

RESEARCH ARTICLE

Effectiveness of the Microlux/DLTM Chemiluminescence Device in Screening of Potentially Malignant and Malignant Oral Lesions

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Abstract

Background: To evaluate the effectiveness of Microlux/DL with and without toluidine blue in screening of potentially malignant and malignant oral lesions. **Materials and Methods:** In this diagnostic clinical trial clinical examination was carried out by two teams: 1) two oral medicine consultants, and 2) two general dentists. Participants were randomly and blindly allocated for each examining team. A total of 599 tobacco users were assessed through conventional oral examination (COE); the examination was then repeated using Microlux/DL device and toluidine blue. Biopsy of suspicious lesions was performed. Also clinicians opinions regarding the two tools were obtained. **Results:** The sensitivity and, specificity and positive predictive value (PVP) of Microlux/DL for visualization of suspicious premalignant lesions considering COE as a gold standard (i.e screening device) were 94.3%, 99.6% and 96.2% respectively, while they were 100%, 32.4% and 17.9% when considering biopsy as a gold standard. Moreover, Microlux/DL enhanced detection of the lesion and uncovered new lesions compared to COE, whereas it did not alter the provisional clinical diagnosis, or alter the biopsy site. On the other hand, adding toluidine blue dye did not improve the effectiveness of the Microlux/DL system. **Conclusions:** The Microlux/DL seems to be a promising adjunctive screening device.

Keywords: Chemiluminescence - microlux/DL - screening - potentially malignant

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Introduction

The prevalence of oral cancer is estimated to be 500 000 new cases every year around the world, which is about 3% of all malignancies, squamous cell carcinoma (SCC) comprises 96% of oral cancer (OC) (Siegel et al., 2012). OC is ranked one of the sixth most frequent malignancies in Asia. Nearly 274,300 new OC cases occur each year. High incidence rates are reported from developing nations situated in South-Central and South-East regions like India, Pakistan, Bangladesh, Taiwan and Sri Lanka. In India and Pakistan 8-10% of all cancers occur in the oral cavity (Sunny et al., 2004; Bhurgri, 2004, 2005), with an incidence rate of more than 10 per 100,000 (Bhurgri et al., 2003; 2006).

The overall 5-year survival rate for oral cancer is about 50% among the worst of all cancer death rates (Johnson et al., 2011). Poor prognosis of oral cancer could be attributed to late stage diagnosis, field of cancerization and second primary tumors (Seoane-Romero et al., 2011). Public awareness of oral cancer as compared with other cancers is low and this contributes to delay in diagnosis (Bhatti

et al., 1995). High incidence is particularly observed in Asian countries with a cultural practice of chewing quid, tobacco chewing along with smoking and alcohol are the main reasons for the increasing incidence rate of OC. Low socioeconomic state and nutritional deficient diet lacking vegetables and fruits contribute towards the risk. In addition, viral infections, such as HPV and oral hygiene, are other important risk factors. The incidence of OC is increasing in most Asian countries; hence, it is important to undertake programs to prevent and control OC by screening for early diagnosis (Rao et al., 2013).

Screening is the application of a test to distinguish people who are symptom free from those having the disease (Wilson and Jungner, 1968). Conventional oral exploration COE (visual and palpation examination) constitutes the gold standard screening study for oral precancer and cancer; the specific technique for the detection of cases is the biopsy and histopathological diagnosis (Seoane and Diz, 2010). The British Dental Association and FDI recommend that systematic visual screening examination should be carried out on every new patient (Warnakulasuriya et al., 2007). Clinical features

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of suspicious potential malignant oral lesions include sharp or distinct margins, a red lesion, a non homogenous white lesion, persistent ulceration larger than 1 centimeter (Rethman et al., 2010).

Several adjunctive aids have been developed for oral cancer screening (Patton et al., 2008). Among which are toluidine blue dye, Oral CDX® brush biopsy kits, salivary diagnostics and optical imaging systems. More importantly, the efficacy of all adjunct methods to visual inspection, with an exception to toluidine blue, has been studied as diagnostic tool not as screening test (Kujan and Sloan, 2013).

