**Journal of Current Research in Oral Surgery**
2023, Volume 3, Page No: 84-90

Available online at: www.tsdp.net

**ISSN: 3062-3480**

**Original Article**

# Toluidine Blue versus Acetic Acid Vital Staining for Detection of Oral Squamous Cell Carcinoma: Sensitivity, Specificity, and Immunohistochemical Correlation with p53 and Ki-67

**William J. Carter[1], Ahmed S. Farouk[2], Li Chen[1]***

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Sydney, Sydney, Australia.
[2]Department of Oral Surgery, Faculty of Dental Medicine, Ain Shams University, Cairo, Egypt.

***E-mail** ✉ lichen@163.com

**Received:** 14 May 2023; **Revised:** 14 August 2023; **Accepted:** 16 August 2023

## ABSTRACT

The objectives of this research were to assess how accurately toluidine blue and/or vinegar can identify oral cancer through screening, and to determine whether clinical staining outcomes using these agents correspond to the expression of the tumor marker p53 and the proliferation marker Ki67. The study included 87 individuals presenting with lesions suspected to be oral squamous cell carcinoma. Lesions were treated with toluidine blue and/or vinegar prior to biopsy. All samples were histopathologically diagnosed and processed immunohistochemically for p53 or Ki67. The findings showed that toluidine blue produced a sensitivity of 93% and specificity of 46%, while vinegar showed 85% sensitivity and 81% specificity. A significant association was found between vinegar results and Ki67 expression ($p = 0.019$). Although p53 levels differed between toluidine-blue–positive and –negative tissues, this association was not statistically significant. Overall, vinegar demonstrated lower sensitivity but greater specificity than toluidine blue for oral cancer screening, and its clinical results aligned with Ki67 expression at the cellular level.

**Keywords:** Toluidine blue, Acetic acid, Cell Carcinoma, Immunohistochemical, Ki67, p53

**How to Cite This Article:** Carter WJ, Farouk AS, Chen L. Toluidine Blue versus Acetic Acid Vital Staining for Detection of Oral Squamous Cell Carcinoma: Sensitivity, Specificity, and Immunohistochemical Correlation with p53 and Ki-67. J Curr Res Oral Surg. 2023;3:84-90. https://doi.org/10.51847/cWfmtu2uCR

## Introduction

Oral malignancies remain a major global concern [1]. Oral squamous cell carcinoma (OSCC) represents the dominant type occurring in the mouth [2]. In Thailand, the current five-year survival rate of 20%–30% remains unsatisfactory [3]. Detecting disease early is crucial for improving patient outcomes. Toluidine blue is a commonly used diagnostic dye for suspected lesions worldwide [4, 5]. Its proposed action is based on its affinity for dysplastic cells with increased nuclear density [6]. Another method under consideration is the use of 5% acetic acid, previously adopted for cervical and oral cancer screening [7]. These reactions appear more prominent in malignant epithelium due to elevated nuclear and protein content [8]. Screening tools become more reliable when their results correspond to cellular biomarkers used for cancer identification. Numerous researchers have highlighted p53, a tumor-suppressor-gene product, as a promising marker for oral cancer detection [9, 10]. p53 is responsible for regulating the cell cycle and apoptosis [11], and abnormalities in this pathway can promote carcinogenesis. Mutated p53 leads to protein accumulation within nuclei [12]. One study indicated that toluidine-blue–positive cells displayed allelic loss on chromosome 17p, where p53 is located [13]. It has also been reported that p53 mutations tend to appear in later stages and may relate to invasive OSCC behavior [14]. Therefore, examining the link between p53 expression and toluidine-blue positivity may help clarify the usefulness of this dye

Ki67, another marker of interest, reflects cellular proliferation and is present only in dividing cells [15]. In normal tissue, Ki67 is restricted to the basal layer, whereas in malignant epithelium it becomes apparent across all layers [16]. Since cancerous tissues contain increased genetic and protein material, vinegar-positive staining can be verified by measuring Ki67. As Ki67 expression rises significantly in poorly differentiated OSCC and with greater dysplasia [17], vinegar could have prognostic utility if its results correlate with Ki67.

