

**ASSESSMENT OF ORAL MUCOSA IN NORMAL,
PRECANCER AND CANCER USING
CHEMILUMINESCENT ILLUMINATION, TOLUIDINE
BLUE SUPRAVITAL STAINING AND ORAL
EXFOLIATIVE CYTOLOGY**

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CERTIFICATE

This is to certify that this dissertation titled "ASSESSMENT OF ORAL MUCOSA IN NORMAL, PRECANCER AND CANCER USING CHEMILUMINESCENT ILLUMINATION, TOLUIDINE BLUE SUPRAVITAL STAINING AND ORAL EXFOLIATIVE CYTOLOGY" is a bonafide work done under our guidance by Dr. M. RAJMOHAN during his postgraduate study period between 2002 to 2005 in the Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, Chennai.

This dissertation is submitted in partial fulfillment for the award of the degree of **Master of Dental Surgery in Branch IV - Oral and Maxillofacial Pathology** of The Tamilnadu Dr. M. G. R. Medical University.

It has not been submitted (partially or fully) for the award of any other degree or diploma.

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INTRODUCTION

Oral cancer is usually first diagnosed when it becomes symptomatic and approximately two thirds of the patients present with developed advanced disease, regional metastasis and consequently poor prognosis.²⁴ Oral precancers associated with habits which occur in the oral cavity include leukoplakia and oral submucous fibrosis. 8 – 10% of these eventually progress to malignancy. The risk of development of carcinoma within an area of leukoplakia is 5 times higher than in those without leukoplakia. Malignant transformation has been reported in 43% of dysplastic leukoplakia cases.¹⁰ It is therefore important to identify these lesions early to enable management.

Conventional visual inspection and palpation of oral soft tissues for the early detection of premalignant or malignant changes have their limitations. The adjunctive application of technology to highlight such lesions may increase the diagnostic yield.¹⁸

A number of techniques have been developed to supplement clinical examination and improve the diagnosis of early oral malignancy.²⁴ They include (a) chemiluminescent illumination (ViziLite™, a trademark of Zila®, Inc., Phoenix, USA), (b) toluidine blue supravital staining test and (c) oral exfoliative cytology.

(a) ViziLite™ is an oral examination device that is claimed to improve identification, evaluation and monitoring of oral mucosal abnormalities in those with increased risk of oral cancer. The specific ViziLite™ wavelength is absorbed by normal cells and reflected by abnormal cells due to their

higher nuclear-cytoplasmic ratio. As a result, atypical mucosal abnormalities appear bright white.⁵⁶

(b) The topical application of toluidine blue, an acidophilic, metachromatic nuclear stain has been used in the *in vivo* evaluation of neoplastic changes of the cervix (Richart, 1963) and the oral cavity (Shedd et al, 1967, Myers, 1970). Areas of carcinoma have a strong affinity for the dye, whereas the normal mucosa does not. This response permits detection of small and early lesions and also permits their surface delineation.⁵⁴

(c) Oral exfoliative cytology examines the morphological characteristics of exfoliated or scraped off superficial cells of the oral mucosa. The exfoliated cells are stained by Papanicolaou stain.² Exfoliative cytology is of diagnostic references in ulcerated oral carcinomas and erosive leukoplakias and is of importance in mass screening programme or where biopsy is not feasible.

As with all cancers, early detection is the key to successful treatment and reduction in morbidity. Use of exfoliative cytology and toluidine blue *in vivo* application for selection of biopsy sites were widely reported in the literature. There is paucity of information regarding the use of ViziLite™ as an aid in early detection of mucosal abnormalities particularly in identifying potential malignant lesions. The present study was done to compare the usefulness and validity of ViziLite™, toluidine blue *in vivo* application and oral exfoliative cytology with the gold standard of biopsy.

REVIEW OF LITERATURE

Toluidine blue:

Supravital staining methods have long been used as an adjunct in the early diagnosis of malignant lesions. In 1928, **Schiller** reported the use of Lugol's iodine solution (iodine and potassium iodide) in carcinoma of the

cervix uteri. Normal epithelium of the portio stains brown, whereas carcinomatous tissue does not take up the stain. **Morgenroth** did not obtain reliable results by using the Schiller test.²³

Toluidine blue, an acidophilic, metachromatic dye belonging to the thiazine group, selectively stains acid tissue components (sulfate, carboxylate and phosphate radicals) such as DNA and RNA and its molecular weight is 305.84.

