AUTOFLUORESCENCE/A CLINICAL TRIAL: A NEW HOPE FOR EARLY DETECTION OF ORAL CANCER & ORAL POTENTIALLY MALIGNANT DISORDERS

[1]UMAIR KHAN, BDS, C-Radiology (UK), MSc Oral Medicine (UCL, EDI, UK), MIAOMT (USA), MBSOM (UK)
[2]ANUM AZIZ, BDS, [3]MYRA AHMED, BDS, [4]MARIAM RABIA, BDS, [5]BUSHRA MAZHAR, BDS
[6]HURIYA IFTIKHAR, BDS, [7]ZAINAB RIZVI, BDS, [8]HAJRA IMTIAZ, BDS, [9]MAHAM CHEEMA, BDS
[10]FATIMA KHALID, BDS

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In conclusion, although all 22 patients showed loss of fluorescence/fluorescence visualization loss (FVL) i.e. producing a black shadow on autofluorescence, the sensitivity of VELscope for the detection of dysplastic lesions was found to be 86%. In Group II (Oral potentially malignant disorders group) patients who showed no clinical sign of speckling (mixed red and white patches or clinical indication of histopathological presence of dysplasia) or neoplastic changes, none of them showed fluorescence visualization loss (FVL) or a black shadow on VELscope examination thus confirming the certainty of autofluorescence with its relation to absence of dysplasia of clinical non-neoplastic lesions by giving the result of FVR (fluorescence visualization retained, hence concluding its sensitivity for non-dysplastic lesions or for oral potentially malignant disorders having no clinically visible neoplastic change at the time of patient's registration into the clinical trial (patient's initial acquaintance), to be 100%. 3 out of a total of 13 patients (3/13=23%) of Group II agreed to undergo biopsy for histopathology in order to detect any neoplastic transformation (dysplasia, CIS and OSCC) histopathologically and none of the patients showed presence of dysplasia or neoplasia on H & E staining thus further supporting the spectroscopic tissue fluorescence imaging results of VELscope in relation to oral potentially malignant disorders having no clinical sign of neoplasia. In Group III (benign lesions/conditions group) neither did the patients show any clinical sign of malignancy nor did the VELscope (Autofluorescence) show FVL. Thus concluding the sensitivity of autofluorescence

[1] Assistant Professor and Head of Division of Oral & Maxillofacial Medicine, Dental Diagnostics and Dental Radiology, Fatima Memorial Hospital College of Dentistry, Lahore. Res: 93-K, Street-4, Phase 5, DHA Lahore. Email: dr.u.khan.1@hotmail.com, Contact number: Cell: 03009638625
[2-10] House officers Oral Medicine Department, FMH

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TABLE 6: DISEASE SPECIFIC PROFILE OF THE PATIENTS

Total number of patients = 92			
No. of patients	**Disease**	**Gender**	**Mean Age (years)**
15	Lichen planus	3 Males & 12 Females	43
9	Oral submucous fibrosis	4Males & 5 Females	37
2	Chronic hyperplastic candidosis	2 Males	48.5
4	*Oral squamous cell carcinoma	3 Males & 1 Female	40.2
3	Oral trauma	2 Males & 1 Female	44.5
1	Haematinic deficiency	1 Female	42
1	Smokeless tobacco keratosis	1 Male	47
16	Coated tongue (Bacteria & Candida)	6 Males & 10 Females	40±5
4	Frictional keratosis	I Male & 3 Females	35.6
1	Telangiectasia	1 Female	33
3	Mucositis	3 Females	35
1	Geographic tongue	1 Male	27
1	Median rhomboid glossitis	1 Male	33
1	Pyostomatitis vegetans	1 Female	24

*Idiopathic OSCC

TABLE 7: AUTOFLUORESCENCE RESULTS IN RELATION TO FOUR GROUPS OF THE PATIENT

Group 1	Diagnosis	Cases (n)	Clinical Examination	Histopathological Assessment	Autofluorescence			
					FVL	FVR	Se	Sp
Dysplasia/ Neoplasia group	Oral lichen planus	7	All +ve for speckling	6 +ve & 1 –ve for neoplasia	7	0	86%	—
	Oral submucous fibrosis	4	All +ve for speckling	All +ve for neoplasia	4	0	100%	—
	Chronic hyperplastic candidosis	2	All +ve for speckling	All +ve for neoplasia	2	0	100%	—
	Oral squamous cell carcinoma (Idiopathic)	4	All +ve for speckling	2 +ve & 2 –ve for neoplasia	4	0	50%	—
	Oral trauma	3	All +ve for speckling	All +ve for neoplasia	2	1	67%	33%
	Haematinic deficiency	1	+ve for speckling	+ve for neoplasia	1	0	100%	—
	Smokeless tobacco keratosis	1	+ve for speckling	+ve for neoplasia	1	0	100%	—
Group 2	Diagnosis	Cases (n)	Clinical Examination	Histopathological Assessment	FVL	FVR	Se	Sp
Oral potentially malignant disorders group	Oral lichen planus	8	All -ve for speckling	Only 2 patients' biopsy done (Both –ve for neoplasia)	1 with white hue (plaque)	7	100%	88%
	Oral submucous fibrosis	5	All –ve for speckling	Only 1 patient's biopsy done (–ve for neoplasia)	5 (with bright white hue)	0	100%	0%
Group 3	Diagnosis	Cases (n)	Clinical Examination	Histopathological Assessment	FVL	FVR	Se	Sp
Benign lesions/ conditions group	Coated tongue	9	Classical	—	9	0	100%	—
	(Bacteria) Coated tongue (Candida) Frictional keratosis	7 4	Classical 3 Plaque & 1 linear	— —	7 4	0 0	100% 100%	— —
	Geographic tongue Median rhomboid glossitis	1 1	Classical Classical	— —	1 1	0 0	0% 0%	— —
	Mucositis	3	Classical	—	3	0	100%	—
	Pyostomatitis vegetans Telangiectasia	1 1	Classical Prominent	— —	1 1	0 0	100% 0%	— —
Group 4 Control group	Clinically normal oral mucous membrane	30	Thorough Introral examination	—	0	30	0%	100%