Our earlier work showed differences in p53 expression in vinegar-positive lesions [18]. However, to date, no additional studies have combined clinical staining outcomes with immunohistochemical markers in OSCC. Accordingly, this research aimed to evaluate toluidine blue and 5% acetic acid for detecting premalignant oral lesions and to analyze the associations between these screening techniques and the expression levels of p53 and Ki67.

## Materials and Methods

*Study population*

Eighty-seven individuals with clinically identified precancerous or OSCC lesions were enrolled. Participants were recruited from Rajvithi Hospital and the Faculty of Dentistry, Chulalongkorn University Hospital, Bangkok, Thailand. All participants provided informed consent, and the study was approved by the relevant ethics committees.

*Clinical application of toluidine blue and vinegar*

An oral medicine specialist documented the clinical characteristics, photographed the lesions, and determined the areas for evaluation. Participants were randomly assigned to three groups. Toluidine blue was applied in 33 cases. A separate group of 30 patients received 5% acetic acid (vinegar). Both staining agents were used in a third group of 24 patients."**

For toluidine blue application, the lesion was first wiped with a cotton swab moistened with 1% acetic acid. A separate applicator containing toluidine blue was then pressed onto the site for 30 s. Afterward, a third swab dipped in 1% acetic acid was used to clear the excess dye. Patients rinsed afterward. A lesion that turned blue was classified as positive, while one that showed no color shift was classified as negative. The region was photographed and an incisional biopsy was obtained from the blue-stained zone.

For the acetic acid procedure, vinegar-soaked gauze was placed over a clean, dry lesion for one minute. Any visible alterations were documented and photographed. An area that became opaque white was considered positive. Lesions that remained unchanged or turned only translucent white were considered negative. Biopsy was taken from the portion turning opaque.

In the group of 24 participants receiving both agents, vinegar was used first, followed by toluidine blue. A positive interpretation required that the lesion display both an opaque-white change and blue staining. A negative result indicated absence of this dual pattern. Biopsies were taken from the region showing both changes. If the blue-stained and opaque-white areas did not overlap, biopsies were performed on each of the two sites. When no color alteration occurred, sampling was done from the central portion of the lesion.

*Immunohistochemical investigation*

Tissues were routinely processed, sectioned, and stained with hematoxylin and eosin for the final histopathological assessment. In cases treated with toluidine blue, the next serial section was used for p53 immunohistochemistry [19]. In cases treated with vinegar, the adjacent section was stained for Ki67. The primary antibody was monoclonal anti-Ki67 (MIB-1, diluted 1:100; Dako, Denmark). The Envision Plus kit (Dako, Denmark) served as the secondary detection system. Color development was produced using 0.03% DAB. Ki67-positive nuclei were counted under 400× magnification, with three representative fields per slide selected. Two investigators independently quantified the cells, and the mean value was used. Positive controls were tissues known to exhibit high Ki67 expression, and negative controls lacked the primary antibody. Samples from the 24 patients treated with both staining agents were excluded from immunohistochemical evaluation.



*Statistical analyses*

Sensitivity and specificity were computed for toluidine blue and/or vinegar in detecting oral malignancy. Fisher's exact test was used to examine the association between vinegar screening outcomes and final histopathological diagnoses. The Mann–Whitney test compared p53 or Ki67 labeling percentages between positive and negative staining groups. A p-value $< 0.05$ denoted statistical significance.

## Results and Discussion

As summarized in **Table 1**, the study enrolled 87 individuals (56% male, 44% female) aged 25–86 years (mean $61.5 \pm 12.38$ years). Lesions occurred most often on the lateral tongue (31.0%), buccal mucosa (20.7%), and floor of the mouth (18.4%). Toluidine blue was applied to 67 lesions, and 58 of these yielded positive staining; 52 of these 58 were confirmed as disease

positive by histopathology **(Table 2)**. Among the 9 lesions staining negative with toluidine blue, 4 were histologically diagnosed as positive. Thus, toluidine blue demonstrated 92.86% sensitivity and 45.45% specificity.

Vinegar was applied to 83 lesions, with 56 showing a positive response **(Table 3).** All 56 were verified as disease positive under histopathology. Among the 27 vinegar-negative lesions, 9 showed disease on final diagnosis. Therefore, the sensitivity of vinegar was 85.25%, and specificity was 81.82%.