Kurnick recommended toluidine blue staining as a rough screening test for nucleic acids. This staining property of metachromasy is due to the presence of repetitive phosphate groups in the nucleic acids and is dependent on pH and temperature. The recommended pH is 6.0 to 7.0. The temperature should not exceed 30⁰C, above which the metachromatic property diminishes in strength.²³

Toluidine blue is usually regarded as a nuclear stain and the *in vivo* test is based on the fact that dysplastic and anaplastic cells contain quantitatively more nucleic acids than normal tissue. Toluidine blue stains to a depth of two to ten cell layers. The fact that the intercellular canals are wider than in normal epithelium might facilitate the penetration of the dye in the tumor tissue.²³

Richart (1963), a pioneer of *in vivo* staining, reported a method of staining for *in vivo* delineation of dysplasia and carcinoma in situ of the uterine cervix. He compared the results of toluidine blue and Schiller's iodine staining in 200 patients with these tissue changes. He concluded that the Schiller test was unreliable, but the toluidine blue technique delineated the distribution of neoplastic epithelium in 95% of his patients.⁴³

Niebel and Chomet utilized the staining technique of Richart and concluded that toluidine blue staining was an accurate method. Richart and

Niebel made no mention of false-negative findings. **Iwano *et al*** tested this reagent in 18 patients with unusual mucosal epithelium. They reported that all carcinomas responded positively to the dye, but inflammatory ulcers and erosions also gave positive reactions.⁴⁵

Topical staining of skin lesions with toluidine blue was used by **Sugarman *et al*** to detect the presence of premalignant and malignant squamous cell lesions.²³

Bahn *et al* reported that epithelial dyskeratosis in chemically induced carcinoma of the hamster cheek pouch could be recognized and delineated with toluidine blue. **Shedd *et al*** and **Myers** have also reported the usefulness of the toluidine blue technique in delineating neoplastic change.⁴⁵

Minra and Ohba proposed that toluidine blue, a cationic dye, binds with DNA or nucleohistone in two ways. One method is by intercalation, another by stacking or aggregation. The dye attaches to the phosphate bonds of DNA or nucleohistone. The efficiency of the dye technique depends upon the amount of DNA present, which relates to the number and size of the superficially located nuclei in the tissues to which the toluidine blue is applied. Toluidine blue will combine with many polyanions, including phosphates, sulfates and carboxylates.⁴⁵

The toluidine blue method was found by **Tarkkanan *et al*** to be a valuable aid in the diagnosis of bronchogenic carcinoma. Mucin producing goblet cells give false positive responses. But, staining of mucin was reported to be less regular and less intense than staining of nucleic acids.²³

Koburg and Steinbach used the technique for determining the site and extent of malignant epithelial lesion in patients with leukoplakia and chronic inflammation of the larynx. False positive reactions were noticed in case of respiratory epithelium, abrasions, ulcers, granulation tissue,

irradiation necrosis and trapping of the dye in papillary keratoses. False negative reactions were obtained only in case of carcinoma beneath an intact mucosa. The dye is not taken up in the presence of leukoplakia and keratoses. Carcinoma in situ and superficial carcinoma are consistently stained. **Thomsen and Thomsen** considered that a negative result of the staining test was a strong evidence against the presence of carcinoma, but they found the test unreliable in carcinoma in situ in contrast to other investigators.²³

Strong MS, Vaughan CW and Incze JS (1968)⁵³ did the study to evaluate the efficacy of toluidine blue. Toluidine blue is a member of the thiazine group of metachromatic dyes. It is soluble in water (upto 3.5%) and alcohol (upto 0.5%), carcinoma in situ and areas of infiltrating carcinoma are stained a deep royal blue. Normal mucosa, except for the debris coated areas of the dorsum of the tongue and areas of leukoplakia do not stain by toluidine blue. They revealed from 5 μ frozen sections that the stain is not picked up primarily by the nuclei to a depth of about three to four cells. They stated that it outlines the surface margins of the tumor and is superior to cytologic techniques because the information is more immediate and accurate.

Sabes WR, Singer RE and Kuhn T (1972)⁴⁵ determine the effectiveness of toluidine blue *in vivo* staining test as an adjunct to biopsy in the diagnosis of chemically induced dysplasias and carcinoma of the hamster cheek pouch. The left cheek pouch of each of the 40 animals was painted twice weekly with 0.05 ml of 0.5% 7,12 dimethyl benz (α) anthracene (DMBA) in lipid petrolatum applied on a cotton swab. The right cheek pouch was painted with lipid petrolatum alone. The left and right pouch of each animal received 1% aqueous solution of toluidine blue application. The

mucosal surface was decolorized by carefully blotting with a 1% acetic acid solution. They concluded that with increased age of the lesions, there was a reversal of the ratio of false positive (retained stain but histologically negative) to false-negative (non-staining but histologically positive) toluidine blue. *In vivo* staining technique, utilizing toluidine blue as an adjunct to biopsy in diagnosis of neoplastic change in the epithelium was unreliable.

Reddy CRRM, Ramalu C, Sundareswar B et al (1973)⁴² used *in vivo* macroscopic staining of the oral cavity lesions with toluidine blue for the purpose of delineating carcinomas, to identify precancerous lesions and to choose sites for biopsy. Normally anterior 2/3 of the tongue and parts of soft palate will also stain for toluidine blue. They found that 11% were false positive and 13% were false negative. They stated that toluidine blue is used to delineate areas of carcinoma or dysplasia and also suggested that it could be useful for mass surveys of oral cancer.

