

Research Report

Screening of oral premalignant lesions in smokers using toluidine blue

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ABSTRACT

Background: A smoker is associated with the risk of developing oral premalignant lesions due to the carcinogenic contents in cigarette. Toluidine blue is a basic chromatic dye used in screening the presence of premalignant lesions due to its ability to detect acidic components in cells and tissues. **Purpose:** This study was purposed to observe the outcomes of toluidine blue staining on oral mucosa of smokers and non smokers and to find out whether quantity and duration of smoking affect the final results of toluidine blue staining. **Methods:** Forty male subjects, aged 20-60 years old were involved in this study, consisted of 10 heavy smokers, 10 moderate smokers, 10 light smokers and 10 non smokers. Subjects were instructed to rinse their mouths with mineral water for 20 seconds followed by acetic acid 1% for another 20 seconds. Toluidine blue stain was applied in excess and left on site for 1 minute. Subjects were instructed to rinse with acetic acid 1% and sufficient water consecutively for 20 seconds each. The areas of oral mucosa that stained blue were captured with intraoral camera and transferred to the computer unit. The staining procedure was repeated after 14 days. **Results:** Chi-square test showed that toluidine blue positive staining dominates the smokers group. Regression and correlation test indicate that Toluidine blue staining is more obvious in subjects who consume more cigarettes. **Conclusion:** It was concluded that oral mucosa of smokers absorbed more toluidine blue than that of non smokers and retention of toluidine blue is affected by quantity and duration of smoking.

Key words: cigarette, oral mucosa, toluidine blue, smokers

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INTRODUCTION

Tobacco usage has become a major health problem worldwide since it causes many harmful effects, such as causing serious systemic effects and damaging tissues of the mouth.^{1,2} In many places, smoking is practiced mostly in the form of cigarette, however, other forms of tobacco, including smokeless tobacco, cigars, and pipes, are also used. Every single bar of cigarette contains more than 4000 chemical substances, including more than 60 carcinogens. Some of them are nicotine, tar, carbon monoxide and hydrogen cyanide.^{3,4} There is good evidence that tobacco in all forms is carcinogenic in the upper aerodigestive tract, including the mouth.^{5,6} The disruption of normal cellular and molecular mechanisms would appear to be the target of tobacco ingredients.⁷

The strong association between cancers of the oral cavity and pharynx with tobacco use is well established. Epidemiological studies show that the risk of developing oral cancer is five to nine times greater for smokers than for non smokers.⁸ The duration of smoking habits and the number of cigarettes consumed daily were significantly associated with the presence of premalignant lesions.⁹

In spite of its lower incidence as compared to other cancers, oral cancer has a low survival rate, largely because the disease is often not diagnosed until it is advanced. Public awareness of oral cancer is also low and this contributes to delay in diagnosis.^{10,11} However, the diagnosis of oral cancer is challenging due to its multitude of ill-defined, variable appearing, controversial and poorly understood lesions that appear in the mouth.¹²

Toluidine blue (tolonium chloride), an acidophilic, metachromatic dye belonging to the thiazine group, is a vital tissue dye which exhibits differential uptake into tissue, resulting in metabolically active areas of lesions being stained a deep blue.¹³ When reacting with the tissues, toluidine blue selectively stains acid tissue components (sulfate, carboxylate and phosphate radicals) such as DNA and RNA. There is no evidence of chemical reaction with any component of the cell within the reactions.¹⁴ Oral premalignant lesions that stained with toluidine blue consistently contained loss of chromosomal genetic information, termed loss of heterozygosity.¹⁵

Previous studies showed that the sensitivity and specificity of toluidine blue were quite reliable in revealing early oral premalignant lesions and monitoring recurrences of oral carcinoma.^{16,17} Toluidine blue-positive staining correlated with clinicopathologic risk factors and high-risk molecular patterns. Oral premalignant lesions which stained positively with toluidine blue showed a consistently higher frequency of loss of heterozygosity for chromosome arms.¹⁵ A question needs to be answered is whether toluidine blue can be used to screen premalignant lesions which would likely occurred in smokers.

The objectives of this study were to screen the premalignant lesions using toluidine blue staining on oral mucosa of smokers and non smokers and to find out whether quantity and duration of smoking would effect the screening results. This study is expected to provide sufficient scientific information about toluidine blue staining as a screening tool to detect the presence of oral premalignant lesions in smokers and non smokers, with attention to quantity and duration of smoking.

MATERIALS AND METHODS

This study involved 40 men whose ages range from 20 to 60 years. Subjects were divided into 4 groups: Group I consisted of 10 heavy smokers (consume more than 15 cigarettes daily), Group II consisted of 10 moderate smokers (consume 10-15 cigarettes daily), Group III consisted of 10 light smokers (consume less than 10 cigarettes daily) and Group IV consisted of 10 non smokers. All smokers have smoked at least 5 years prior to the study. The sampling technique is purposive sampling.