Both toluidine blue and vinegar were used on 27 lesions; 23 displayed positive results to both agents **(Table 4).** Of these, 22 were disease positive histologically. Five lesions showed negative reactions, and 1 of these was confirmed positive. Combined use yielded 95.65% sensitivity and 80% specificity. Fisher's exact test showed significant associations between staining outcomes and final diagnoses for both toluidine blue ($p = 0.000$) and vinegar ($p = 0.004$).

**Table 1**. Characteristics of the patients.

| Characteristic | Value / Distribution |
|---|---|
| Total number of patients | 87 |
| Sex distribution | |
| Male | 49 (56.3%) |
| Female | 38 (43.7%) |
| Male : Female ratio | 6.5 : 5 |
| Age range (years) | 25 – 86 |
| Mean age ± SD (years) | 61.5 ± 12.38 |
| Anatomical location of lesions* | |
| Lateral tongue | 27 (31.0%) |
| Buccal mucosa | 18 (20.7%) |
| Floor of mouth | 16 (18.4%) |
| Lower lip | 9 (10.3%) |
| Soft palate | 7 (8.0%) |
| Hard palate | 5 (5.7%) |
| Alveolar ridge | 5 (5.7%) |



**Table 2.** Summary of findings from toluidine blue (TB) testing in comparison with histopathology (67 samples from 54 individuals).

| | Histopathological Diagnosis | | Total |
|---|---|---|---|
| | Disease Positive (OSCC) | Disease Negative | |
| **Toluidine blue result** | | | |
| Positive | 52 (True Positive) | 6 (False Positive) | 58 |
| Negative | 4 (False Negative) | 5 (True Negative) | 9 |
| **Total** | **56** | **11** | **67** |

a Conditions classified as disease-positive include dysplasia, carcinoma in situ, and squamous cell carcinoma.
b Conditions categorized as disease-negative include hyperplastic changes, inflammatory lesions, and normal mucosa
c Sensitivity = 52/56 = 92.86%; Positive predictive value = 52/58 = 89.66%.
d Specificity = 5/11 = 45.45%; Negative predictive value = 5/9 = 55.56%.

**Table 3**. Outcomes of screening with 5% acetic acid compared with tissue diagnoses (83 specimens from 57 participants).

| | Histopathological Diagnosis | | Total |
|---|---|---|---|
| | Disease Positive (OSCC) | Disease Negative | |
| **Acetic acid (vinegar) result** | | | |
| Positive | 52 (True Positive) | 4 (False Positive) | 56 |
| Negative | 9 (False Negative) | 18 (True Negative) | 27 |
| **Total** | **61** | **22** | **83** |

a Dysplasia, carcinoma in situ, and squamous cell carcinoma were grouped as disease-positive.
b Hyperplasia, inflammation, and normal epithelium were treated as disease-negative.
c Sensitivity = 52/61 = 85.25%; Positive predictive value = 52/56 = 92.86%.
d Specificity = 18/22 = 81.82%; Negative predictive value = 18/27 = 66.67%.

**Table 4.** Combined application of toluidine blue (TB) and 5% acetic acid with corresponding histopathology (28 samples from 24 subjects).

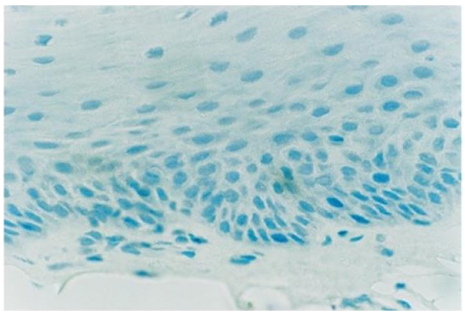
| | Histopathological Diagnosis | | Total |
|---|---|---|---|
| | Disease Positive (OSCC) | Disease Negative | |
| **Combined toluidine blue + acetic acid result** | | | |
| Both tests positive | 22 (True Positive) | 1 (False Positive) | 23 |
| At least one test negative | 1 (False Negative) | 4 (True Negative) | 5 |
| **Total** | **23** | **5** | **28** |

a Disease-positive = dysplasia, carcinoma in situ, and squamous cell carcinoma.
b Disease-negative = hyperplasia, inflammatory tissues, and normal mucosa.
c Sensitivity = 22/23 = 95.65%; Positive predictive value = 22/23 = 95.65%.
d Specificity = 4/5 = 80.00%; Negative predictive value = 4/5 = 80.00%.