Reddy CRRM, Mouli KC and Kameswari VR (1975)⁴¹ evaluated 1673 people living in 3 villages for their smoking habits and examined their palates. They found 30 people having ulcers by using reverse smoking. These ulcers were stained by toluidine blue and followed yearly once for the next 3 yrs. They found that all carcinomas, palatal nevi and dysplastic areas stained blue. The results showed that toluidine blue positive ulcers could either continue to be positive or become carcinomas or heal. The toluidine blue negative ulcers could heal up or rarely become positive ulcers.

Shedd DP, Hukill PB, Balm S et al (1967)⁴⁸ examined 62 patients, in which, 42 were known / suspected epidermoid cancers and 20 were non-neoplastic lesions. They stated that all the dysplastic lesions showed positive reaction. The intensity of the blue color was less than that which develops in

carcinoma in situ. All the carcinoma in situ cases showed positive staining response. Patients with recurrent or persistent cancer after irradiation, stain blue. They concluded that *in vivo* staining could be used for (1) recognition of carcinoma in situ or small, early invasive carcinoma, (2) determination of the margins of such tumors and (3) evaluation of areas of field cancerization.

Strong MS, Vaughan CW and Incze J (1970)⁵² used toluidine blue to detect carcinoma in situ, early infiltrating carcinoma and the exact margins of an established carcinoma in the larynx. They concluded that leukoplakia and keratoses do not fix dye. Carcinoma in situ or superficial carcinoma will constantly absorb the stain and indicate the site from which a positive biopsy may be taken. It has proved to be reliable in the larynx as it has been in the oral cavity and pharynx.

Srivatsava YC and Mathew MN (1971)⁵¹ conducted a study on random selection of 200 patients with chronic oral ulcerative lesions who were above the age of 30 years. The oral cavity was cleaned with 1% acetic acid. 1% aqueous solution of toluidine blue was applied to the entire exposed surface. Excess stain was removed by 1% acetic acid. Toluidine blue dye is generally recognized as a nuclear stain. They concluded that the non-neoplastic epithelium also stain blue but these areas are much more diffuse and blend into adjacent epithelium gradually, but the areas of neoplasia tend to be sharply demarcated and stain more intensely and uniformly.

Vahidy NA, Zaidi SHM and Jafarey NA (1972)⁵⁴ used topical application of toluidine blue for the detection of carcinoma in 1,190 cases with oral lesions. It gave a positive result in 546 cases with 131 false positives and a negative result in 484 cases with 66 false negatives. In 160 cases, the results of the test were doubtful. The sensitivity of the test after

excluding the doubtful results is 86% and specificity is 76% from their study.

Lundgren J, Olofsson J and Hellquist H (1979)²³ compared the *in vivo* toluidine blue staining and histopathological findings. They used 2% aqueous solution of toluidine blue and excess stain was removed by washing with saline and drying with a cotton swab. A four-grade scale was used (negative, 1+, 2+ and 3+). In malignant specimens, 91% exhibited positive staining with toluidine blue and 9% did not stain at all (false negative). In benign specimens, 48% showed false positives and 52% showed positive staining. Their results showed that the sensitivity of the toluidine blue test was 91% for malignant lesions. The specificity of the staining reaction was 52% in benign lesions. They concluded that the toluidine blue test certainly does not offer an alternative to the histological examination of a clinically suspected area; it may serve as a complementary diagnostic aid in microlaryngoscopy.

Gyesko A and Holczinger L (1974)¹⁴ used toluidine blue as a staining method for nucleoli of ascites tumor cells in cytopathology. The smears were stained by toluidine blue solution at 70⁰C for 1-2 minutes. The slides were rinsed 3 times for 1 minute each in the acid buffer. They suggested that this technique is rapid, reproducible and seems to be selective for nucleoli details and to determine the correct number of nucleoli or nucleolar fragments in each nucleus.

Sogaard H, Baandrup U and Poulsen EH (1976)⁵⁰ investigated 60 patients and divided into 3 groups; Group A (30 patients with cervical smear showed dysplasia / carcinoma in situ), Group II (15 patients with negative stain test) and Group III (15 pregnant women). Their results showed that in Group A, toluidine blue test were positive in 3 benign and 25 malignant

lesions and negative in 2 benign lesions. In Group B, toluidine blue test was positive in 3 and negative in 12 benign lesions. In Group C, toluidine blue test was negative in 15 patients. They concluded that the sensitivity is 100% and the specificity is 81% from their study.

Mashberg A (1981)²⁶ conducted a study to determine the feasibility of using a toluidine (toluidine blue) mouth rinse as a routine procedure after thorough clinical examination to discover undetected cancers. He examined 105 asymptomatic oral lesions (51 carcinomas and 54 non-malignant lesions). His results showed that 2% were false negatives with toluidine blue application, 5.9% with the toluidine blue mouth rinse and 9.3% were false positives with toluidine blue application, 7.4% with the toluidine blue mouth rinse. Four inapparent, second primary cancers, unobserved for application were delineated by the toluidine blue rinse. He concluded that toluidine blue rinse might be used to detect unobserved, asymptomatic oral mucosal cancers after clinical examination. After a positive rinse stain, a subsequent positive toluidine blue application stain 10 –14 days later mandates a biopsy of the lesion.

