Introduction

Oral cancer is a significant threat to public health all over the world, especially in South and Southeast Asia, where betel use is very popular. In Taiwan, it accounts for an average of 1,436 deaths annually, being the 5th highest cause of death owing to its malignancy. Despite advances in the detection and treatment of many other malignancies, the overall survival rate of oral cancer has remained disappointingly low for the last 10 years and was reported as less than 50% in the literature. Not surprisingly, this reflects the fact that the treatment outcome depends not mainly on the improvement of surgical techniques but on the characteristics of the cancer itself. According to recent statistical data reported in Taipei Veterans General Hospital in Taiwan, the 5-year survival rate of stage I oral cancer patients was 66.2%, while for stage IV, the survival rate declined to only 22.2%. The paper suggested that the early detection of oral cancers and treating patients in their early stages were the most important factors in improving the survival rate.

Periodic clinical examination of the oral cavity is the mainstay for early detection of oral cancers. It was shown to reduce mortality from oral cancer by 32% in high-risk individuals. Additionally, using adjunctive aids such as toluidine blue (also referred to as toluidine chloride) has been widely accepted to improve the effectiveness in large-scale screening for oral cancer diagnosis. However, it is hazardous if swallowed, and was shown to have toxicity to fibroblasts. Another kind of dye material, methylene blue, has a similar chemical structure and exhibits similar physicochemical properties to toluidine blue. It is less toxic to the human...
body and has recently been proposed for screening
some gastrointestinal or prostate tumors. The applica-
tion of this material in detecting oral lesions has so far
not been addressed. The objective of this study was to
evaluate the sensitivity and reliability of in vivo staining
with methylene blue as a diagnostic adjunct in screening
for oral malignant or precancerous lesions.

Methods

Subjects
This project was approved by the institutional review
board of Taipei Veterans General Hospital (VGH),
with informed consent signed by patients. Fifty-eight
patients at the Oral and Maxillofacial Surgery, Depart-
ment of Dentistry, Taipei-VGH between October
2002 and August 2003 with the presence of abnormal
oral manifestations were included in this study. All the
patients had no history of oral cancer or previous oral
surgery. The patients’ clinical profiles such as gender,
age, and habits of betel use were collected. All the
patients were subjected to a systematic oral examina-
tion with clinical diagnosis as: (1) homogeneous leuk-
oplakia: white, uniform, flat lesion with a smooth,
wrinkled, or corrugated surface, not able to be scraped;
(2) nonhomogeneous leukoplakia: white lesion with
irregular and exophytic surface; (3) erythroplakia: red
lesion with ill-defined margin; (4) ulceration: localized
and superficial lesion that does not heal after local
treatment. The control group comprised 20 dental
students from National Yang-Ming University who
volunteered and who were randomly enrolled, with
a mean age of 22 years. All volunteer students had
no habits of betel quid chewing, smoking, or alcohol
drinking.

Gargling solution
A set of methylene blue dye system includes 2 bottles
of solution. The dye rinse solution (Bottle A) contains
active ingredient methylene blue 1%, with the addition
of 1% malachite, 0.5% eosin, glycerol, and dimethylsul-
foxide. Pre- and post-rinse solution (Bottle B) contains
1% lactic acid, raspberry flavor, and purified water.

Staining procedure
The application of methylene blue was as follows.
A 5-minute teeth brushing procedure was required
before testing. All patients rinsed their mouths with
Bottle B for 20 seconds to remove food debris and
excess saliva and to provide a consistent oral environ-
ment. The mucosa of the target area was gently dried
with gauze and power air spray to ensure that the
lesion was not contaminated with saliva. Patients gar-
gled and rinsed with 1% methylene blue dye (Bottle A)
for 20 seconds, then expectorated. Patients then rinsed
again with Bottle B for 20 seconds to wash out the
excess dye. The pattern of dye retention was assessed
by the intensity of stain retention on the lesion. Local,
stippled, patchy and deep blue stains were marked as
positive (+) reaction. Wide, shallow or faint blue stains
were marked as negative (−) reaction. For equivocal
staining, Bottle B solution was applied with cotton
rolls to wipe out the staining surface. If the blue stain
was washed out, negative reaction was recorded and
vice versa. If the patient had a highly suspicious lesion
that was not all stained by the solution, the patient was
instructed to revisit within 14 days to repeat the test
in order to reduce the false-negative rate. The results
of methylene blue dye staining were recorded with
photographs, and biopsy was performed simultane-
ously in the suspected lesions to compare the accuracy
of the diagnostic capability of methylene blue.

