a group of growth factors, since cancer is caused by uncontrolled proliferation of cells, which is itself induced by abnormalities of cell cycle regulatory mechanisms or activation of growth factors [3].

P53 tumour suppressor gene is located on the short arm of human chromosome 17 and encodes for a phosphoprotein that has dual activity on normal cells: It inhibits cell proliferation by arresting it at the G1-phase after DNA damage, and it induces apoptosis after genotoxic damage. It is thought that both mechanisms suppress tumor growth [4].

Epidermal growth factor receptor (EGFR) is a 170-KDa transmembrane glycoprotein whose gene is located on chromosome 7 p12. It is a member of family of tyrosine kinase (TK) growth factor receptors, a group of proteins whose aberrant activity plays a key role in cell growth and neoplastic progression [5].

Cyclin D1, together with other cyclins, are the rate-limiting controllers of the G1-phase progression in mammalian cells and are expressed in the G1- and S-phases of the cell cycle. Overexpression of cyclin D1 results in loss of cell cycle control and accelerates progression through the G1-phase [6].

Ki-67 is a nuclear protein expressed in the G2 and M phases of actively dividing cells [7]. This antigen is a proliferation marker that correlates with the presence and severity of epithelial dysplasia [8,9]. This a retrospective study aimed at testing the expression of the different biomarkers in relation to the clinical & histopathological characteristics of the tongue cancer cases trying to detect the prognostic markers that can anticipate the behavior of the tongue cancer and consequently would have a significant role in determining the therapeutic strategy for these patients.
**Material and Methods**

Ethical permission application via the Human Biomaterials Research Centre HBRC at Imperial NHS Charing Cross Hospital to collect the specimens of tongue cancer cases in the period from July 2004 up to August 2008 (37 patients). The histopathological reports were reviewed and the following clinical data were obtained: Age, sex, localization of disease, and further development.

**Preparation of samples:**

Tumor tissues were identified for each case using the archived diagnostic slide as reference and the areas marked on the slides to ensure the presence of tumor in the respective blocks. Five serial sections, 5µm thick, were cut; onto Super-Frost plus coated slides, one of them was stained with Haematoxylin and Eosin (H&E). The remaining slides were used for immunohistochemistry. The H&E stained slides were reviewed to ensure there was a representative tissue and to facilitate the comparison with the immune stains.

Thirteen cases were excluded because there was no sufficient tumor in block for further immunohistochemical evaluation, so only 24 patients were included. Upon follow-up, tumors recurrence was reported in 11 cases (45.8%).

**Histology:**

Histologically, 4 cases were carcinoma in situ, or invasive tumors (20 cases). Invasive carcinomas were graded into grade I (well differentiated n=5); grade II, moderately differentiated (n=10); and grade III, poorly differentiated tumors (n=5), in cases of combined grades the higher grade was considered.

**Immunohistochemical staining:**

Immunohistochemical analyses were performed to assess the expression of cell cycle-related proteins: P53, EGFR, cyclin D1 and the proliferation marker Ki-67. The primary mouse monoclonal antibodies, respective clones, working dilutions, sources and evaluation criteria are listed in Table (1). Automated immunohistochemistry was done using the Leica bond Max which is a closed system utilizing a polymer detection system and cover tiles to facilitate even distribution over the sections. Dewaxing of the sections and antigen retrieval was carried out on the machine and the slides were counterstained in hematoxylin. Paraffin sections on glass slides can be placed directly onto the machine. Either ER1 (Epitope retrieval 1) or ER2 can be used as antigen retrieval solutions.

**Assessment of immunohistochemistry:**

Assessment of staining results was carried out independently from the histological classification of specimens. All slides were reviewed by two observers under light microscopy. A minimum 1000 cells in 5 high power fields were evaluated in each section. In cases of P53, cyclin D1 and Ki67 only nuclear staining was evaluated. EGFR cell membrane immunostaining with cytoplasmic enhancement was observed. The results were expressed as continuous variables range, mean and standard deviation. The expression assessment of all proteins and the cutoff points used were based on previous studies (Table 1).

