Comparison and Evaluation of the Reliability of Oratest and Generic Toluidine Blue in the Detection of Oral Malignancy and Premalignancy

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Abstract:
Statement of Problem: Early diagnoses of oral malignancies and pre-malignancies have direct effect in the outcome of these lesions. Therefore the techniques for early diagnosis have been considered as useful method in practice.

Purpose: The aim of this study was to evaluate the reliability of Oratest and Generic Toluidine Blue (GTB) in the detection of oral malignancy and premalignancy.

Materials and Methods: The study group consisted of 30 patients with potentially malignant epithelial lesions and superficial oral ulcerations suggestive of malignancy; of these, 7 patients with oral squamous cell carcinomas (OSCCs) were considered as positive controls and 7 patients with benign epithelial lesions as negative controls. These cases were selected from patients who were referred to the Cancer Institute, Tehran University of Medical Science (TUMS), Department of Oral Medicine, Faculty of Dentistry, TUMS and Department of Oral Medicine, School of Dentistry, Azad University of Medical Science. All lesions (n=49) submitted to both rinses (with intervals of at least 24 hours), followed by biopsy and histological analysis.

Results: The sensitivity of Oratest was 93.6%, the specificity 80%, the positive predictive value 76.6% and the negative predictive value 94.7%. These results for GTB were 84.8%, 81%, 77.7% and 86.3% respectively.

Conclusion: Staining with Oratest and GTB is highly reliable for the detection of insitu and invasive carcinomas and as an adjunct to clinical judgment and not a substitute for biopsy. The results of Oratest are more reliable than GTB especially in the detection of dysplastic lesions.

Key Words: Oratest; Toluidine Blue; Oral squamous cell carcinoma

INTRODUCTION
Oral squamous cell carcinoma (OSCC), the most common oral malignancy, often presents a clinical challenge in diagnosis to the clinicians, particularly in its early stages of development [1]. Unfortunately, less than 40% of cancer lesions are diagnosed at this stage, because these lesions are usually asymptomatic and exhibit only minimal mucosal changes [2]. OSCC is usually first
diagnosed when the lesion is obviously invasive or when the patient experiences pain, functional limitation, or regional lymphadenopathy [3].

A number of techniques have been developed in order to improve the diagnosis of oral malignancy in its early stages. During the 1960s, Toluidine Blue O (TB) was used in vivo for the first time to stain malignant epithelium, while normal tissues failed to retain this dye [4]. Afterwards this technique, named vital staining, has been applied for over 30 years to aid the detection of early OSCC, delineation of surgical fields for biopsy sites, detection of second primary cancers, and recognition of post surgical and post irradiation tumor recurrence.[2]. Toluidine blue is now commercially available in a ready-to-use kit (Oratest: ZILA Europe inc. UK), which is used as the test substance in the present study.

The purpose of this study was to assess the reliability of Oratest and Generic Toluidine Blue (GTB) in the detection of oral epithelial dysplasia, insitu carcinoma and OSCC.

MATERIALS AND METHODS
Thirty patients with clinically detectable oral premalignant lesions and oral cancers were selected from patients who were referred to the Department of Oral Medicine, Faculty of Dentistry, Tehran University of Medical Sciences (TUMS), Cancer Institute, TUMS and Department of Oral Medicine, School of Dentistry, Azad University of Medical Science from December 2001 to February 2003. Twenty one of the patients were men with a mean age of 59.8 years, and nine were women with a mean age of 55.5 years. Seven patients with histologically documented OSCC were considered as positive-staining cases and 7 patients with clinically documented benign epithelial lesions (reticular lichen planus, epulis fissuratum and Heck’s disease) were considered to be negative.

A thorough head, neck and oral cavity examination was performed for all patients by an Oral Medicine specialist. The diagnosis was based on the evaluation of location, size, morphology, color and surface characteristics of the suspected lesions. All lesions were photographed by a digital camera (Coolpix 7500, Nikon, Japan).

After clinical diagnosis, the lesions were submitted to Oratest® Tolonium chloride mouth rinse (Oratest: ZILA Europe inc. United Kingdom) according to the technique recommended by the manufacturer. Location, size, morphology, color and surface characteristics of the suspected lesions that had retained the blue color of the Oratest were recorded in a diagram and also photographed. The GTB was prepared following the recommendation of Mashberg [5,6] and the staining procedure was repeated at least 24 hours later, using the same technique. Site(s) stained by the dye were re-photographed and recorded in a diagram separately.