Light-based oral cancer screening aids are used based upon the assumption that abnormal metabolic or structural changes have different absorbance and reflectance properties. Commercially available light-based systems include Vizilite Plus with TBlue system (Zila Pharmaceuticals, Phoenix, Arizona, U.S.), Microlux/DL (AdDent Inc, Danbury, Connecticut) and Orascoptic DK (Orascoptic, Middleton, WI) (Mehrotra and Gupta, 2011).

For Microlux the oral cavity is examined with a battery-powered light-emitting diode (LED) fiberoptic source that provides a blue-white (440-nanometer range) illumination. Few studies have been published evaluating its effectiveness (McIntosh et al., 2009).

Toluidine blue, is a metachromatic dye that binds to DNA, highlighting, potentially malignant oral lesions since the early 80s (Kujan and Sloan, 2013). It has also been suggested that toluidine blue may delineates lesion margins, accelerate the decision to biopsy, and guide biopsy site selection (Epstein and Guneri, 2009).

The aim of the current study is to evaluate the effectiveness of Microlux/DL with and without toluidine blue in screening of potentially malignant and malignant oral lesions.

Materials and Methods

Study population

Sample size calculation was done prior to the study according to the prevalence of smoking among Jeddah adults (Bassiony, 2009), the prevalence of premalignant lesions among smokers (Talole and Patki, 2006), and the estimated target population, with a 95% confidence level and 0.05 acceptable level of error, accordingly the sample size estimated was 388, which was rounded to 600 to decrease type I error and improve power of the study (599 patients were included in the study, 450 male and 149 female with mean age of 34.8 year). The calculation was done according to Statcal Epi Info version 6. The study protocol was reviewed and approved by the local ethical committee at King Abdulaziz University Faculty of Dentistry (KAUFD). Different sections of Jeddah, Saudi Arabia. Participants were selected from population clusters such as those found in major and private hospitals during hospital health events, companies, university and schools comprising students, staff and employee as well as workers from different factories. Eligible participants provided written informed consent prior to participation. Men and women who were ≥18 years of old and tobacco user was the only inclusion criteria of the research which

was voluntarily and done between 2011 to 2013.

Study protocol

This study is a diagnostic clinical trial in which the examination was carried out by two teams: 1) two oral medicine consultants, 2) two general dentists, all the examiners were trained and calibrated to acceptable consistency (95%) in the dental clinics. Participants were randomly and blindly allocated for each examining team (291 and 308 patients were examined by oral medicine consultants and general dentists respectively). Within each team the Microlux/DL device and toluidine blue examination were performed by examiner B who was independent and blinded from the results of the conventional oral examination (COE) performed by examiner A.

Examination technique

All participants were 1) interviewed and received a survey for demographic data, medical history and oral cancer risk factors as well as habits as tobacco chewing, smoking and alcohol consumption, 2) a comprehensive head and neck examination, 3) COE under the attached light of a portable dental chair using disposable dental mirrors and in case of lesion detection the criteria as size, ease of visibility, border distinctness, and presence of satellite lesions were recorded (Farah and McCullough, 2007). Room lights were dimmed, the oral cavity was examined and any visually identified lesion was evaluated using the Microlux/DL diffused light illumination kit (Microlux/DLTM, AdDent Inc., Danbury, CT, USA) (McIntosh et al., 2009), after the recommended 1% acetic acid solution for 60-s rinse procedure .

Oral examinations for toluidine blue (TB) was done by 1% acetic acid wash. 1% solution of toluidine blue rinse for 30 seconds. Excess stain was eliminated by applying 1% acetic acid for 30 seconds. Lesions were examined by Microlux/DL+TB to see the size of retained stained areas. Interpretation of the stain was done as mentioned by Mashberg (1980).

After performing all the procedures A provisional clinical diagnosis, Microlux/DL diagnosis and Microlux/DL plus toluidine blue diagnosis were recorded for each lesion The clinician perception regarding each technique was assessed based on the following questions: (a) Did the technique enhance detection? (b) Did the technique uncover new lesions? (c) Did the technique change the provisional clinical diagnosis? (d) Did the technique alter the site of the biopsy? (McIntosh et al., 2009)

All visualized suspicious lesions were then biopsied using punch biopsy under local anesthesia at KAUFD clinics for definite histopathological diagnosis. Fixed tissue was stained with haematoxylin and eosin and interpreted by independent oral pathologist consultant who was blinded to the examination's results. a photo documentation was obtained prior to the surgical biopsy of the cases.