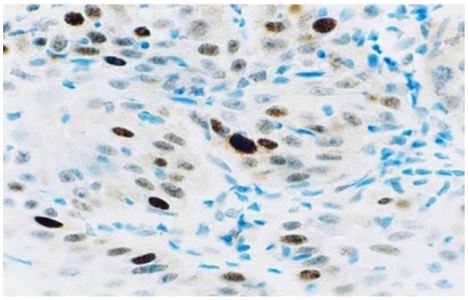
*Immunohistochemistry*

**Figure 1** illustrates p53 staining patterns. In normal oral mucosa, staining was barely detectable **(Figure 1a).** Dysplastic areas displayed isolated positively marked cells **(Figure 1b).** Squamous cell carcinoma demonstrated abundant stained cells arranged in cord-like configurations **(Figure 1c).** Although TB-positive sites showed a mean p53 expression of $4.93 \pm 1.32\%$ versus $1.49 \pm 0.97\%$ in TB-negative lesions, this contrast failed to reach significance ($p = 0.198$) **(Table 5).**

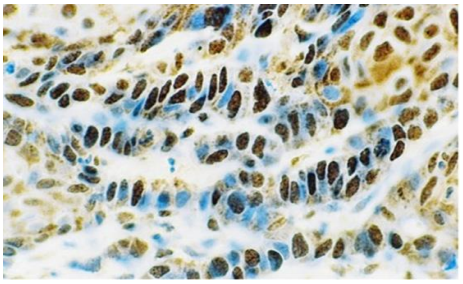
The Ki67 staining results are presented in **Figure 2**. Normal mucosa exhibited minimal labeling **(Figure 2a)**. Dysplastic samples showed distinct suprabasal staining **(Figure 2b)**. In carcinoma specimens, Ki67-positive nuclei appeared throughout the entire epithelium **(Figure 2c)**. Increasing levels of Ki67 positivity accompanied more severe diagnoses—from normal mucosa (1/33%), to hyperplasia/inflammation (4/44.4%), to dysplasia/carcinoma in situ (9/81.8%), and finally to squamous cell carcinoma (24/88.9%) **(Table 6).** The rise in staining percentages followed the same gradient **(Figure 3).** Vinegar-positive lesions averaged $3.23 \pm 0.58\%$ Ki67-positive cells, versus $1.45 \pm 0.45\%$ in vinegar-negative tissues, and this difference was statistically significant ($p = 0.018$) **(Table 7)**.



a)



b)



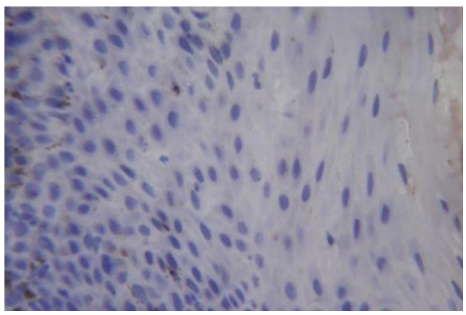
c)

**Figure 1**. p53 immunostaining in (a) normal mucosa, (b) dysplasia, and (c) squamous cell carcinoma.

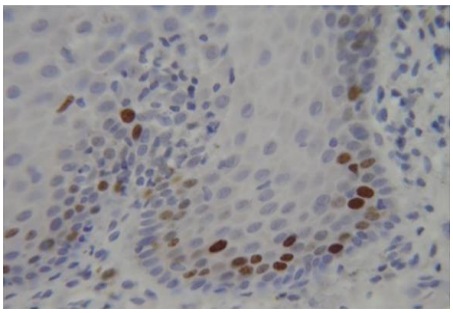
**Table 5**. p53-positive cell percentages stratified by TB result.

| Toluidine Blue Staining Result | Percentage of p53-Positive Cells (Mean ± SE) | p-value |
|---|---|---|
| Positive | 4.93 ± 1.32 | 0.198 |
| Negative | 1.49 ± 0.97 | |