**Statistical analysis:**

Data were statistically described in terms of mean±standard deviation (±SD) and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using t-Test and one way analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons. For comparing categorical data, Chi square (x²) test was performed. Exact test was used instead when the expected frequency is less than 5. p-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

**Results**

**Clinicopathological data:**

Clinicopathological parameters are summarized in Table (2). The study sample consisted of 15 males (62.5%) and 9 females (37.5%) with an age
range 38 to 84 years (mean 65.4 years). Clinically, 18 patients were at least 60 years. Most cases presented by an ulcer (50%). The majority of the cases (62.5%) were seen in the lateral side of tongue. All cases originated from base of tongue were males and those from anterior surface were females, (p=0.03). Moderately differentiated carcinoma was the most frequent among studied cases (10/24) (Figs. 1,2). Seventeen cases were T1 (70.8%). Recurrent tumor was seen in 11/24. (45.8%). Tumor recurrence showed no significant correlation with age, sex, and tumor grade. Tumor recurrence was observed in all cases (100%) originated from base of tongue which was of statistical significance versus other sites (p=0.02). In addition, all stage II and III cases recurred in comparison to stage I where less than half of its cases developed tumor recurrence (p=0.03), Table (3).

Evaluation of immunohistochemistry:

The positivity rate of the selected immunostains P53 (Fig. 3), EGFR (Fig. 4), cyclin D1 (Fig. 5), and ki-67 (Fig. 6) in the total sample is shown in (Table 2) which reveals positive immunostaining in most of the cases. The mean values of their expressions were evaluated as continuous variables and they were divided into negative and positive cases according to chosen cutoff points. Their correlations with clinicopathological parameters are presented in Tables (4,5).

Fig. (1): Whole mount of H&E stained section from tongue resection specimen showing the invasive squamous cell carcinoma in the centre (arrow) and the uninvolved mucosa either side of the tumour.

Fig. (2): Invasive, keratinising moderately differentiated squamous cell carcinoma arising from surface epithelium (H&E x40) (keratin indicated by arrow).

Fig. (3): Expression of p53 in the tumour cell nuclei at the periphery of the islands and in basal surface epithelium (Immunostained section X40).

Fig. (4): Diffuse strong expression of EGFR in the tumour and in basal surface epithelium (Immunostained section X40).

Fig. (5): Nuclear expression of cyclin D1 in basal epithelium and in tumour islands (Immunostained section X40).

Fig. (6): Nuclear expression of Ki67 in basal epithelium and in tumour islands (Immunostained section X40).
Table (2): Patients, tumour characteristics and biomarkers expressions.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>24 (100)</td>
</tr>
</tbody>
</table>
| Age:
  <60               | 6 (25)          |
  >60               | 18 (75)         |
| Sex:
  Male             | 15 (62.5)       |
  Female            | 9 (37.5)        |
| Tumor localization:
  Lateral surface of the tongue | 15 (62.5) |
  Anterior          | 3 (12.5)        |
  Posterior and dorsum | 3 (12.5)     |
  Base              | 3 (12.5)        |
| Histological grade:
  Carcinoma in situ | 4 (16.7)        |
  Well differentiated | 5 (20.8)       |
  Moderately differentiated | 9 (37.5) |
  Poorly differentiated | 6 (25)        |
| Stage:
  Stage 0          | 4 (16.7)        |
  Stage I           | 17 (70.8)       |
  Stage II          | 2 (8.3)         |
  Stage III         | 1 (4.2)         |
| Tumor recurrence:
  No               | 13 (54.2)       |
  Yes              | 11 (45.8)       |
| Biomarkers positivity:
  P53              | 17 (70.8)       |
  Cyclin D1         | 18 (75)         |
  EGFR              | 24 (100)        |
  Ki-67             | 5 (20.8)        |

N: Number, EGFR: Epidermal growth factor receptor, PI: Proliferation index.