Mashberg A (1983)²⁵ conducted a study on 179 asymptomatic lesions, 134 patients had undergone toluidine chloride (toluidine blue) application and rinse. In toluidine blue application results showed that false negative rate was 2.5% and false positive rate was 12%. By using toluidine blue rinse, the false negative rate was 11.1% and false positive rate was 9.2%. He concluded that use of toluidine chloride rinse was suggested as a screening agent for discovery of asymptomatic carcinomas that are undetected by routine soft tissue examination.

Silverman S, Migliorati C and Barbosa J (1984)⁴⁹ examined 132 patients who were suspected of having oral carcinomas or precancerous

(dysplastic) lesions. They used 1% aqueous solution of toluidine blue for 30 seconds followed by tap water rinse and then blotted with 1% acetic acid. Biopsy had taken from all patients. They concluded that the overall accuracy of the toluidine blue test was 91%. The majority of errors involved false positives in the group of benign lesions.

Miller RL, Simms BW and Gould AR (1988)³¹ did the experiment on 22 Golden Syrian hamsters. The animals were randomly divided into 2 groups. Group A consisted of 14 animals. The right buccal pouch of each group A animal was painted with 0.5% DMBA dissolved in heavy mineral oil (USP). Each pouch was painted thrice a week for 11 weeks. The left buccal pouches were painted with heavy mineral oil alone on the same schedule. Group B consisted of 8 animals. Each animal received an application of 50% v/v turpentine and mineral oil solution (TLP) to both the right and the left buccal pouches. This procedure was also carried out thrice a week for 11 weeks. TLP was continued on a daily basis for an additional 3 weeks period.

At the end of the 14 weeks, all animals were anaesthetized with ketamine HCl. Each buccal pouch was rinsed with 1% acetic acid for 30 seconds and subsequently rinsed with tap water for 60 seconds. Each pouch was swabbed with 1% toluidine blue for 60 seconds. All pouches were decolorized by using 1% acetic acid solution rinse for 30 seconds. Their results showed that no clinical lesions were observed in any group B buccal pouches. In group A, 10 animals retained the toluidine blue stain. Most of the papillary white lesions failed to retain toluidine blue.

They suggest that toluidine blue was of little value in detecting premalignant lesions in the hamster buccal pouch. A false negative rate for premalignant lesions was 92.5% and for malignant lesions, it was 27.8%.

Rosenberg D, Cretin S, Bronx NY *et al* (1989)⁴⁴ did meta-analysis, an analytical technique that uses raw data from previous researches, to estimate the efficacy of screening for oral cancer with tolonium chloride (TCl). They analyzed 16 studies. They stated that the sensitivities were in the range of 86% to 100% with a mean of 96.7%. The specificities were in the range of 70% to 100% with a mean of 90.8%. They suggested that if tolonium chloride is used to screen high-risk populations, the likelihood of a false negative finding is extremely low and false positive results will be relatively numerous.

Epstein JB, Scully C and Spinelli JJ (1992)¹¹ conducted a study, which included 59 patients with possible oral premalignant lesions and oral cancers. Toluidine blue and Lugol's iodine were applied for all patients with cotton bud. The results of this study indicate sensitivity of 92.5% and specificity of 63.2% for toluidine blue. These results suggest that the use of both tissue stains appears to be worthy of consideration. They concluded that the use of toluidine blue and Lugol's iodine as an adjunct to sound clinical judgement, is of value in the diagnosed at risk patients and assists in selecting the best site for biopsy.

Hobdy-Henderson KC (1993)¹⁷ wrote about safety of tolonium chloride as a mucous membrane dye. He said that there was no report of toxicity but patients experienced nausea, vomiting, tenesmus, dysuria and bluish tint to the skin. Intravenous doses of Tc 7-10mg / kg of body weight, have resulted in electrocardiographic changes, hyperpnoea and agitation. He mentioned that the median lethal dosage was 27.56mg / kg body weight, in mice and death was related to cardiac or respiratory failure. He concluded that tolonium chloride appears to have no carcinogenic effect in animals.

Mathews RA (1995)²⁸ reviewed the toluidine blue efficacy for staining squamous cell carcinoma. He said that the use of this dye by inexperienced persons could result in many false positive results. He suggested that repeated staining was useful to decrease false positives. He concluded that toluidine blue staining provides an opportunity to identify asymptomatic oral cancers at a very early stage.

Warnakulasuriya KAAS and Johnson NW (1996)⁵⁵ conducted a study on 102 patients with unconfirmed oral mucosal lesions. All patients rinsed 10ml of toluidine blue solution for 1 minute and excess stain was removed by water rinse. Sensitivity was 100% and no false negatives for oral carcinomas. In oral epithelial dysplasias, sensitivity was 79.5% and false negative rate was 20.5%. The overall specificity of this technique was 62%. They suggested that this method was valuable for surveillance of high-risk patients.