Biopsy
Incision biopsy was performed in the most obvious
staining area of the suspicious lesion of patients under
local anesthesia. If there was no dye uptake in the
lesions, the biopsy specimen was taken from the area
judged by a specialist’s experience. The specimens
were then fixed in 10% neutral buffered formalin and
processed in the pathology laboratory for initial rou-
tine pathologic diagnosis.

Histologic examination
All the specimens were microscopically evaluated by
pathologists who were blind to the results of methyl-
ene blue stain. The pathology reports of the lesions
were classified as: (1) benign lesions including epithelial
hyperplasia, lichen planus, hyperkeratosis; (2) precancer-
ous lesions including verrucous hyperplasia, dysplasia;
and (3) malignant lesions including verrucous carci-
noma and squamous cell carcinoma.

Data analysis
The pathologically proven cancers and precancerous
lesions were the targets of screening. The results of
positive/negative uptake of methylene blue in each
lesion were correlated with the histopathologic diag-
nosis. Statistical analysis was performed, including sensi-
tivity, specificity, positive and negative predictive values.
The association of methylene blue uptake and patho-
logic diagnosis among the precancer/cancer group,
benign group, and normal group were analyzed using
Fisher’s exact test. A $p$ value of less than 0.05 was
considered significant.
Results

Subject characteristics
Seventy-eight people (58 patients, 20 students who volunteered) were enrolled in this study. The patients’ ages (patient group) ranged from 31 to 82 years (41 ± 15 years), with the ratio of male to female being 51:7. The students’ ages (control group) ranged from 20 to 24 years. Two-thirds of patients (n = 38) had a history of betel quid chewing, and 52 patients had a history of cigarette smoking. The suspected lesions were distributed over the buccal mucosa (n = 25), tongue (n = 16), gingivae (n = 9), lip (n = 3), floor of the mouth (n = 2), palate (n = 2), and retromolar trigone (n = 1).

In the control group, as methylene blue dye was not used to examine the oral cavity, it was necessary to verify that the dye would not be retained on normal mucosa. The results demonstrated that there was no retained dye in the control group.

Methylene blue staining related to grade of pathology
The clinical and histopathologic diagnosis of oral lesions and the results of staining are shown in Table 1. The pathologic grade was classified as benign lesions, precancer lesions and cancer lesions as previously described in the methods section. The following statistical terms were used to describe and analyze the relationship between the grade of pathology and the uptake of methylene blue staining.

Sensitivity represents the proportion of histologically proved cancer/precancerous lesions which are detected by positive methylene blue staining. In the current study, 26 of 29 pathologically proved cancer/precancerous lesions were positive with deep and focal methylene blue staining (Figure 1B). The overall sensitivity was 90%. Among the 3 false negative cases, 2 were clinically presented as chronic ulcers with induration over the tongue and were later proved as squamous cell carcinoma after biopsy, and 1 was a homogeneous leukoplakia on the buccal mucosa with a pathologic diagnosis of epithelial dysplasia. They were stained with a faint blue color (Figure 2B).

Specificity suggests the proportion of pathologic benign lesions, neither precancerous lesions nor cancers, which are correctly identified as negative staining of methylene blue (Figure 3B). In our study, 20 of 29 benign lesions showed negative staining; thus, the specificity was 69%.

The results of staining with methylene blue for all lesions correlated well with the pathologic diagnosis and are summarized in Table 2. Fisher’s exact test showed significant differences among cancer/precancerous lesions, benign lesions, and normal control groups (p < 0.05). Overall, the positive predictive value was 74% (26/35), and the false predictive value was 87% (20/23).

Discussion
Oral cancer is very common in Southeast Asia, including Taiwan. Areca nut chewing is the main etiologic factor inducing carcinogenesis in oral mucosa. Individuals with all the habits of smoking, drinking and areca nut chewing were reported to have 123 times the risk of

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Homogeneous leukoplakia (n = 36)</th>
<th>Nonhomogeneous leukoplakia (n = 11)</th>
<th>Erythroplakia (n = 5)</th>
<th>Ulcerations (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histologic diagnosis</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Malignancy (n = 16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>10</td>
<td>–</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Precancerous lesion (n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysplasia</td>
<td>4</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Benign lesion (n = 29)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>–</td>
<td>9</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>3</td>
<td>7</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Total (n = 58)</td>
<td>17</td>
<td>19</td>
<td>11</td>
<td>5</td>
</tr>
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</table>

Table 1. Clinical and histologic diagnosis of oral lesions and results of staining
people without such habits. Therefore, oral cancer screening of high-risk individuals is very important in these countries.