Table (3): Correlation of tumor recurrence with clinicopathological characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Recurrence</th>
<th>No recurrence</th>
<th>p value</th>
</tr>
</thead>
</table>
| Age:
  <60 (n=6)         | 2 (33.3%)  | 4 (66.7%)     | 0.4     |
  >60 (n=18)         | 9 (50%)    | 9 (50%)       |         |
| Gender:
  Male (n=15)       | 7 (46.7%)  | 8 (53.3%)     | 0.9     |
  Female (n=9)       | 4 (44.4%)  | 5 (55.6%)     |         |
| Tumor localization:
  Lateral (n=15)    | 6 (40%)    | 9 (60%)       | 0.07    |
  Anterior (n=3)     | 2 (66.7%)  | 1 (33.3%)     |         |
  Posterior and dorsum (n=3) | 2 (66.7%) | 1 (33.3%) | 0.02* |
  Base (n=3)         | 3 (100%)   |               |         |
| Tumor grade:
  Carcinoma in situ | 2 (50%)    | 2 (50%)       | 0.8     |
  Well differentiated | 2 (40%)    | 3 (60%)       |         |
  Moderately differentiated | 4 (44.4%) | 5 (55.6%) |         |
  Poorly differentiated | 3 (50%)   | 3 (50%)       |         |
| Tumor stage:
  Stage 0           | 2 (50%)    | 2 (50%)       |         |
  Stage I            | 6 (35.3%)  | 11 (64.7%)    | 0.03**  |
  Stage II           | 2 (100%)   |               |         |
  Stage III          | 1 (100%)   |               |         |
| Biomarkers:
  P53
    >10% (n=7)       | 3 (42.9%)  | 4 (57.1%)     | 0.8     |
    >10% (n=17)     | 8 (47.1%)  | 9 (52.9%)     |         |
  Cyclin D1:
    <50% (n=6)      | 4 (71.4%)  | 2 (28.6%)     | 0.1     |
    50% (n=18)      | 7 (35.3%)  | 11 (64.7%)    |         |
  EGFR:
    >10% (n=0)      | 11 (45.8%) | 13 (54.2%)    | 0.6     |
    >10% (n=24)     | 11 (45.8%) | 13 (54.2%)    |         |
  Ki-67:
    >0% (n=19)     | 8 (42.1%)  | 11 (57.9%)    | 0.4     |
    >0% (n=5)       | 3 (60%)    | 2 (40%)       |         |

P53 immunostaining. P53 was expressed in 17 cases (70.8%) with its mean value ranged from (0 up to 100%). P53 immunoexpression mean values were not significantly associated with the clinical variables analyzed: Sex (p0.1) and age of patients (p0.1) as well as tumor site (p0.5), stage (p0.9) and recurrence (p0.4). The mean of its expression was significantly higher in poorly differentiated tumors (94±6.6) (p=0.02). Considering p53-positive and p53-negative cases, no statistically significant differences were found concerning age (p0.7) and sex (p0.2) of patients, different sub-sites (p0.7), tumor stage (p0.2), or recurrence (p0.8). Positive p53 immunostain was significantly correlated with poorly differentiated tumors.

Cyclin D1 expression:

Cyclin D1 was expressed in all cases range from 10-95%. Overexpression (>50%) was detected in 18 (75%) specimens. In those sections that included adjacent normal and dysplastic mucosa, uniform staining for cyclin D1 was observed in the majority of cases, predominantly in the suprabasal epithelial cells. However, the staining in adjacent mucosa was generally of reduced intensity compared with invasive carcinoma. Cyclin D1 expression mean and overexpression did not correlate with age, sex, sub-site, pathological stage or tumor recurrence (p>0.05). However, cyclin D1 was significantly expressed in moderately and poorly differentiated tumors.

EGFR expression:

EGFR was expressed in all cases (100%) with moderate or strong intensity, its mean ranged from 75 up to 100%. In tumor the expression involved all the epithelial layers (with more intensity at the periphery) while in normal oral mucosa it was localized to the basal cell layer. Its expression mean revealed no significant correlation with sex, age, tumor grade, stage or recurrence (p>0.05). Considering EGFR-positive and EGFR negative cases, all cases were positive with no statistical significant differences found concerning age, sex, different sub-sites, tumor grade, stage and recurrence (p>0.05).

Ki-67 expression:

The Ki-67 proliferation index (PI) ranged from 5 to 85%. There was no correlation between Ki-67 proliferation index and age (p0.3), tumor grade (p0.6), stage (p>0.4), recurrence (p0.3). In order to clarify whether the Ki-67 index is related with clinicopathological prognostic parameters, tumors were divided into two groups high Ki-67 PI (>50%) and low Ki-67 PI (<50%). There was no correlation between Ki-67 PI and age, sex (p>0.5). However, lateral side of tongue, histological grade II tumors
and stage I as well as non recurrent tumors were significantly associated with low ki-67 PI (p = 0.02), (p=0.03), (p=0.007) and (p=0.01) respectively.

**Evaluation of the biological markers in relation to each other:**

There was no association between all studied markers with each other, the only exception was the significant correlation observed between cyclin D1 overexpression and nuclear accumulation of p53, and data are presented in Table (6). Of the tumors that overexpressed cyclin D1, 77.7% had concurrent increase in p53 accumulation in contrast to the cyclin D1 negative tumors, where only half of them were p53 positive (p=0.01).