The pattern of dye retention was assessed by the intensity of staining: sites with dark royal blue coloring, stippled staining or minimal stain-retention were considered as positive, and areas which did not demonstrate any kind of blue staining were considered as having a negative staining pattern. [5,6] The biopsy sites(s) were selected on the basis of clinical appearance and dye retention. Areas retaining stain were biopsied. In locations where staining was negative, clinical judgment directed the biopsy. Various sites, representative of the entire specimen, were selected for biopsy from lesions larger than 1 cm. The biopsy specimens were submitted to routine procedures of Hematoxylin-Eosin staining and were examined by a pathologist who was blinded to the results of tissue staining.

The results of the clinical and histological diagnosis were compared to the staining results. For statistical analysis the sensitivity,
specificity and the positive and negative predictive values were calculated separately for each rinse according to the method proposed by Rosenberg and Cretin [2], and were then compared.

RESULTS
Twenty five of the 44 studied patients were smokers (21 smoked paper cigarettes, 4 smoked hand-rolled cigarettes) and five regularly consumed alcohol. Totally 49 lesions were found in 44 cases. Different staining patterns were observed in different cases (Figures 1-4). The clinical diagnosis of the lesions and the staining results are presented in Table I. All superficial ulcerations, OSCCs and recurrent tumors retained stain. Non-homogenous leukoplakia and erosive/ulcerative lichen planus retained the stain with a higher frequency than did the homogenous leukoplakia. There was no retention of stain in reticular lichen planus and homogenous leukoplakias.

The histological diagnosis and the results of staining are demonstrated in Tables II and III. The lesions were histologically classified as follows: 1- S.C.C, also including insitu carcinoma, 2- Epithelial dysplasia, which was classified into mild, moderate and severe, 3- Lichen planus, and 4- other benign lesions. All malignant lesions retained stain, indicating a 100% sensitivity of Oratest and Generic Toluidine blue for the detection of in-situ and invasive carcinomas.

Of the five epithelial dysplasias, GTB detected two dysplastic lesions (1 mild and 1 moderate), while Oratest detected four (2 mild, and 2 moderate) of these lesions, demonstrating a staining sensitivity of 40% for G.T.B and 80% for Oratest.

In the total sample analysis, 23.3% of the results were false–positive and 5.2% were false–negative for Oratest, while G.T.B resulted in 22.2% false- positive and 13% false negative cases.

The sensitivity of staining with Oratest was 93.6% (Confidence interval, CI, 95%: 86.7% to 100%), the specificity 80% (CI 95%: 68.8% to 91.2%), the positive predictive value (PPV) was 76.6% (CI 95%: 64.7% to 88.5%) and the negative predictive value (NPV) was 94.7% (CI 95%: 88.4% to 100%). But G.T.B demonstrated a sensitivity of 84.8% (CI 95%: 74.6% to 95%), a specificity of 81% (CI 95%: 70% to 92%), a PPV of 77.7% (CI 95%: 66% to 89.4%) and a NPV of 86.3% (CI 95%: 76.7% to 95.9%).

DISCUSSION
The samples investigated in this study was composed of 14 (28%) premalignant lesions, 17(34%) clinically suspected malignancies, 7(14%) superficial oral ulcerations suggestive of malignancy, and 7(14%) benign lesions, demonstrating that this work included different forms of lesions which a practitioner could experience in his/her professional practice.

To our knowledge, the present study may be the only report in the English literature on the evaluation and comparison of Oratest and Generic Toluidine blue (GTB).

Various investigations with an exception of Rosen et al [7], have demonstrated that Toluidine blue has a high sensitivity in its detection of malignant oral lesions; values vary from 84 to 100% [2-6]. In this study, staining was seen to be highly efficient in the detection of carcinomas (in-situ and moderate and poorly differentiated), exhibiting a sensitivity of 100%, because no false negative results occurred among the lesions histologically diagnosed as carcinomas.

Recently, Warnakulasuiya and Johnson [8] confirmed that O.S.C.C could be detected with a sensitivity of 100% by using a commercial rinse (Orascan) containing Toluidine blue as its active ingredient.

Epstein et al [9], evaluated the utility of Toluidine blue application in facilitating the recognition and diagnosis of clinically evident
### Table I: Clinical diagnosis of oral lesions and results of staining

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>N (%)</th>
<th>Toluidine Blue</th>
<th>Oratest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-homogenous leukoplakia</td>
<td>4(8.1)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Homogenous leukoplakia</td>
<td>3(6.1)</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Patchy leukoplakia</td>
<td>1(2)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Reticular lichen planus</td>
<td>4(8.1)</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Erosive/ulcerative lichen planus</td>
<td>5(10)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Erythroplakia</td>
<td>1(2)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>S.C.C. (including seven documented cases)</td>
<td>13(26)</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>Superficial ulceration</td>
<td>7(14)</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Recurrent tumor</td>
<td>4(8.1)</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Epulis fissuratum</td>
<td>2(4)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Heck’s disease</td>
<td>1(2)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Post operative follow-up</td>
<td>4(8.1)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>49(100)</td>
<td>32</td>
<td>17</td>
</tr>
</tbody>
</table>