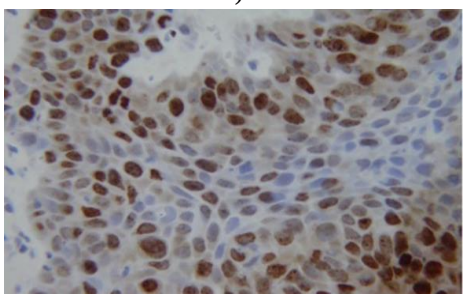
a Evaluated with the Mann–Whitney test.



a)



b)



c)

**Figure 2.** Ki67 staining in (a) normal mucosa, (b) dysplastic epithelium, and (c) squamous cell carcinoma.

**Table 6.** Number of Ki67-positive samples according to the histopathological classification.

| Histopathological Diagnosis | Number of Specimens | Ki-67 Positive Specimens |
|---|---|---|
| Oral squamous cell carcinoma | 27 | 24 (88.9%) |
| Epithelial dysplasia and carcinoma in situ | 11 | 9 (81.8%) |
| Epithelial hyperplasia and chronic inflammation | 9 | 4 (44.4%) |
| Normal oral mucosa | 3 | 1 (33.3%) |

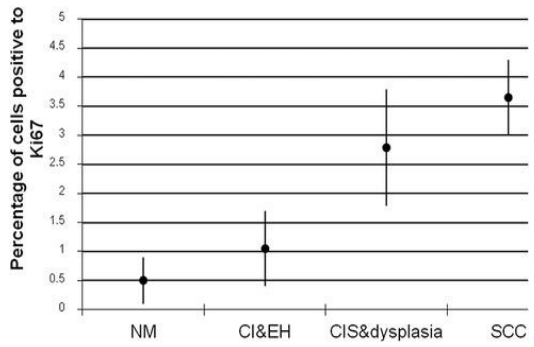



**Figure 3**. Ki67-positive cell percentages ± SD for each tissue group (NM = normal mucosa; CI = chronic inflammation; EH = epithelial hyperplasia; CIS = carcinoma in situ; SCC = squamous cell carcinoma).

**Table 7**. Ki67-positive cell percentages according to vinegar staining results.

| Acetic Acid (Vinegar) Staining Result | Percentage of Ki-67-Positive Cells (Mean ± SE) | p-value |
|---|---|---|
| Positive | 3.23 ± 0.58 | 0.019 |
| Negative | 1.45 ± 0.45 | |

a Statistical comparison made with the Mann–Whitney test.

Participants averaged 61.8 ± 11.8 years of age, and the male-to-female ratio (6:5) was similar to previous literature [1, 20]. Betel-nut use remains frequent among older Thai women [18], likely contributing to the proportion of female patients in this study. The anatomic distribution of lesions was mostly consistent with earlier reports [1, 20], except for the comparatively high involvement of the buccal mucosa (23%).

Compared with earlier work on toluidine blue for oral cancer screening [21–24], our sensitivity values were similar, but specificity was markedly reduced. This reduced specificity may be linked to the small number of benign controls and inclusion of every blue-tinged lesion, even lightly stained regions. Past studies noted that light royal-blue staining often lacks diagnostic relevance [25]. Vinegar, in contrast, showed higher sensitivity and specificity here than values typically documented for cervical screening [7, 8, 26–28]. When both agents were used sequentially on the same lesion, diagnostic performance improved further, yielding 96% sensitivity and 80% specificity.

The comparison between oral cancer screening outcomes using toluidine blue or vinegar and the final pathological findings showed that both agents tended to highlight malignant or dysplastic areas more readily than healthy tissue. This supports the usefulness of toluidine blue and 5% acetic acid as tools for early detection. Because vinegar produced a sensitivity similar to that of toluidine blue but demonstrated superior specificity, further research on its application for identifying premalignant oral changes in underserved rural settings is recommended.