Portugal LG, Wilson KM, Biddinger PW et al (1996)⁴⁰ did a prospective study of 50 patients undergoing surgical resection of squamous cell carcinoma of the upper aerodigestive tract. After removal of the tumor, intraoperative staining of the remaining unresected mucosa was performed using 1% toluidine blue solution. Irrigation with 1% acetic acid is performed to remove any excess stain. A finding was considered positive if the area stained dark blue. All margins were evaluated by frozen sections. They said that false positive staining resulted from traumatic handling of mucosal tissue. Breaks in the mucosal lining result in exposure of the submucosa, which can take up the dye. They suggested that toluidine blue is a valuable complement to present methods of assessing margin status by providing immediate visual information.

Porter SR and Scully C (1998)³⁹ recommended two-stage toluidine blue staining procedure. In the first stage, instruct the patient to rinse 1% acetic acid solution for 20 seconds, apply toluidine blue solution for 20 seconds and removes excess stain by 1% acetic acid for 20 seconds. In the second stage, to reduce the risk of inappropriate diagnosis the staining procedure must be repeated after 10-14 days. They said that the false diagnosing of oral cancer was 1.2 %– 9.1% and likelihood of not staining cancer was 0% – 11.1%.

Epstein JB, Oakley C, Millner A *et al* (1997)¹⁰ did toluidine blue staining in 81 patients who were previously treated with cancer. All the lesions underwent histopathological evaluation. They said that toluidine blue application was more accurate (68%) than clinical examination (59%) and sensitivity of toluidine blue application was 100% than with clinical evaluation (78%). No false negative results were seen with the use of toluidine blue. They concluded that toluidine blue application is an important adjunct to the clinical examination because it may increase the clinical suspicion of the examiner and also assist in identifying sites, which need biopsy and delineating margins of the lesion.

Martin IC, Kerawala CJ and Reed M (1998)²⁴ did the experiment on 14 specimens undergoing surgery as primary treatment of intraoral mucosal squamous cell carcinoma. The extent of invasive malignant disease and surrounding epithelial dysplasia was defined by both visual inspection and the application of toluidine blue vital stain. No false positives or false negatives resulted with invasive carcinomas. A sensitivity of 100% obtained with invasive malignant disease. False negative staining rates of 42% for carcinoma in situ and 58% for moderate or severe dysplasia were also observed. They suggest restricting the use of vital staining to selective cases.

Allen CM (1998)¹ wrote about toluidine blue staining for detection of oral cancer and precancerous lesions. He said that this procedure might be useful in the hands of clinicians who were experts in evaluating oral malignancy and premalignancy and the high degree of false negative results obtained with dysplastic mucosal lesions may seriously mislead clinician and affected patients. He concluded that there is no substitute for a thorough clinical examination of the oral mucosa.

Ephlos H and Mashberg A (1999)⁹ stated that toluidine blue is an adjunct to thorough clinical examination in the early detection of asymptomatic carcinomas of the oral cavity and oropharynx. It is useful for screening high-risk patients and also guides the clinician to the most appropriate biopsy site and facilitates the delineation of tumor margins in the operating room. This vital staining technique is only one element of a much-needed program to promote early detection of oral and oropharyngeal cancer.

Chromoendoscopy refers to staining of endoscopic tissues or topical application of chemical stains or pigments to alter tissue appearances and improve localization, characterization or diagnosis.

Canto MI (1999)⁶ evaluated four vital stains that had been used in patients with Barrett's esophagus: (1) Lugol's iodine solution, (2) Methylene blue, (3) Toluidine blue and (4) Indigo carmine.

1.5% to 4% Lugol's iodine solution was sprayed on the esophagus and within minute the normal whitish squamous mucosa will change to dark brown or greenish brown as a result of binding of the iodine to glycogen in nonkeratinized stratified squamous epithelium. Cells that are inflamed, dysplastic or malignant will not stain with Lugol's iodine. The sensitivity was 89%, specificity was 93% and accuracy was 91%.

Toluidine blue has been used to diagnose inflammatory and malignant cells because of their increased nuclear- cytoplasmic ratio. The sensitivity was 98%, specificity was 80% for diagnosing Barrett' esophagus. Indigo carmine is a blue contrast stain and is not absorbed by cells. A 0.1% solution of indigo carmine was sprayed on columnar mucosa after delineation of the squamo-columnar junction with Lugol's iodine. These areas were examined by high magnification endoscope.

Methylene blue is a vital stain taken up by actively absorbing tissues such as small intestine and colonic epithelium. It will not stain squamous or gastric mucosa. It has a sensitivity of 94%. He concluded that methylene blue appears to be highly accurate for selective staining of specialized columnar epithelium, which define Barrett's esophagus. Lugol's iodine and toluidine blue staining have not been shown to improve the diagnosis of dysplasia or cancer.