![Fig 1: A: Large leukoplastic lesion on left buccal mucosa, B: Staining with oratest shows intensivestippled area. GTB shows same pattern. Diagnosis: S. C. C](image)

### Table II: Histological diagnosis of oral lesions and results of staining

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>N(%)</th>
<th>Toluidine Blue</th>
<th>Oratest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign lesions</td>
<td>21(42)</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>4(8.1)</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>19(38)</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>Epithelial dysplasia</td>
<td>5(10)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>49(100)</td>
<td>27</td>
<td>22</td>
</tr>
</tbody>
</table>
Fig 2: A: Post operative follow–up for previous S.C.C of tongue & floor of the mouth No Clinical lesion delineated, B: After GTB application, area did not stain. Oratest application shows irregular stippled stain. Subsequent biopsy confirms Mild dysplasia.

Fig 3: A: Leuko erythroplastic lesion on left mucosa, B: GTB and oratest application shows same pattern. Buccal Point of Dark royal blue stain diagnosed as carcinoma in-situ. Unstained area was mild dysplasia. (False negative).

Fig 4: A: Superficial ulceration on buccal mucosa which remain for 2 months, B: GTB. Oratest application shows intensive staining. Diagnosis No Tumor was found. (False–positive)
lesions in patients previously treated for cancer and undergoing post monitoring. Toluidine blue stain identified all in situ carcinomas and invasive malignant lesions, whereas clinical examination identified 78% of the in situ carcinomas or invasive malignant lesions. Other researches also demonstrated the 100% sensitivity in detection of carcinomas [5,6,10,11].

The variable ability of Toluidine blue to identify premalignant lesions has been shown in previous studies [4-6,8,10,11]. Unlike malignancies; premalignant lesions yield a variety of color reactions following staining. Well-defined evaluation criteria are not yet established for this purpose. For example, Reddy et al [12] defined a positive result as an area staining greater than 2mm in diameter. In 160 out of 1190 patients investigated by Vahidy et al [11], the test result was reported doubtful. Warnakulasuriya and Johnson [8] reported that only 9 out of 24 white lesions and 37 out of 66 red/spackled/nodular lesions retained the dye, confirming that staining was inconsistent with clinical judgment. Hyperkeratotic lesions among Asian subjects are reported by others to reject the stain [11]. It is noteworthy that in two earlier studies on white Caucasian patients, all premalignant and dysplastic lesions retained the dye, giving 100% sensitivity [10,13].

Among the lesions that had microscopic dysplasia in Warnakulasuriya and Johnson’s [8] study, 28/39 (74%) had stained positive and two had a doubtful result.

Onofre and colleagues [3] reported that among 6 epithelial dysplasias, 3 (1 mild and 2 moderate) did not retain the stain.

In the present study, among the lesions diagnosed as epithelial dysplasia (n=6), four (1 mild and 3 moderate) did not retain GTB, while only 2 (1 mild and 1 moderate) did not stain with Oratest. According to Warnakulasuriya and Johnson [8], false negative results may occur as a result of a lack of objective criteria for the evaluation of stain uptake. Mashberg [14] reported that lesions with limited dysplasia or atypia did not intensely retain the stain.

Although the false negative was found in this study, the use of GTB and Oratest should not be discontinued, because it was shown that all in situ and invasive carcinomas had retained the stain.

Previous studies demonstrated a great variation in the specificity of staining by Toluidine blue ranging from 40% to 100% [15-22]. A possible explanation for the lower specificity results obtained in some studies may be that their final conclusions had been drawn on the basis of staining results acquired in the first visit and not 7 to 14 days after the first consultation. This may cause a large number of false-positive results, generally produced by the retention of stain in inflammation and traumatic areas. To decrease

