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# Thymidine Labeling Index in Laryngeal Squamous Cell Carcinoma

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**Abstract:** The aim of this study was to investigate the prognostic value of the thymidine labeling index (TLI) in laryngeal squamous cell carcinoma (SCC). The TLI in tumor tissue and adjacent healthy tissue was assessed prospectively in 31 patients and these values were correlated with age, TNM, grade and recurrence statistically. The tissues (tumoral and adjacent tumor-free tissue) that were obtained during surgery were labeled with $^{3}$H-Thymidine. TLI was calculated as the number of labeled tumor cells * 100 / total number of tumor cells. A statistically significant difference was observed between tumor tissue (mean TLI value = 17.62± 6.39%) and adjacent tumor-free tissue (mean TLI value = 9.16±3.32%) ($p<0.05$), but there was no significant relationship between TLI and age, TNM, grade and recurrence. It was concluded that TLI in the laryngeal SCC was not a suitable prognostic marker for follow-up and accurate therapeutic planning due to methodological and tumor dependent reasons.

**Key Words:** Thymidine labeling index, laryngeal squamous cell carcinoma, prognostic factors

## Introduction

As yet, sensitive and specifically prognostic markers have not been found in squamous cell carcinoma of the larynx. The TNM classification system is often cited as the single most important prognostic factor. However, within stages, the biological behavior of the tumor can vary widely, so individualized prognostic markers are needed for accurate therapeutic planning and follow-up (1).

In recent years, the proliferative activity of tumors has been considered a potential indicator of biological behavior. Several previous studies have suggested that a kinetic cell cycle parameter of the $^{3}$H-Thymidine labeling (TLI) value at the time of surgery is a valid prognostic indicator of the proliferative rate of some solid tumors (2). TLI is one of the standard techniques for determining the kinetic activity of tumors (other techniques include static and dynamic flow cytometry, identification of the Ki-67 antigen, and detection of bromodeoxyuridine labeled cells using a monoclonal antibody). Radioactive thymidine incorporates into DNA and its cellular absorption is measured autoradiographically (2). In the literature in English (1980-2000), only two studies make specific reference to a labeling index with tritiated thymidine in the SCC of the larynx in a sufficiently large sample of cases (1,3).

In this study, our aim was to correlate TLI values in laryngeal SCC patients with age, TNM, recurrence, pathological grade and prognosis.

## Materials and Methods

Thirty-one patients with primary laryngeal SCC who were treated at the Department of Otolaryngology Head and Neck Surgery of the Istanbul Faculty of Medicine during 1994-1997 were included into the study. All of the patients were male and aged between 38 and 79 years (median age 56). Localizations of the tumors in the larynx were the supraglottic region in 54.8% (n=17), the glottic region in 29% (n=9), the subglottic region in 6.5% (n=2) and the transglottic region in 9.7% (n=3). Tumors that pass through the paraglottic space clinically and radiologically and are superior and inferior to the ventricle are defined as transglottic tumors (4).

All patients were initially treated with surgery. These operations were transoral epiglottectomy in one patient, supraglottic laryngectomy in five patients, laryngofissure

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cordectomy in three patients, vertical hemilaryngectomy in two patients, total laryngectomy in twenty patients, and a type of neck dissection performed simultaneously in twenty-four cases. Nodal disease in the neck was detected in nine neck dissection specimens (29%) by postoperative pathological examination and radiotherapy was given to nine patients (29%) in addition to their surgical therapy. After the initial treatment, a loco-regional recurrence developed in four patients (13%) in the first year of the follow-up.

TNM classifications were made according to AJCC (1992) (5).