Our data also demonstrated that the visible alterations triggered by vinegar during screening corresponded with notable differences in Ki67-positive cell counts between vinegar-reactive and non-reactive samples. Although p53 expression appeared higher in toluidine blue–positive tissues than in negative ones, this difference was not statistically meaningful. One explanation may be the challenge of distinguishing definite positive staining (deep blue) from weak, misleading discoloration (light blue), as a recent study noted that pale tonal changes lack diagnostic value [25]. When Ki67-positive cells were evaluated

according to final diagnostic categories, a clear trend emerged: more advanced lesions exhibited higher proportions of Ki67-reactive nuclei. Specimens of oral squamous cell carcinoma, carcinoma in situ, and epithelial dysplasia showed significantly greater Ki67 positivity than tissues with minimal or no pathological change. These findings indicate that the immunohistochemical grading of premalignant and malignant oral lesions aligns with Ki67 labeling. Overall, the results further support incorporating p53 and Ki67 assessments into strategies for treatment planning and predicting clinical outcomes in oral cancer.

**Acknowledgments:** We would also like to extend our heartfelt gratitude to Anocha Suesuwan for her help with data collection and to Kevin Tompkins for his assistance in reviewing this manuscript.

**Conflict of Interest:** None

**Financial Support:** None

**Ethics Statement:** The studies involving humans were approved by The Committee on Investigations Involving Human Subjects of Faculty of Dentistry, Chulalongkorn University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## References

1. Silverman S Jr. Oral cancer. 5th ed. Hamilton: BC Decker; 2003.
2. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990s. Int J Cancer. 1999;80:827–41. doi:10.1002/(SICI)1097-0215(19990315)80:6<827::AID-IJC6>3.0.CO;2-P
3. Vatanasapt V, Sriamporn S. Oral cavity. In: Deerasamee S, Martin N, Sontipong S, editors. Cancer in Thailand vol II, 1992-1994, IARC technical report No. 34. Lyon: IARC; 1999. p. 26–9.
4. Martin IC, Kerawala CJ, Reed M. The application of toluidine blue as a diagnostic adjunct in the detection of epithelial dysplasia. Oral Surg Oral Med Oral Pathol. 1998;85:444–6. doi:10.1016/S1079-2104(98)90071-3
5. Rosenberg D, Cretin S. Use of meta-analysis to evaluate tolonium chloride in oral cancer screening. J Oral Surg. 1989;67:621–7. doi:10.1016/0030-4220(89)90286-7
6. Rajmohan M. Assessment of oral mucosa in normal, precancer and cancer using chemiluminescent illumination, toluidine blue supravital staining and oral exfoliative cytology [Dissertation]. Chennai: Talmilnadu Dr. M.G. R Medical University; 2005.
7. Sankaranarayanan R, Wesley R, Thara S, Dhakad N, Chandralekha B, Sebastian P, et al. Test characteristics of visual inspection with 4% acetic acid (VIA) and Lugol's iodine (VILI) in cervical cancer screening in Kerala, India. Int J Cancer. 2003;106:404–8. doi:10.1002/ijc.11245
8. Sankaranarayanan R, Wesley R, Somanathan T, Dhakad N, Shyamalakumary B, Amma NS, et al. Visual inspection of the uterine cervix after the application of acetic aicid in the detection of cervical carcinoma and its precursors. Cancer. 1998;83:2150–6. doi:10.1002/(SICI)1097-0142(19981115)83:10<2150::AID-CNCR13>3.0.CO;2-0
9. Rich AM, Kerdpon D, Reade PC. P53 expression in oral precancer and cancer. Aust Dent J. 1999;44:103–5. doi:10.1111/j.1834-7819.1999.tb00209.x
10. Kaur J, Srivastava A, Ralhan R. Overexpression of p53 protien in betel- and tobacco-related human oral dysplasia and squamous cell carcinoma in India. Int J Cancer. 1994;58:340–5. doi:10.1002/ijc.2910580305
11. Warnakulasuriya KAAS, Johnson NW. Expression of p53 mutant nuclear phosphoprotien in oral carcinoma and potentially malignant oral lesions. J Oral Pathol Med. 1992;21:404–8. doi:10.1111/j.1600-0714.1992.tb01028.x
12. Mielcarek-Kuchta D, Olofsson J, Golusinski W. P53, Ki67 and cyclin D1 as prognosticators of lymph node metastases in laryngeal carcinoma. Eur Arch Otorhinolaryngol. 2003;260(10):549–54. doi:10.1007/s00405-003-0651-6
13. Ibrahim SO, Lillehaug JR, Johannessen AC, Liavaag PG, Nilsen R, Vasstrand EN. Expression of biomarkers (p53, transforming growth factor alpha, epidermal growth factor receptor, c-erbB-2/neu and the proliferative cell nuclear antigen) in oropharyngeal squamous cell carcinomas. Oral Oncol. 1999;35(3):302–13. doi:10.1016/S1368-8375(98)00120-1
14. Shahnavaz SA, Regezi JA, Bradley G, Dubé ID, Jordan RC. P53 gene mutations in sequential oral epithelial dysplasias and squamous cell carcinomas. J Pathol. 2000;190(4):417–22.