Kerawala CJ, Beale V, Reed M *et al* (2000)²⁰ did a prospective study of 11 patients undergoing surgery as primary treatment for intraoral mucosal squamous cell carcinoma. Mucosal surfaces were stained immediately preoperatively. Resections were performed to include a 1 cm margin of tissue of either normal clinical appearance or absent toluidine blue staining. All 14 resected margins were free from invasive squamous cell carcinoma. Totally 16 areas of carcinoma in situ or dysplasia were identified at tissue margins, all of which occurred in clinically normal mucosa that had failed to stain with toluidine blue. They concluded that toluidine blue might be an adjunct in identifying invasive tumor at mucosal resection margins. It would appear to be of no benefit in delineating positive resection margins due to carcinoma in situ or severe dysplasia and hence it may be of little value in reducing the incidence of local recurrences.

Onofre MA, Sposta MR, Narorro CM *et al* (2001)³⁷ selected 50 patients with potentially malignant epithelial lesions (PMELs) and superficial oral ulcerations suggestive of malignancy. The biopsy sites were selected on the basis of the clinical appearance of the lesion and the staining result. All retained stain, indicated 100% sensitivity of staining for detection of carcinoma in situ and invasive squamous cell carcinoma. In epithelial dysplasia, sensitivity was 50%. The overall specificity was 67%. The positive predictive value was 43.5% and negative positive value was 88.9%. They concluded that staining with toluidine blue is highly reliable for the detection of in situ carcinoma and invasive carcinoma. It is an adjunct to clinical judgment and not a substitute for either clinical judgment or biopsy.

Chemiluminescent light (ViziLite™):

Sankaranarayanan R, Wesley R, Somanathan T *et al* (1998)⁴⁶ compared the performance of unmagnified naked eye visual examination of the uterine cervix after application of 3-4% acetic acid termed “VIA” (Visual Inspection with Acetic acid) with cytology in detecting cervical lesions. For this study, they included 3000 women and tests were performed by trained female cytotechnicians. First, they collected smear for cytologic examination followed by application of 3-4% acetic acid on the cervix using a thick cotton swab. The cervix was examined after 1-2 minutes under adequate light directed from a halogen lamp or torch for the determination of any dull or bright white (aceto- white) areas.

Acetic acid causes dehydration of the cells and some surface coagulation of cellular proteins, thereby reducing the transparency of the epithelium. These changes are more pronounced in dysplastic epithelium due to a higher nuclear density and consequent high concentration of protein. Recognition of the “aceto- white” of the cervical epithelium with

naked eye constitutes a positive VIA test. This study compared the performance of VIA and cervical cytology in detecting cervical carcinoma and its precursors.

Subjects with a positive VIA or with a positive cervical smear were further subjected to diagnostic investigation by colposcopy and biopsy. The results showed that VIA was positive in 9.8% and cytology was positive in 10.2%. The sensitivity, the specificity and the positive predictive value were 90.1%, 92.2% and 17.0% respectively for VIA. The sensitivity, the specificity and the positive predictive value were 90.1%, 92.2% and 17.0% respectively for cytology. They concluded that VIA and cytology had very similar performance in detecting moderate dysplasia or more severe lesions.

Huber MA, Bsoul SA and Terezhalmay GT (2004)¹⁸ did a pilot study on 150 patients with good general health and were free of any significant dental disease. A conventional oral soft tissue examination was performed by visual inspection under incandescent overhead and halogen dental illumination, as well as palpation to detect abnormal epithelial changes. Patients were instructed to rinse with 1% acetic acid solution for 11 minutes and then asked to expectorate. The chemiluminescent capsule was activated (ViziLite™). Ambient lights in the room were dimmed and the oral soft tissue examination (visual inspection) was repeated under chemiluminescent illumination to detect abnormal epithelial changes. A brush biopsy or a conventional scalpel biopsy was performed to compare with ViziLite™ results.

Epithelium with an altered nuclear cytoplasmic ratio reflected the diffuse, low-level, blue-white chemiluminescent light and appeared sharply demarcated from the adjacent normal epithelium. Patients showed “aceto-

white” and were considered as “positives”. Absence of such findings was considered as “negatives”.

Results showed that leukoedema, leukoplakia and erythroplakia were positive for chemiluminescent illumination test. Other lesions like linea alba, cheek biting, stomatitis areata, tori, nicotine stomatitis, snuff pouch and traumatic ulcer were negative. They suggested that the diffuse, low level, blue- white chemiluminescent light is absorbed and imparts a “blue hue” to normal tissues. In epithelium with excessive keratinization, hyperparakeratinization, significant inflammatory infiltrate and altered nuclear cytoplasmic ratio appeared “aceto- white”. The sensitivity and the specificity of this technology were not discussed.