TLI was determined in tumor samples and macroscopically tumor-free mucosa from the same laryngeal region at the time of surgery (later confirmed by pathological examination). These samples were divided into small fragments (volume 1-3 $mm^3$) and immediately incubated in 20% fetal calf serum, 100 u/ml penicillin, 10 mg/ml streptomycin and 6 mci/ml $^3$H-Thymidine (M 199 solution, Medium 199 Gibco). Then the fragments were washed with 0.9% NaCl and embedded in paraffin. Three-micron sections were covered with Ilford K-2 emulsion. After a three-day period of exposure, the autoradiographs were developed using Kodak D19 b solution and stained with Hematoxylin-eosin. Cells were considered to be labeled when there were five or more grains over the nucleus under light microscopic investigation with immersion objective. The scoring of labeled cells was limited to tumoral cells on the periphery of each fragment, and on the average of 2000 cells were counted per tumor. TLI was calculated as the number of labeled tumor cells * 100 / total number of tumor cells.

Histological grading was done according to differentiation (well differentiated – Grade I, moderately differentiated – Grade II, poorly differentiated – Grade III).

The relations between TLI and age, grade, TNM, stage and recurrence were tested using the paired Student's t test and non-parametric tests (Tukey's HSD, Mantel-Haenszel, Pearson) under SPSS for Windows 6.1 (SPSS Inc., Chicago, IL).

## Results

TLI was assessed in the tumor samples (all of the cases, n=31) and the adjacent tumor-free tissue as a control (n=16). The geometric means of the TLI in tumor samples (abbreviated TLI (T) ) and TLI in control samples (abbreviated TLI (C) ) were found to be 17.62±6.39% and 9.16±3.32% respectively. This difference was statistically significant ($p<0.05$).

### Age and TLI

All of the patients were grouped according to age as follows: 40-50, 50-60, 60-70 and above 70 (Table 1). A linear correlation was observed between TLI(T) and age, but it was not statistically significant.

### Tumor localization and TLI

The average TLI(T) and TLI(C) values were, respectively, 19.9% and 10.7% in the supraglottic region, 14.6% and 7.4% in the glottic region, 15.3% and 7.4% in the subglottic region and 11.6% and 6.7% in transglottically spreading tumors (Figure 1). Although TLI(T) values in supraglottic localization were higher than those in the other regions, the difference between them was not statistically significant.

### TNM and TLI relation

1. T and TLI: The average TLI(T) and TLI(C) were, respectively, 17.2% and 9.5% in T1 tumors, 16.2% and 10.7% in T2 tumors, 20.3% and 10.7% in T3 tumors and 16.3% and 7.8% in T4 tumors (Figure 2). Significant correlation was not detected.
2. N and TLI: The average TLI(T) was 17.4% in pathological nodal invasion cases (N (+)) (9 of 31 cases) and 17.9% in pathologically node–free cases (N(-)) (Figure 3). The difference between N(+) and N(-) TLI(T) values was insignificant.
3. M and TLI: No distant metastasis was detected in our cases.

In addition to these analyses, localization was also divided into subgroups and the TNM-TLI relation was assessed in these groups.

Table 1. Age and TLI relation.

| Age | N | Average TLI(T) |
|---|---|---|
| 40-50 | 8 | 13.6 |
| 50-60 | 8 | 16.4 |
| 60-70 | 12 | 19.8 |
| Older than 70 | 3 | 22.6 |

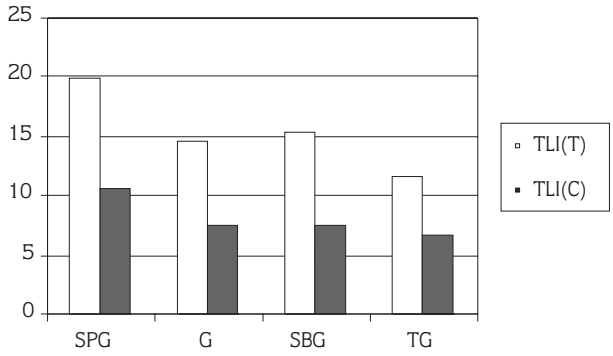


Figure 1. Localization and TLI relationship.
SPG: Supraglottic, G: Glottic, SBG: Subglottic, TG: Transglottic, TLI(T): TLI Tumor, TLI(C): TLI Control.