<table>
<thead>
<tr>
<th>Analyzed Lesions</th>
<th>Sensitivity of Toluidine blue N(%)</th>
<th>Sensitivity of Oratest N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>19/19(100)</td>
<td>19/19(100)</td>
</tr>
<tr>
<td>- In situ</td>
<td>3/3(100)</td>
<td>3/3(100)</td>
</tr>
<tr>
<td>- Moderately differentiated</td>
<td>8/8(100)</td>
<td>8/8(100)</td>
</tr>
<tr>
<td>- Poorly differentiated</td>
<td>8/8(100)</td>
<td>8/8(100)</td>
</tr>
<tr>
<td>Epithelial dysplasia</td>
<td>2/5(40)</td>
<td>4/5(80)</td>
</tr>
<tr>
<td>- Mild</td>
<td>1/2(50)</td>
<td>2/2(100)</td>
</tr>
<tr>
<td>- Moderate</td>
<td>1/3(33)</td>
<td>2/3(66)</td>
</tr>
<tr>
<td>Benign lesions</td>
<td>19/25(76)</td>
<td>18/25(72)</td>
</tr>
</tbody>
</table>
the number of false-positive results and consequently increase the specificity of staining. Mashberg 6 recommends that all irritating and inflammatory factors should be eliminated in the lesions that retain stain. These lesions should be reevaluated and stained after 7 to 14 days, and if the stain is retained once more, they should be considered as suspicious of carcinoma. Trials which followed this recommendation, generally recorded a higher specificity (88-94%) [5,6,15,16]. A larger number of false-positive results and a decrease of specificity are demonstrated in inflammatory and ulcerative lesions [5,10,11,17]. Previous studies have recorded a range of values for specificity of the dye retention to differentiate benign lesions which may result from the wide range of clinical lesions included in this category in the trial designs [18-22]. The lowest specificity recorded so far is 40%.

In the current study, Oratest revealed 29.6% false-positive cases with a specificity of 73.1%; while GTB showed a specificity of 76% and 24% false-positive cases. One of the false-positive results was among the non-homogenous leukopkias, 5 among ulcerative lesions, 1 among clinically diagnosed S.C.C, 2 among recurrent tumors and 4 among erythematous reactions, demonstrating that 80% of these results occurred in lesions with ulceration or erythema. The higher level of false-positive results may increase the necessity to perform a larger number of biopsies in potentially malignant epithelial lesions (PMELs) and superficial ulcerations suspicious of malignancy. This may be beneficial because all PMELs and superficial ulcerations suspicious of malignancies that do not respond to treatment should be submitted to microscopic analysis 3. In addition, the diagnosis of PMELs, based only on clinical appearance, may lead to a misdiagnosis and therapeutic errors.

In this study, results showed that the lesions which stained with Oratest, demonstrated a 76.6% probability of having areas with epithelial dysplasias, insitu carcinoma or invasive carcinomas, while GTB showed a 77.7% probability. The negative predictable value indicated that lesions that did not retain the Oratest stain demonstrated a 94.7% probability of not having areas of epithelial dysplasia while GTB’s negative predictable value was 86.3%.

CONCLUSION
Within the limitation of this study, it can be concluded that staining with Oratest and GTB is highly reliable for the detection of insitu carcinoma and invasive carcinoma. From our point of view, staining with Oratest and GTB is an adjunct to clinical judgment and not a substitute for either judgment or biopsy. The results of Oratest appeared to be more reliable than GTB, especially in detection of dysplastic lesions. Staining should be routinely used as a method to assist in the choice of biopsy sites and in the follow-up of oral cancer patients. We believe that Oratest (Oratest: ZILA Europe inc. United Kingdom) serves the important purpose of accelerating biopsy, particularly in persistent lesions, and allows the selection of areas of the lesions more likely to be demonstrated as malignancies or dysplasias.

AKNOWLEDGMENT
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REFRENCES
2- Rosenberg D, Cretin S.Use of meta-analysis to evaluate toluidine chloride in oral cancer
ارزیابی و مقایسه تکراری‌ذیری استفاده از Oratest و تولوئیدن بلوی ذراتی و پیش‌بندی‌های حفره دهان

م. اسلامی ۱، ع. کهیان ۲، مطهری ۳، ف. آقاهسینی ۴، شرف‌زاده ۵

چکیده

قبلا: تشخیص بدکننده‌های دهان در مرحله اولیه در بستر بیمارستان بسیار مؤثر است به‌طوری که در این مطالعه، فعالیت‌های روش‌های مطالعه‌ای در حال اجرا بودند. اثربخشی: طبق ارزیابی تکراری‌ذیری Oratest و تولوئیدن بلوی ذراتی در تشخیص بدکننده‌های دهان و پیش‌بندی‌های حفره دهان انجام شد.

روش تحقیق: این مطالعه در بیمارستانهای ایران در مرحله اولیه در بستر بیمارستان به‌طور مبهم انجام شد.

یافته‌ها: پژوهش سپاسی تشخیص Oratest و Toluinity به‌طور مبهم در درصد ۳۲ به‌طور مبهم انجام شد. اثربخشی: طبق ارزیابی تکراری‌ذیری Oratest و تولوئیدن بلوی ذراتی در تشخیص بدکننده‌های دهان و پیش‌بندی‌های حفره دهان انجام شد.