**Table (4):** Correlations between means of p53, EGFR, cyclin D1, and ki-67 protein expression and clinicopathological characteristics in squamous cell carcinoma of the tongue.

<table>
<thead>
<tr>
<th>Clinicopathological characteristic</th>
<th>Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P53 (%)*</td>
</tr>
<tr>
<td><strong>Age:</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>80.3±47</td>
</tr>
<tr>
<td>≥70</td>
<td>60.5±41</td>
</tr>
<tr>
<td><strong>Gender:</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>73±36.5</td>
</tr>
<tr>
<td>Female</td>
<td>54±51.6</td>
</tr>
<tr>
<td><strong>Tumor localization:</strong></td>
<td></td>
</tr>
<tr>
<td>Lateral surface</td>
<td>66.1±43</td>
</tr>
<tr>
<td>Anterior</td>
<td>98.3±41.1</td>
</tr>
<tr>
<td>Posterior and dorsum</td>
<td>65±56.3</td>
</tr>
<tr>
<td>Base</td>
<td>50±45.8</td>
</tr>
<tr>
<td><strong>Tumor grade:</strong></td>
<td></td>
</tr>
<tr>
<td>Carcinoma insitu</td>
<td>52.5±55</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>68±40.7</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>51.1±47.8</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>94.1±46.6</td>
</tr>
<tr>
<td><strong>Tumor stage:</strong></td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>52.5±55</td>
</tr>
<tr>
<td>Stage I</td>
<td>63.2±42.4</td>
</tr>
<tr>
<td>Stage II</td>
<td>95±7</td>
</tr>
<tr>
<td>Stage III</td>
<td>100±0</td>
</tr>
<tr>
<td><strong>Recurrence:</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>65.9±41.7</td>
</tr>
<tr>
<td>No</td>
<td>70±43.6</td>
</tr>
</tbody>
</table>

*: Mean. EGFR: Epidermal growth factor receptor. PI: Proliferation index.

**Table (5):** Correlations between studied biomarkers and clinicopathological characteristics in squamous cell carcinoma of the tongue.

<table>
<thead>
<tr>
<th>Clinicopathological characteristics</th>
<th>Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P53 &gt;10% (n=17)</td>
</tr>
<tr>
<td><strong>Age:</strong></td>
<td></td>
</tr>
<tr>
<td>&lt;60 (n=6)</td>
<td>4</td>
</tr>
<tr>
<td>≥70 (n=18)</td>
<td>13</td>
</tr>
<tr>
<td><strong>Gender:</strong></td>
<td></td>
</tr>
<tr>
<td>Male (n=15)</td>
<td>12</td>
</tr>
<tr>
<td>Female (n=9)</td>
<td>5</td>
</tr>
<tr>
<td><strong>Tumor localization:</strong></td>
<td></td>
</tr>
<tr>
<td>Lateral (n=15)</td>
<td>10</td>
</tr>
<tr>
<td>Anterior (n=3)</td>
<td>3</td>
</tr>
<tr>
<td>Posterior and dorsum (n=3)</td>
<td>2</td>
</tr>
<tr>
<td>Base (n=3)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Tumor grade:</strong></td>
<td></td>
</tr>
<tr>
<td>Carcinoma insitu (n=4)</td>
<td>2</td>
</tr>
<tr>
<td>Well differentiated (n=5)</td>
<td>4</td>
</tr>
<tr>
<td>Moderately differentiated (n=9)</td>
<td>5</td>
</tr>
<tr>
<td>Poorly differentiated (n=6)</td>
<td>6</td>
</tr>
<tr>
<td><strong>Tumor stage:</strong></td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>2</td>
</tr>
<tr>
<td>Stage I</td>
<td>12</td>
</tr>
<tr>
<td>Stage II</td>
<td>2</td>
</tr>
<tr>
<td>Stage III</td>
<td>1</td>
</tr>
<tr>
<td><strong>Recurrence:</strong></td>
<td></td>
</tr>
<tr>
<td>Yes (n=13)</td>
<td>9</td>
</tr>
<tr>
<td>No (n=11)</td>
<td>8</td>
</tr>
</tbody>
</table>

N: Number. EGFR: Epidermal growth factor receptor. PI: Proliferation index.
Table (6): Relation between studied biomarkers.