For a large-scale community screen, some dye materials help to identify abnormal mucosa tissue which raise oral examiners’ attention and refer the patients with suspicious lesions to oral surgeons for further examinations. This vital staining method was used at first in medicine for detecting cervical dysplasia and carcinoma in situ in the 1960s. Niebel and Chomet were the pioneers who used dye material to detect oral cancer in 1964. Toluidine blue dye is known as 1 of the diagnostic adjuncts to detect oral cancer/precancerous lesions. The efficacy of this technique has been evaluated in many reports with diverse results. It has yielded sensitivities between 72% and 100%, and specificities between 45% and 67%, in detecting suspicious malignancies. However, the Material Data Safety Sheet indicates that toluidine blue dye is probably toxic by ingestion, and it is seldom used for detecting cancers in other parts of the human body.

Methylene blue is another recently proposed dye for in vivo staining used in endoscopic examination. Its application has been reported recently in detecting some gastrointestinal abnormalities such as Barrett's esophagus, gastric cancer, prostate cancers, and also bladder cancer. The exact mechanism for the uptake of methylene blue dye in epithelial cells is still not very clear, but it resembles toluidine blue dye in its acidophilic characteristic and may penetrate into cells with an abnormal increase in nucleic acid, thus resulting in different uptake between normal and highly dysplastic/malignant cells.

Usage of the methylene blue technique in detecting oral cancer or precancerous lesions has not been reported thus far. Among all the statistical values, sensitivity rate and false-negatives are the most important in evaluating the efficacy of certain diagnostic tools for detecting abnormal lesions. In the current study, 26 of 29 pathology-proven precancer/cancer lesions showed positive staining with deep and focal methylene blue dye. Overall, 90% sensitivity (88% for malignancy and 92% for precancerous lesions) was reported, with a false-negative rate of 10%. Compared to the 72–100% sensitivity reported in the previous studies, these values indicated that using methylene blue dye in the
detection of cancer or precancerous lesions is acceptable. As for false-negatives, we consider that the ambiguous light blue stains which may be misinterpreted as negative but clinically suspicious of malignancy still need further biopsy to prove the diagnosis pathologically.

In the aspect of specificity, we obtained a value of 69% (20/29) with a resulting false-positive rate of 31%. The 9 false-positives were homogeneous leukoplakia \((n=3)\), nonhomogeneous leukoplakia \((n=4)\), erythroplakia \((n=1)\), and an ulceration \((n=1)\). The high false-positive rate was discussed to be related to the retention of stain in inflamed and trauma areas.\(^{19}\) Other causative factors may include the irregular, papillary or digital surfaces of the lesions, which may cause the mechanical retention of dye, contamination of saliva and plaque, retention of dye material in papilla of the tongue or minor salivary gland ducts over the mucosa. The high number of false-positives in this study means that more patients received biopsies. Nevertheless, rational management for patients with suspected oral lesions who have either a positive or negative methylene blue stain remains biopsy of the lesion.

Applying this method to screen high-risk patients with the habits of betel quid chewing or smoking, a large group of individuals may include those with obvious oral lesions and those with normal oral mucosa. To study these people and to re-evaluate the efficacy of methylene blue in detecting oral cancers/precancerous lesions, a large proportion of the people with normal oral mucosa will lower the rate of false-positives and result in higher specificity. Although we had a control group with fully normal oral mucosa, there was a flaw in the experimental design that these students had no habits of betel quid chewing and histories of smoking. However, individuals who had these habits without lesions were also not very suitable to be our control group because performance of biopsy in normal mucosa would not be ethical.

In conclusion, this study shows that methylene blue staining has nearly 90% sensitivity in detecting oral cancers or precancerous lesions. Considering its low toxicity and the fact that it is cheaper than toluidine blue, it may be convenient to substitute it for toluidine blue in large-scale oral screening of high-risk
Nevertheless, the pathology report from biopsy is still the gold standard to accurately diagnose the lesion before a treatment modality is determined.

Acknowledgments

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Table 2. Efficacy of methylene blue application in pathologically proved cancer/precancerous lesion

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Cancer/Precancer (n = 29)</td>
<td>26 (90)*</td>
<td>3 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Benign (n = 29)</td>
<td>9 (31)</td>
<td>20 (69)†</td>
<td></td>
</tr>
<tr>
<td>Normal (n = 20)</td>
<td>0 (0)</td>
<td>20 (100)</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

Positive predictive value 26/35 (74)
Negative predictive value 20/23 (87)

*Sensitivity; †specificity.

References

2. Stell PM, McCormick MS. Cancer of the head and neck: are we doing any better? Lancet 1985;2:1127.