Oral exfoliative cytology:

Significant advances in the detection of cancer and precancerous lesions have been made after **Papanicolaou** originally described his procedure for the early diagnosis of the carcinoma of the cervix.³ **Weinmann** (1940) was the first person to study the keratinization of the oral mucosa in smears taken from the oral cavity.² The main practical value of cytology lies in the diagnosis of malignant tumors. **Montgomery and Von Haam** were the first in 1951, followed by **Pomeranz and Stahl** in 1953 to use the cytodiagnostic method in cancer and precancerous lesions of the mouth, which has been later routinely employed by **Silverman, Becks and Farber** (1958), **Pape** (1959), **Fasske et al** (1960).²

Berg and Ross (1969) reviewed oral cytology and reported false negative reports ranging from 0 to 29%. **Folsom et al** (1972) reported a false negative rate of 31%. This is due to improper sampling, technical errors, misinterpretation of the specimen and bias. Studies investigating the reliability of the procedure have reported that the accuracy ranges from 69%

to 100% for malignancy. The accuracy has been reported as low as 6% for premalignant lesions.³

Oral smears can be used when patients refuse a biopsy or if they are considered to have risk for surgery. It can help to screen lesions in patients who have had radiation therapy or large lesions. **Blozis GG** (1972)³ stated that exfoliative cytology can be a useful and valuable diagnostic aid if utilized in specific situations on appropriate lesions.

Banoczy J (1976)² wrote about the value of exfoliative cytology. In advanced oral carcinomas, the diagnosis might be already established clinically, the effectiveness of exfoliative cytology might be extremely good, but the importance of method in the detection of carcinoma is far less. It may act as an important diagnostic aid in early oral carcinomas with ulcerated surfaces. Early oral carcinomas with non-ulcerated surfaces are not suitable for cytodiagnosis. A poor degree of reliability was found in specimens taken from lesions of the vermilion border of the lips and the attached gingiva. These sites are more keratinized and less shedding of cells than other areas of the oral mucosa.

The use of exfoliative cytology in diagnosing malignant transformation in oral precancerous lesions is still controversial, because the keratinized layer in oral leukoplakia did not interfere with the good results of cytodiagnosis. False negative cytologic results in hyperkeratotic leukoplakia were 63.9%. Higher efficacy of cytology in detecting malignancy in the erosive leukoplakia was 76.6%. He concluded that exfoliative cytology is effective in early-ulcerated oral carcinomas and erosive leukoplakias. Clinically unsuspected early oral carcinomas might be detected by the use of exfoliative cytology and in extensive lesions, the site of the biopsy might be selected.

Cahn LR (1963)⁴ mentioned that the oral exfoliative cytology is an important diagnostic adjunct for detection of early cancer. It is also useful to detect of viral infections. Some of these cells may have a trailing cytoplasm, making them resemble the “tadpole” cell often considered pathognomonic of malignancy. Hereditary benign intraepithelial dyskeratosis, Darier’s disease and white sponge nevus of Cannon show cells that would be highly suspicious by exfoliative cytology method. He concluded that exfoliative cytology is a most important part of the diagnostic armamentarium.

Ingram Rc, Krantz S, Mendeloff J *et al* (1963)¹⁹ did the experiment on 422 lesions from 405 patients. Cytological smears were made from all lesions. A total of 27 oral carcinomas were found. 17 of these were clinically overt cases of cancer. Cytological results were positive in 15 cases and in 2 cases, false negative result was observed. The remaining 10 were clinically unsuspected early carcinomas, in which, 8 cases yielded positive cytological findings, 2 cases did not. They concluded that cytology used with proper clinical examination and follow up is a valuable adjunct in the early detection of small oral carcinomas.

Helsper JT and Sharp GS (1964)¹⁶ developed a method of collecting exfoliative cytology from the oral cavity by the use of Gey’s balanced salt solution as a mouth wash. This solution would preserve the cells prior to the actual smear and microscopic examination with less distortion than normal saline. Following the clinical oral examination, 1 ounce of Gey’s solution is given to the patient and is instructed to rinse his mouth thoroughly for 60 seconds. The specimen is collected and sent to the laboratory, where it is centrifuged and a smear is made from the sediment. The slide is stained by the Papanicolaou technique. They suggested that this method might be used

to detect abnormalities of the mucous membranes, including carcinoma and carcinoma in situ.

Ogden GR, Cowpe JG and Green MW (1989)³⁴ assess the effect of radiotherapy on the DNA distribution and cytomorphology of normal oral mucosa. Smears were obtained from normal buccal mucosa, which was in the field of exposure, for the irradiation of malignant tumors. The smears for DNA estimation were fixed in methanol, formalin and acetic acid in the ratio of 85: 10: 5. The smears underwent Feulgen hydrolysis. The smears for cytomorphological assessment (measurement of nuclear area and cytoplasmic area) were fixed in equal parts of 95% ethanol and diethyl ether and then stained with the Papanicolaou stain. They concluded that any changed DNA state arising in normal buccal mucosa as a consequence of irradiation returns to a diploid state within 4 to 6 weeks of radiotherapy. These high doses of irradiation, while being capable of inducing dysplasia seem to have no lasting effect on DNA distribution. Because increased nuclear and cytoplasmic area, which returned to normal values after completion of treatment.