<table>
<thead>
<tr>
<th>Molecular markers</th>
<th>Cyclin D1</th>
<th>EGFR</th>
<th>Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>P53:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=7)</td>
<td>4*</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Positive (n=17)</td>
<td>14*</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Cyclin D1:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=6)</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Positive (n=18)</td>
<td>18</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>EGFR:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative (n=0)</td>
<td>18</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Positive (n=24)</td>
<td></td>
<td></td>
<td>19</td>
</tr>
</tbody>
</table>

N: Number. EGFR: Epidermal growth factor receptor. PT: Proliferation index. *p-value=0.01

Discussion

Squamous cell carcinoma of the tongue is a common malignancy and despite advances in its therapy, which have improved quality of life, survival rates have remained static for many years. Mortality from this disease remains high because of the development of distant metastases and the emergence of local and regional recurrences [16]. The aim of this study was to identify histopathological and immunohistochemical criteria in biopsies from tongue SCC that may help identifying the patients with a high risk of local recurrence or metastasis following initial surgical excision of the tumors.

All the markers chosen in this study are intimately related to the cell cycle and consequently related to the growth of the tumors. P53, cyclin D1, EGFR and Ki-67 expressions were investigated as possible markers in tongue SCC. Thirty seven cases were evaluated and 24 cases were included, 15 males and 9 females. Male predominance and patients mean age were in agreement with published studies [17-19]. The lateral tongue was the most frequently involved anatomical sub-site in the current study as well as that reported by Kokemueller et al., [19]. Moderately differentiated tumors as well as T1 tumors were the most frequent among studied cases [19,20]. Less than half of our patients suffered from locoregional recurrence and this was in accordance with literatures which are quoted between 16 and 42% [21,25].

Tumor recurrence was observed in 50% of patients older than 60 while one third of patients younger than 60 showed tumor recurrence and almost half of males and females showed tumor recurrence (statistically insignificant). Recurrence in older age was reported by Jerjes et al., [18]. In contrast Vargas et al., [26] reported higher rate of recurrence in young female patients in their study. In the current work all tumors originated from base of tongue were males and those from anterior surface were females with significant difference between these two sites. Similarly, male predominance of base of tongue tumors was reported [27]. Furthermore, tumors originated from base of the tongue developed tumor recurrence and this was of statistical significant difference versus other tongue sites. SCC originated from base of tongue was reported to be more aggressive than tumors that arise from mobile tongue [19]. Moreover, base of tongue SCC and oral tongue SCC (OTSCC) cases ought to be separated because base of tongue cancer show more similarities to tonsillar cancer than OTSCC [15,27]. Recurrence of tumors originated from base of tongue is largely explained by tumor’s site influence on nodal metastasis, as well as the surgeon’s ability to achieve clear resection margins may be restricted by accessibility to the tumor primary site [28]. However, Dahlgren et al., [27] reported mostly equal rate of recurrence of tumors originated from tongue base and mobile tongue.

In the present work, tumor grade had no significant correlation with tumor recurrence similar to Hosel et al., [22]. In contrast Wang et al., [25] and An et al., [29] reported significant relationship between tumor grade and locoregional recurrence, this difference may be explained by different sample size. A lower recurrence rate was related to T1 versus TII and III tumors which was in agreement with An et al., 2008 [29]. In contrast, Wang et al., [25] reported no significant correlation between tumor stage and recurrence.

P53 was expressed in 70% of studied cases. In the literature the percent of positivity for p53 was variable depending on tumor site whether it is from tongue or different head and neck sites; Sa et al., [30] reported similar positivity rate in tongue SCC. No significant correlations were observed between p53 expression and age, and sex of patients, grade
and stage of tumors similar to other studies [31,20,32]. However, in the present work all cases of grade III tumors expressed p53 which was significantly different from other grades and insitu tumors. In the literature, the relation between p53 expression and recurrence of head and neck SCC is still controversial. In the present study, p53 expression showed no significant correlation with tumor recurrence similar to some previous studies [3,33,34]. Though earlier, Shin et al., 1996 [10] reported p53 as a good predictor of overall disease free survival in patients with head and neck SCC.