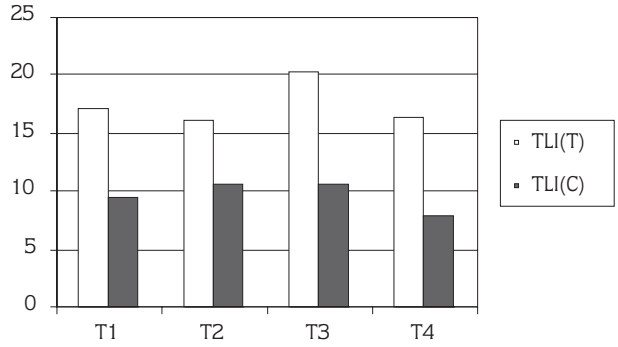


Figure 2. T and TLI relationship.
T: Primary tumor, TLI(T): TLI in tumoral tissue, TLI( C): TLI in control tissue.

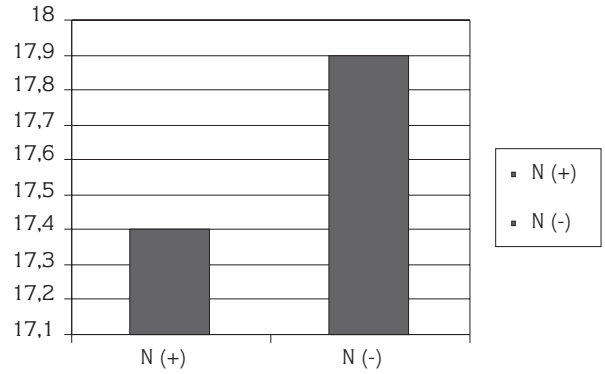


Figure 3. Nodal status and TLI relationship.
N(+): Nodal involvement, N(-): Node-free disease.

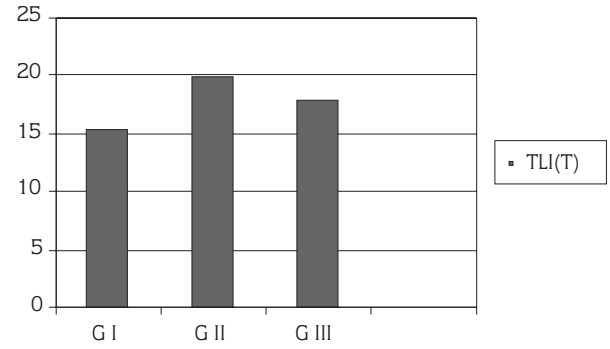


Figure 4. Grade and TLI relationship.
GI: Grade I, GII: Grade II, GIII: Grade III.

1. Supraglottic region: The average TLI(T) and TLI(C) in T1 (n=1) were 15.7% and 14.4% respectively. TLI(T) and TLI(C) values were 17.7% and 12.3% in T2 (n=5) tumors, 21.4% and 12.9% in T3 (n=7) tumors, 21.2% and 8.5% in T4 (n=4) tumors. The average TLI(T) was 18.1% in nodal disease positive cases (n=5) and 20.8% in nodal disease negative cases (n=12). No significant difference was detected in the supraglottic region.
2. Glottic region: The average TLI(T) and TLI(C) in T1 (n=3) were 15.8% and 4.9% respectively. TLI (T) and TLI(C) values were 17.3% and 11% in T2 (n=2), 17.7% and 7.3% in T3 (n=3) and 11.3% and 6.7% in T4 (n=1) tumors. The TLI(T) value was 17.7% in nodal disease positive cases (n=3) and 15.6% in nodal disease negative cases (n=6). A statistical correlation was not found.
3. Because of the limited number of patients, the transglottic and subglottic localizations were not assessed.

### Histological grading and TLI relation

Histologically, eleven patients were grade I, thirteen patients grade II and seven patients grade III. The TLI(T) value was 15.3% in grade I cases, 19.8% in grade II cases and 17.9% in grade III cases (Figure 4). A statistical difference was not found.

### Recurrence and TLI relation

Loco-regional recurrence was seen in 4 of 31 patients after six, eight and twelve months following the initial treatment. The average TLI(T) value was 12.6%. The average TLI value in recurrent cases was not higher than the total average of TLI(T).