Subjects were asked for their consents as the study samples and to answer the standardized smoking questionnaires. Then, the anamnesis was accomplished by reviewing the medical and dental histories, oral and systemic health, lifestyles and habits. The staining procedure was preceded by a complete and thorough clinical intraoral examination. Subjects accepted brief elaborations about the process before rinsing their mouths with mineral water for 20 seconds to clean up the debris and followed by acetic acid 1% as pre-rinse solution for another

20 seconds. The oral mucosa surface was then dried with gauze. Toluidine blue stain was applied in excess and left on site for 1 minute. In order to eliminate excess stain, the oral mucosa was again rinsed with acetic acid 1% and sufficient water consecutively for 20 seconds each.¹⁹ The areas of oral mucosa that stained blue were captured with intraoral camera and transferred to the computer unit. Inflammation, irritation and ulcers would take up the stain. Because the test carried a risk of false positives, a second test was required to minimize the risk. The recommended protocol, thus included another 14 days later to give inflammatory lesions an opportunity to heal.²¹

RESULTS

If one or more regions in the mouth were persistently stained blue during the first and the second staining, the results were considered positive, otherwise, the staining that did not meet the criteria were considered negative. The pictures of positive and negative staining were displayed in figure 1 and 2, respectively.

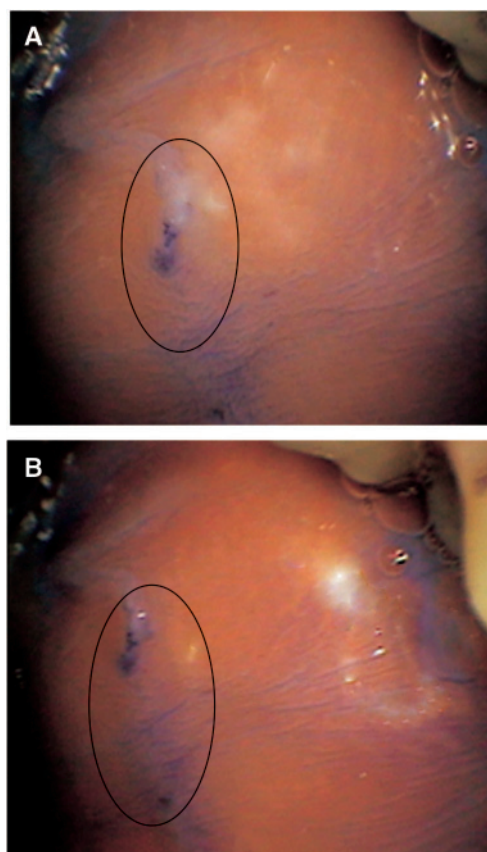


Figure 1. Photographs of the toluidine blue positive staining. The same region was persistently stained blue during the first (A) and second (B) staining.