Statistical analysis

The collected data was analyzed using SPSS version 16.0 (SPSS, Chicago, USA). And P- value less than 0.05

was considered statistically significant. The test validity were calculated for each examination technique including: sensitivity, specificity, positive and negative predictive values with likelihood ratios, where the histopathological results served as gold standard. Contingency coefficient was used for testing agreement of the two methods in provisional diagnosis; Wilcoxon Signed Ranks was used for comparison of the distribution of lesion size and ease of visibility and McNemar test used for comparison by border distinctness. Chi-square test was used for comparison of consultants and general dentists with exact P as indicated.

Results

This study was conducted on 599 individuals, they were 450 males and 149 females, the mean age was 34.8 years, figure 1 illustrates the participants habits regarding tobacco use and alcoholic drinks as well as other habits.

Table 1 shows a highly significant agreement between COE and both Microlux/DL with and without toluidine blue staining in visualizing and detection of suspicious

lesions, however using Microlux/DL+TB yields a better ease of visibility and border distinctness compared to COE (p<0.05). Fifty three suspicious lesions were detected by COE, while 52 and 51 lesions were detected by Microlux/DL and Microlux/DL+TB respectively. but only 39 lesions were biopsied for histopathological examination as the biopsy were scheduled 2 weeks after clinical examination at KAUFD out patients clinics . the dropped out cases were 5 lesions disappeared (due to stop of smokeless habit), 2 patients refused the biopsy and 7 patients failed to follow-up.

Table 2 demonstrate the validity of both screening techniques in detection of clinical suspicious lesions compared to COE and histopathology it shows that Microlux/DL has better sensitivity (94.3) and specificity (99.6) while Microlux/DL+TB has (88.7) and (99.3) .Considering histopathology as the gold slandered for diagnosis of dysplasia the sensitivity of both techniques was 100% while the specificity dropped to 39.4 and 35,3 for Microlux/DL and Microlux/DL+TB respectively .

To assess the effect of the examiner experience, the

Table 1. Comparison of COE, Microlux/DL and Microlux/DL+TB in Visualizing Suspicious Lesions

	COE No (%)		Microlux/DL No (%)		p	Microlux/DL+TB No (%)		p
All lesions								
No	546	(91.2)	547	(91.3)	Contingency coefficient= 0.87 (0.000)*	548	(91.5)	Contingency coefficient= 0.84 (0.000)*
Yes	23	(8.8)	52	(8.7)		51	(8.5)	
Smokeless keratosis	30	(5.0)	27	(4.5)		24	(4.0)	
Leukoplakia	12	(2.0)	13	(2.2)		24	(4.0)	
Erythroleukoplakia	4	(0.7)	6	(1.0)		16	(2.7)	
Erythroplakia	1	(0.2)	-	(0.0)		1	(0.2)	
OSF	3	(0.5)	5	(0.8)		4	(0.7)	
Lichenoid lesion	2	(0.4)	-	(0.0)		-	(0.0)	
Proliferative leukoplakia	1	(0.2)	-	(0.0)		-	(0.0)	
Median size (mm ²)	30		20		Wilcoxon Signed Ranks Z=0.78 (0.435)	20		Wilcoxon Signed Ranks Z=0.61 (0.540)
Ease of visibility	Mean (SD)	3.08 (0.83)	3.23 (0.90)		Wilcoxon Signed Ranks Z=1.06 (0.287)	30.73 (0.7)		Wilcoxon Signed Ranks Z=2.34 (0.019)*
	Median	3	3		McNemar (Exact p=1.000)	4		McNemar (Exact p=0.0001)*
Border distinctness	Sharp	17 (32.1)	19 (36.5)			33 (64.7)		
	Diffuse	36 (67.9)	33 (63.5)			18 (35.3)		
No. of satellite lesions	0	39 (73.6)	42 (80.8)		Wilcoxon Signed Ranks Z=1.29 (0.196)	42 (82.4)		Wilcoxon Signed Ranks Z=1.45 (0.148)
	1	5 (9.4)	5 (9.6)			5 (9.8)		
	2	5 (9.4)	2 (3.8)			3 (5.9)		
	3-4	4 (7.6)	3 (5.7)			1 (2.0)		