doi:10.1002/(SICI)1096-9896(200003)190:4<417::AID-PATH544>3.0.CO;2-G

15. Epstein JB, Zhang L, Poh C, Nakamura H, Berean K, Rosin M. Increased allelic loss in toluidine blue-positive oral premalignant lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;95(1):45–50. doi:10.1067/moe.2003.97
16. Hong MK, Laskin WB, Herman BE, Johnston MH, Vargo JJ, Steinberg SM, et al. Expansion of the Ki-67 proliferative compartment correlates with degree of dysplasia in Barrett's esophagus. Cancer. 1995;75(2):423–9. doi:10.1002/1097-0142(19950115) 75:2<423::AID-CNCR2820750202>3.0.CO;2-5
17. Takkem A, Barakat C, Zakaraia S, Zaid K, Najmeh J, Ayoub M, et al. Ki-67 prognostic value in different histological grades of oral epithelial dysplasia and oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2018;19(11):3279–86. doi:10.31557/APJCP.2018.19.11.3279
18. Bhalang K, Suesuwan A, Dhanuthai K, Sannikorn P, Luangjarmekorn L, Swasdison S. The application of acetic acid in the detection of oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106(3):371–6. doi:10.1016/j.tripleo.2008.01.017
19. Kurokawa H, Zhang M, Matsumoto S, Yamashita Y, Tanaka T, Tomoyose T. The relationship of the histologic grade at the deep invasive front and the expression of Ki-67 antigen and p53 protein in oral squamous cell carcinoma. J Oral Pathol Med. 2005;34(10):602–7. doi:10.1111/j.1600-0714.2005.00358.x
20. Neville BW, Damm DD, Allen CM, Chi AC. Oral & maxillofacial pathology. 4th ed. Philadephia: W.B. Saunders; 2015.
21. Mashberg A. Final evaluation of tolonium chloride rinse for screening of high- risk patients with asymptomatic squamous carcinoma. J Am Dent Assoc. 1983;106:319–23. doi:10.14219/jada.archive.1983.0063
22. Onofre MA, Sposto MR, Navarro CM, Scully C. Assessment of the blue toluidine stain in oral lesions with suspicious of malignancy. J Dent Res. 1995;74:782.
23. Warnakulasuriya KAAS, Johnson NW. Sensitivity and specificity of OraScan toluidine blue mouthrinse in the detetion of oral cancer and precancer. J Oral Pathol Med. 1996;25:97–103. doi:10.1111/j.1600-0714.1996.tb00201.x
24. Onofre MA, Sposto MR, Navarro CM. Reliability of toluidine blue application in the detection of oral epithelial dysplasia and in situ and invasive squamous cell carcinomas. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;91:535–40. doi:10.1067/moe.2001.112949
25. Gandolfo S, Pentenero M, Broccoletti R, Pagano M, Carrozzo M, Scully C. Toluidine blue uptake in potentially malignant oral lesions in vivo: clinical and histological assessment. Oral Oncol. 2006;42:89–95. doi:10.1016/j.oraloncology.2005.06.016
26. University of Zimbabwe/JHPIEGO Cervical Cancer Project. Visual inspection with acetic acid for cervical cancer screening: test qualities in a primary-care setting. Lancet. 1999;353:869–73. doi:10.1016/S0140-6736(98)07033-0
27. Belinson J, Pretorius R, Zhang W, Wu LY, Qiao YL, Elson P. Cervical cancer screening by simple visual inspection after acetic acid. Obstet Gynecol. 2001;98:441–4.
28. Cronje HS, Groesbeck PP, Brumo FC, Amanda DB, Peter D, Roosmarie HB. A comparison of four screening methods for cervical neoplasia in a developing country. Am J Obstet Gynecol. 2003;188:395–400. doi:10.1067/mob.2003.153