In the current work, cyclin D1 showed no statistical significance difference with patient age or sex similar to Mineta et al., [13]. It was significantly expressed in moderately and poorly differentiated tumors. Similarly statistical significant difference in moderately differentiated tumor was reported by Bova et al., [20] and Perisanidis et al., [34]. However, in other series cyclin D1 had no significant correlation with tumor grade in head and neck SCC [3,13,14,35]. Moreover, Saawarn et al., [36] reported expression of cyclin D1 in well differentiated oral SCC and concluded that cyclin D1 expression is associated with better tumor differentiation. The controversy between our results and theirs would be attributed to that they evaluate both percentage and intensity of cyclin D1 expression and calculated summation score as well as their cases were not solely tongue cancer.

Cyclin D1 showed no significant correlation with tumor stage and tumor recurrence in agreement with Shirika et al., [9]. In contrast, Bova et al., [20] reported significant correlation between cyclin D1 expression and disease free survival.

EGFR was universally expressed in the studied cases with no significant correlations between its expression and age, sex, sub-site, tumor grade, stage, and recurrence. The present work confirms the observations of other studies [3,37,38] about the high EGFR expression in oral SCCs which would suggest that an uncontrolled growth in these tumors may be mediated by abnormal EGFR expression. It seems that EGFR overexpression is needed for development of tongue SCC rather than to be correlated with its progression.

High Ki-67 labeling index was observed in 20.8% of studied cases. No statistical significance difference was observed between ki67 expression age and sex of patients as well as tumor sub-sites within the tongue. However cases originated from lateral surface were associated with low ki-67 PI. Kim et al., [39] reported a statistical significant difference in ki-67 expression between tumors originated from base of tongue versus other tongue sub-sites. This may be attributed to the difference in the cutoff point used (in their work 10%). In the current study ki-67 PI showed no statistical correlation with tumor stage or grade, though grade I tumor and stage II were significantly associated with low ki-67 PI. Vicente et al., [14] and Bova et al., [20] reported significant correlations between ki-67 expression and tumor grade but not with stage of tumors. Furthermore, no significant correlation was found between ki-67 PI and tumor recurrence though non recurrent cases were associated with low ki-67. Similarly, Vicente et al., [14] reported no significant correlation between ki-67 expression and tumor recurrence. While Wangsa et al., [15] reported significant correlation between ki-67 expression and tumor recurrence in stage I cancer tongue.

On evaluating the relation of the studied biomarkers to each other, no significant correlations were found between all studied markers. The only exception was positive significant correlation between expressions of p53 versus cyclin D1. Similarly, Bova et al., [20] and Perisanidis et al., [34] reported significant correlation between p53 and cyclin D1, in contrast to Kumar et al., [40] and Lam et al., [32].

In the literature, there is controversy about the relation between the evaluated biomarkers. Some studies reported no significant correlation between studied biomarkers with one another Shiraki et al., [9] (EGFR vs. Cyclin D1 and p53), Sarkis et al., [41] (EGFR vs. P53), Bova et al., [20] (ki-67 vs. p53 and cyclin D1). Other studies reported significant correlation between these studied markers, Vicente et al., [14] reported positive correlations between cyclin D1 and ki-67 overexpression. And Farrar et al., [42] reported significant correlation between p53 and ki-67.

With respect to the present results and above mentioned studies the prognostic significance of p53, cyclin D1, EGFR and ki-67 in tongue cancer is not resolved because most studies in the literature used pooled tumor specimens from different head and neck sites and patients with diversified treatment regimens as well as the use of different cutoff points for evaluation of biomarker positivity [34,35]. Accordingly, these markers were not of great help to define high risk groups of recurrence of tongue SCC. So, it seems reasonable that there are further prognostic factors, which are still unknown and currently are not detectable by histopathological and immunohistochemical techniques.
Conclusion:
Based on our results, it is justified to conclude that tumor originated from tongue base as well as stage II-III tumors have more tendencies to recur. Biomarkers studied suggested that non recurrent tumors, grade II and stage I were associated with low ki67 proliferation index. And expression of p53 and overexpression of cyclin D1 were associated with poorly differentiated tumors. The biomarkers studied are not associated with tumor recurrence in tongue SCC. However further multi-institutional studies on large number of tongue SCC cases and evaluation of these markers on molecular level may be more relevant than immunohistochemistry as well as search for other prognostic histopathological parameters and biomarkers are recommended.

Acknowledgments:
We are indebted to Dr. Ann Sandison, Consultant Histopathologist Imperial College Healthcare NHS Trust, for her valuable comments and discussions.

Conflicts of interest:
There are no conflicts of interest.

References