## Discussion

Analysis of the cell kinetic activity may assist in prognosis and be useful in predicting which individual tumors will respond to therapy, in particular to radiation therapy or chemotherapy (2,6,7). Previous prognostic studies with TLI in laryngeal SCC revealed different results. Table 2 summarizes the comparison between our results and those of other authors. This table demonstrates that the number of our patients in our series was lower than in the series of Chauvel et al. and Matturri et al., but the same as that in those of the other authors. The median age of the series was similar. The average TLI value of the tumor tissue was slightly higher in our group. Our study of the TLI value of the normal laryngeal mucosa adjacent to the tumor was unique. A significant statistical difference was detected between these two tissue values. Only Balzi et al. found a significant relation between TLI and tumor site (T1-T3) (1). Only Matturri et al. detected a statistically significant difference between TLI and the nodal status of the tumor (3). None of the authors found any significant correlation in relation to histological grade. All of the authors determined different significant relationships. The major reasons for these differences fell into four categories: methodological reasons, tissue sampling, histological assessment, and tumor dependent reasons.

### Methodological Reasons:

This group can be assessed under the following headings: laboratory equipment, radioactive material, transport medium and time, culture medium, and experienced specialist. These subjects must be questioned objectively. Ganzer et al. reported an average labeling index varying from 4.1% to 14.6% from tumor to tumor specimen with the same methodology (8).

### Tissue Sampling:

We took our samples from fresh laryngeal tissue immediately after the surgical resection intraoperatively. The samples were obtained from a cancer field that did not contain any necrotizing area. The adequate volume is usually 1-5 mm3. The control samples were taken from the tumor-free area –the same laryngeal region of the tumor- macroscopically (later microscopically). They were immediately sent to the laboratory in the transport medium. Nevertheless, sampling may be done variably especially according to tumor site, volume and depth. According to Greenberg et al., considerable variability was observed in the labeling index at different sites of the same specimen (9). They recommended that an average value be determined from multiple sections. In addition, Holm et al. feel that irregular thymidine incorporation is secondary to poor penetration during one-hour incubation and recommend that suspension techniques be employed (10).

### Histological assessment:

In our laboratory, the value of TLI was determined in labeled cells that were counted from a total of 2000 tumor cells. Chauvel et al. scored 1000 cells per tumor and three fragments per tumor (6). Matturi et al. counted 4000 total tumor cells (3). Balzi et al. assessed 10,000 tumor cells (1). We considered cells containing 5 grains or more to be labeled. Matturi et al. considered six or more grains over the nucleus to be labeled (3). Chauvel et al. used the same criterion as in the present study (6).

### Tumor dependent reasons:

Tumors classified as being at the same stage may have different clinical and pathological behavior. Some tumors have more necrotic, ulcerative areas that limit adequate sampling.

Table 2. Review of the literature and comparison of the results.

| Author | Number of patients | Age | TLI (T) | TLI(C) | T | N | Grade |
|---|---|---|---|---|---|---|---|
| Chauvel et al. (1989) | 48* | 38-83 (median 59) | 11.0 ± 4.5 | (-) | ** | ** | insignificant |
| Balzi et al. (1991) | 31 | 47-72 (median 62) | 13.21 | | T1-T3 significant | insignificant | insignificant |
| Matturri et al. (1997) | 48 | 34-72 (median 59.3) | 14.16 | | insignificant | significant | insignificant |
| Unal et al. (2001) | 31 | 38-79 (median 56) | 17.62±6.39 | 9.16±3.32 | insignificant | insignificant | insignificant |

(*) 48 of the 87 patients had laryngeal squamous cell carcinoma.
(**) The author assessed the TLI-TNM relation according to stage and did not find a significant difference among clinical stages.

## Conclusion

This study did not confirm the previous results concerning correlation between TLI and prognosis, histological grade, TNM and age. However, we observed a significant difference between average tumor tissue TLI and adjacent normal tissue TLI. We feel that the prognostic value of TLI in laryngeal SCC is limited, and that variability in tissue sampling is probably the major reason. Further investigations are needed for the technique and the interpretation of the results.

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