*p<0.05 (Significant)

Table 2. Validity of Different Methods of Detection of Suspicious Lesions Versus COE and Histopathology

		True		False		Sn	Sp	PVP	PVN	Likelihood ratio		McNemar (P)
		+ve	-ve	+ve	-ve					-	+	
Versus COE												
Microlux/DL	Examiner 1	30	258	1	2	93.8	99.6					
	Examiner 2	20	286	1	1	95.2	99.7					
	Total	50	554	2	3	94.3	99.6	96.2	99.5	253.8	0.06	1.000
Microlux/DL+TB	Examiner 1	29	257	2	3	90.6	99.2					
	Examiner 2	18	285	2	3	85.7	99.3					
	Total	47	542	4	6	88.7	99.3	92.2	98.9	126.7	0.11	0.754
Versus histopathology												
COE	Examiner 1	4	5	14	0	100	26.3					
	Examiner 2	20	286	10	0	100	33.3					
	Total	5	10	24	0	100	29.4	17.2	100	1.42	0	0.000*
Microlux/DL	Examiner 1	4	7	12	0	100	36.8					
	Examiner 2	1	4	11	0	100	26.7					
	Total	5	11	23	0	100	32.4	17.9	100	1.78	0	0.000*
Microlux/DL+TB	Examiner 1	4	7	12	0	100	36.8					
	Examiner 2	1	5	10	0	100	33.3					
	Total	5	12	22	0	100	35.3	18.5	100	1.55	0	0.000*

*p<0.05 (Significant); **Fisher's Exact Test indicated no significant difference between the two examiners

Table 3. Dentists’ Perception of the Efficiency of Different Methods for Diagnosing Suspicious Lesions

Category	Yes	Current users	Quit	None	Total	Decline
The method enhances detection	323	53.9	123	20.5	316.12	0.000*
It uncovers new lesions	51	8.5	29	4.8	10.89	0.004*
It alters the provisional clinical examination	32	5.3	39	6.5	0.93	0.63
It alters the chosen Biopsy site**	5	12.8	18	46.2	14.27	0.001*

*p<0.05 (Significant); **No. of patients=39

validity of both techniques was re-evaluated comparing both examiners. Interestingly, oral medicine consultants were more able to detect dysplasia as proven by the histopathology using any technique (4 out of 5 lesions).

Table 3 demonstrates the practitioner perception of both techniques; Microlux/DL significantly enhances lesion detection and uncovers new lesions while Microlux/DL+TB alter more significantly the chosen biopsy site as presented in.

Discussion

The International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) have stressed that third of a predicted 15 million cancer cases in the future can be reduced and more effectively manage another third by implementing effective cancer control and screening strategies (IARC-WHO, 2002; Eaton, 2003).

Unfortunately, most of the publications in oral cancer screening is based on observational, case cohort studies, but there is only single ongoing randomized controlled trail in Kerala, India using visual inspection and their most recent results concluded a sustained reduction in oral cancer mortality during the 15-year follow-up (Sankaranarayanan et al., 2013). The latest update of the ongoing Cochrane systematic review stated that this study is associated with great degree of bias (Brocklehurst et al., 2010). Furthermore, the American Dental Association council on scientific affairs expert panel on screening for oral squamous cell carcinomas has suggested that clinicians should be aware of signs of potentially malignant lesions during routine visual and tactile examinations in all patients, specially for heavy tobacco or alcohol consumers and recommended the need for additional oral cancer screening using adjunctive aids (Rethman et al., 2010).

Oral cancer screening remains controversial (Downer et al., 2004; Bassiony, 2009). More importantly, COE is unable to detect potentially malignant lesions that are present in apparently normal mucosa (Lingen et al., 2008). Histological assessment of tissue biopsy is the gold standard for definitive diagnosis, but it is considered invasive and time consuming (Messadi, 2013). So, a non-invasive technique which detect oral malignant or potentially malignant lesions with high both specificity and sensitivity is needed. Several authors systematically or critically assessed the current oral cancer screening or diagnostic aids in the literature (Lingen et al., 2008; Patton

et al., 2008; Fedele, 2009; Rethman et al., 2010; Kujan and Sloan, 2013; Messadi, 2013). They concluded that, up to date no technique or technology definitely improves oral cancer screening more than COE alone. Moreover, most of the studies have design flaws such as they were using the devices in a “case-finding” way, rather than as true screening tools.