Ogden GR, Cowpe JG and Green MW (1991)³² compared the efficiency of the cytobrush with that of the wooden tongue spatula. The cytobrush has been used frequently in cervical cytology. Wooden spatula is used in oral exfoliative cytology usually. For 26 patients, 2 smears were collected from clinically normal mucosa from four sites in the oral cavity (dorsal tongue, ventral tongue, buccal mucosa and hard plate). The smears were graded for cell yield and dispersion on a three-point scale. Cytobrush produced significantly better dispersion for the dorsal tongue, ventral tongue and buccal mucosa and a better cell yield for tongue surfaces. No significant difference for cell yield or dispersion was found for the hard palate. They

stated that the cytobrush is an effective instrument for use in exfoliative cytology of normal oral mucosa.

Ogden GR, McQueen S, Chisholm DM *et al* (1993)³⁶ took 2 smears from biopsy confirmed oral cancers and from the contralateral mucosal site of 20 patients using a Cytobrush. Using a panel of antikeratin antibodies, the keratins expressed by these cells were identified using a standard immunocytochemical technique and assessed on a 3-point scale. The report establishes that the simple keratins 8,18 and 19 showed the most significant difference between smears taken from biopsy confirmed oral cancers and from normal oral mucosa. The simple keratins (K8, K18) are not expressed in oral mucosa and keratin 19 is limited to the basal cells. They stated that suprabasal expression of keratin 19 is associated with malignancy and keratins 8 and 18 are associated with oral cancer. They concluded that keratins detection within the smears from oral lesions could be valuable in the diagnosis of oral cancer.

Ogden GR, Cowpe JG and Wight AJ (1997)³⁵ wrote about the decline of the use of oral exfoliative cytology in clinical practice due to the subjective nature of its interpretation and only a small number of abnormal cells identifiable in a smear. They reviewed the more recent application of quantitative techniques, together with advances in immunocytochemistry. They considered the influence of the quantitative analysis of cytomorphology, DNA analysis and other tumor markers applied to oral exfoliative cytological smears.

Cytomorphology:

In 1952, **Johnston DG**³⁵ used planimetry to measure the nuclear (NA) and cytoplasmic areas (CA) and nucleus to cytoplasmic ratios (N: C). This planimetry has been compared with the use of image analysis, for both

normal and abnormal mucosa. Image analysis was concluded to be a more appropriate method for evaluating oral smears.

Quantification of nuclear DNA content:

Atkin NB and Richards BM³⁵ established the quantitative analysis of DNA content, based on the Feulgen reaction. This method could be used to distinguish malignant and normal cells. Microspectrometry has been used to assess the DNA distribution of normal and malignant cells. The application of flow cytometry to the analysis of DNA content has been documented.

Identification of other tumor markers:

Cytokeratin expression has been identified in cell imprints, cell culture, fine needle aspiration and cervical exfoliative cytology. Keratin 16 and 19 were frequently expressed in the aerodigestive tract of head and neck cancer patients. They concluded that the sensitivity and the specificity for NA and CA were 76% and 82% respectively. The sensitivity of the DNA quantification alone was 70%. The sensitivity and specificity and positive predictive value for cytokeratin 19 were 95%, 11% and 70% respectively. These results indicate that oral exfoliative cytology may provide an important adjunct in the assessment of patient with a potentially cancerous oral lesion.

Epstein JB, Zhang L and Rosin M (2002)¹² reviewed recent advances in techniques for detecting oral premalignant and malignant lesions. They mentioned about toluidine blue staining, DNA content of oral lesions, exfoliative cytology and molecular analysis of exfoliative cells. They concluded that the use of molecular markers in diagnosis may lead to better survival and less treatment associated morbidity through early recognition and intervention for oral lesions, which are a risk.

SUMMARY & CONCLUSION

In this study, we examined 30 patients. They were divided into 3 groups. Group I composed of 10 normal patients (free of mucosal lesions), Group II 10 patients diagnosed clinically as precancers and Group III 10 patients clinically diagnosed as oral cancers. All the patients were subjected to chemiluminescent illumination test (ViziLite™ test), toluidine blue supravital staining test, oral exfoliative cytology and histopathological examination.

Our conclusions from this study are as follows

1. All normal patients showed Class I cytology and normal epithelium in histopathology but showed negative result to chemiluminescent illumination test and toluidine blue staining test.
2. Chemiluminescent illumination test was sensitive for precancerous and cancerous lesions, which presented as keratotic lesions and red-white lesions.
3. Chemiluminescent illumination test was negative for precancerous and cancerous patients, which presented as erosive lesions.
4. Toluidine blue staining test was reliable in precancerous and cancerous lesions, which present as erosive and red- white lesions.
5. Toluidine blue staining test was negative for keratotic lesions.
6. Chemiluminescent illumination test is relatively reliable than toluidine blue staining test and useful chair side diagnostic test.

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