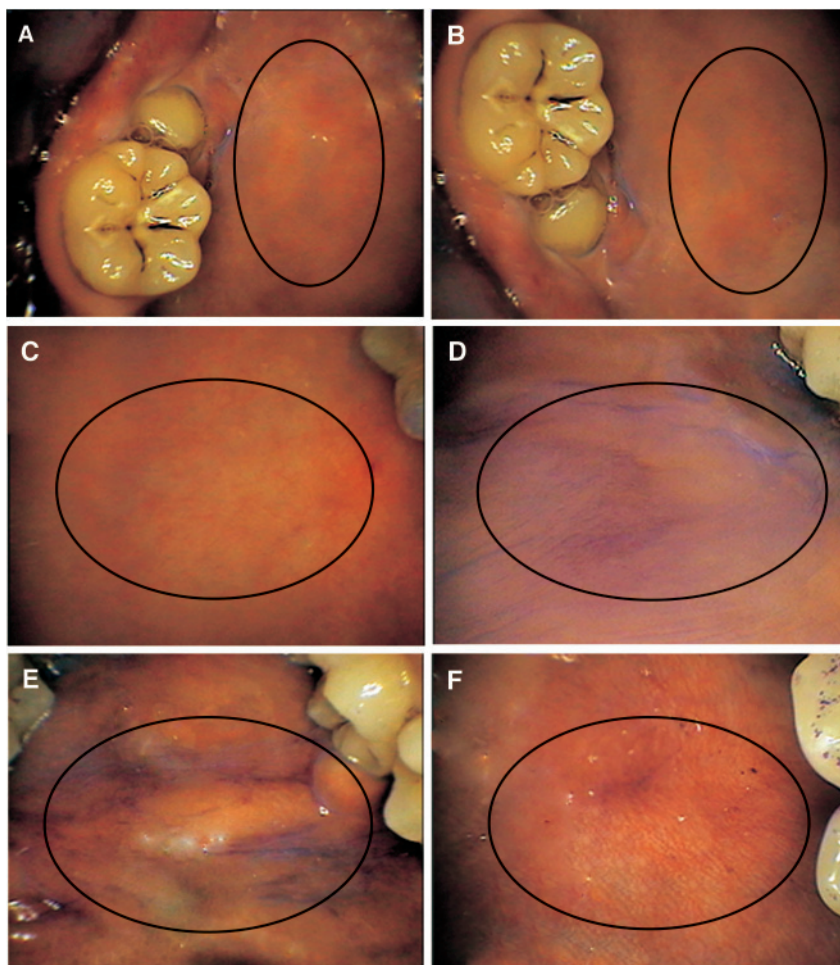


Figure 2. Photographs of the toluidine blue negative staining. No areas was stained during the first (A) and the second (B) staining. No staining in the first (C) and weak staining in the second (D). Equivocal in the first (E) and no staining in the second (F).

The numbers of subjects with positive or negative outcomes were calculated with the number of cigarette smoked daily and the duration of smoking habit. Table 1 showed that positive staining dominates the smoker group. The Fisher's exact statistic (alternative test of Chi-square test) analyzed the data and gave a result that retention of toluidine blue were significantly higher in smokers than that in non smokers. It was demonstrated by Fisher's Exact Test with the significance of 0.003.

Table 1. Result of toluidine blue staining

Categories	Smokers	Non smokers	Total
Positive staining	27	4	31
Negative staining	3	6	9
Total	30	10	40

The total lifetime cigarette consumption was calculated by the following formula:

$$\text{Total cigarette use} = \text{bars/day} \times 365 \text{ days/year} \times \text{years of smoking}$$

The regression and correlation analysis were completed to check the influence of total lifetime cigarette consumption. The regression test showed that the quantity and duration of smoking affected the final result with a significance of 0.026. The Spearman correlation test also proved significant relation between total cigarette use and staining results (sig. = 0.001) with correlation coefficient = 0.510, therefore, interpreted as moderate relation.

The clinical appearance of the toluidine blue staining was not restricted to positive and negative only. In most clinical situations, the probability of staining results lay somewhere between these two extremes. The pattern of dye retention was assessed by the intensity as strong, equivocal, weak, and no staining. Strong staining was rarely found in this study. Weak and equivocal staining were common, and thus might not be ignored. In this study, they were categorized as positive due to an interesting finding that there were no significant differences in the molecular profiles of lesions with strong staining and those with weak staining.¹⁸ Further evaluation of every toluidine blue stain is important, even when the staining is faint.

DISCUSSION

Nearly all smokers included in this study were positively stained, meanwhile, the rate of negative staining was higher among the non smokers group. Statistically, the Fisher's exact test also proven that oral mucosa of non smokers absorbed less stain than that of smokers. The results confirmed that the content of acidic components in oral mucosa of smokers are probably higher than that of non smokers.²²

Referring to the high sensitivity rates of toluidine blue,^{16,21} the results of this study indicated that smokers are more susceptible to develop oral premalignancies. This is supported by the fact that cigarettes contain carcinogenic agents such as tar, carbon monoxide and hydrogen cyanide.³

Intraoral regions that stained positively, when confirmed with biopsy, expressed various histologic changing from mild dysplasia, moderate dysplasia, severe dysplasia, carcinoma in situ and invasive cancer.²¹ Toluidine blue is useful in raising or confirming clinical suspicion. Even after having the test, subjects might still not having the disease. They only have a probability of the disease. Toluidine blue is not more than a screening tool used for early diagnosis of presymptomatic diseases, thus should never be used as a single determinant. Toluidine blue positive lesion may not be ascertained as premalignancy unless confirmed by biopsy. Histopathologic assessment is the most reliable and valid measure of disease, and therefore it is used as the gold standard.

Another supportive finding was that the risk of positive staining increased with the increasing number of lifetime cigarette use. The relative risk of developing oral premalignancy was associated with a positive dose-response relationship.⁹ Although quantity and duration of smoking markedly affect the retention of toluidine blue dye, a lot of factors still need to be considered. Many variables were not under control in this study, such as the oral health, systemic health and lifestyle. These features are likely to contribute more to the staining results. The occurrence of oral cancer is apparently an interaction of many factors, such as genetics, alcohol consumption, smokeless tobacco use, HPV, HIV seropositive, narcotics abuse, sunlight exposure and dietary intake.¹³

In conclusion, the results of this study demonstrate that toluidine blue retention is more noticeable on oral mucosa of smokers than that of non smokers. The quantity and duration of smoking also affect the retention of toluidine blue on oral mucosa. Therefore, it is suggested that toluidine blue staining can be used as a screening tool to detect the presence of oral premalignant lesions in smokers. It is recommended that the next study may include individuals with higher lifetime cigarette consumption as subjects to produce stronger intensity of toluidine blue staining.

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