Few studies evaluated the efficacy of Microlux/DL (McIntosh et al., 2009). To our knowledge, the current study is the first to evaluate Microlux/DL both as a screening device and as a case finding (diagnostic) device (Lingen et al., 2008; Patton et al., 2008).

Results of the current study show that sensitivity and specificity and PVP of Microlux/DL for visualization of suspicious premalignant lesions considering COE as a gold standard (i.e screening device) are 94.3%, 99.6% and 96.2% respectively, when considering biopsy as a gold standard in detection of dysplasia these variables were 100%, 32.4% and 17.9% which indicates that it cannot discriminate between benign and malignant oral mucosal lesions, and does not provide any indication of the underlying pathology of mucosal lesions. Microlux/DL enhances detection of the lesion and uncover new lesions compared to COE, whereas, it does not alter the provisional clinical diagnosis, or alter biopsy site.

Our results are in accordance with other studies (Ram and Siar, 2005; Farah and McCullough, 2007) evaluating the clinical efficacy of Vizilite where sensitivity were 100%, specificity 0%, 14.2%, respectively. Also, it was found that use of ViziLite showed no difference in lesion size, ease of visibility and border distinctness as COE and did not change the provisional diagnosis nor biopsy site (Farah and McCullough, 2007).

However, our results are different from the study performed by McIntosh et al. (2009) where the sensitivity and specificity of Microlux/DL were 77.8% and 70.7%, respectively, but the PPV was 36.84%, they reported that Microlux/DL enhances lesion visibility and border distinctness above COE, no new lesions was uncovered ,nor alteration of the provisional clinical diagnosis, or biopsy site, this could be explained by different methodology used and different settings as in McIntosh et al. (2009) study the two oral diagnosis specialists performed all examinations rather than general dentists while in our study examinations were done by two teams one was oral medicine specialists and the other team was generalists and the patients were randomly examined by any of the teams. Moreover, independent and blind examination protocol was applied within the same team as examiner A was responsible for each COE and examiner B was responsible for each Microlux/DL examination in an attempt to eliminate bias. Although the sensitivity and specificity were not significantly different comparing both oral medicine specialists and general dentists.

On the other hand, In this study adding toluidine blue dye did not improve the effectiveness of the Microlux/DL system, results of the current study showed that sensitivity and specificity and PVP of Microlux/DL+TB for visualization of suspicious premalignant lesions considering COE as a gold standard (i.e screening device) were 88.7%, 99.3% and 92.2% respectively, while they

were 100%, 35.3% and 18.5% . When these variables were measured considering biopsy as a gold standard. Moreover, it did not enhance detection of the lesion or uncover new lesions nor it altered the provisional clinical diagnosis compared to COE, whereas it altered chosen biopsy site more significantly.

Considering the inherent limitations of toluidine blue as a diagnostic test our results are in accordance with other studies (Epstein et al., 1997; Nagaraju et al., 2010; Chaudhari et al., 2013). However, results were different from other studies (Myers, 1970; Mashberg and Feldman, 1988) who observed specificity to be much higher 100% and 95% respectively. Such difference between specificity could be attributed to the study setting, as all these studies were carried out in specialized institutions by experienced clinicians. Gray et al. (2000) concluded that in a primary care setting toluidine blue is ineffective as a screening test due to the low specificity in staining dysplasia, however, in patients at risk for a second primary lesion it can adjunct in the evaluation of oral lesions.

In conclusion, microlux/DL seems to be a promising screening device while conventional oral examination is the gold standard screening tool .Furthermore it is not effective as a diagnostic tool where the diagnostic gold standard for detection of potentially malignant and malignant lesions remains the histopathological examination of biopsy specimens. We acknowledge some limitations of our study as borderline cases were not included which may have an impact on the overall prevalence of premalignant lesions and consequently the validity of the screening test. Also the study was conducted in high risk group where tobacco consumption was high. Finally randomized control studies are required to generalize the findings.

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