for benign lesions to be 62.5% (Table 7). In Group IV (Control group) all patients showed FVR (fluorescence visualization retained) on VELscope hence the specificity of autofluorescence for the controls was 100%.

DISCUSSION

Five-year survival rates for oral cancer have not changed for several decades. Poor survival is at least in part due to the failure in early detection of oral potentially malignant disorders (OPMDs) and oral cancers. To this end improving diagnostic abilities of primary care dentists via specialists (such as Oral Physicians) and also facilitating less interventional investigations in secondary care units remain important cornerstones in the research agenda. This clinical trial was based on the investigation of the utility of autofluorescence as a diagnostic test to evaluate its accuracy in the detection of early stage oral squamous cell carcinomas, oral potentially malignant disorders and benign conditions.

TABLE 8: FORMULAS FOR CALCULATING SENSITIVITY AND SPECIFICITY VALUES OF AUTOFLUORESCENCE

Sensitivity = No. of true positives / Total number of patients in the particular group
OR
Sensitivity = No. of true positives / No. of true positives + No. of false negatives
Specificity = No. of true negatives / Total number of patients in the particular group
OR
Specificity = No. of true negatives / No. of true negatives + No. of false positives
Note:-In case of Group III since the conditions like Median rhomboid glossitis, Geographic tongue and Telangiectasia produce black shadow on autofluorescence which is not indicative of dysplasia as these lesions are never cancerous hence the sensitivity is 0% for them but in case of frictional keratosis, mucositis and coated tongue; autofluorescence does not produce black shadow and neither show FVR therefore the FVL values for these lesions is the same but sensitivity values are 100%.

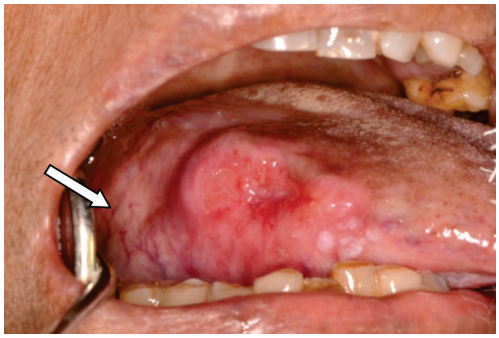

Fig 3: An example of OSCC presenting in the form of a large, deep necrotic ulcer with irregular raised margins surrounded by keratosis on the right lateral border of the tongue

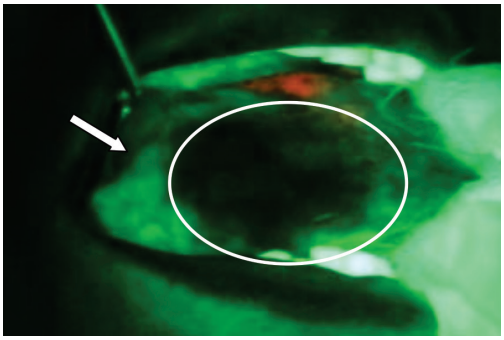

Fig 4: The loss of fluorescence visible in this picture notably over-extends the clinically abnormal margins visible in Fig 3. And another dysplastic lesion ≥ 1cm in the posterior region is visible which clearly appears clinically normal in fig 3.

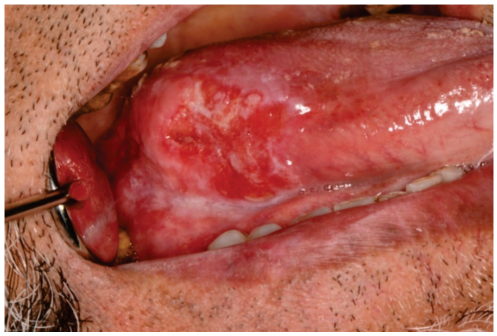

Fig 5: A classical example of Oral squamous cell carcinoma. The mild plaque type keratosis is noteworthy.

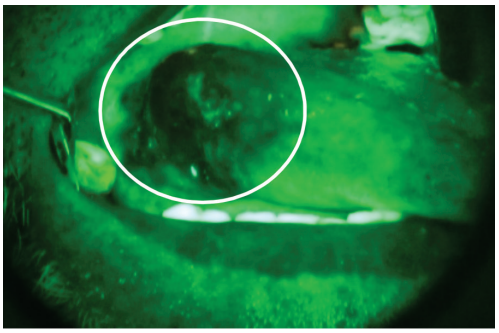

Fig 6: Fluorescence visualization loss (FVL) is predominently producing a black shadow in relation to its clinical counter part shown in fig 5.

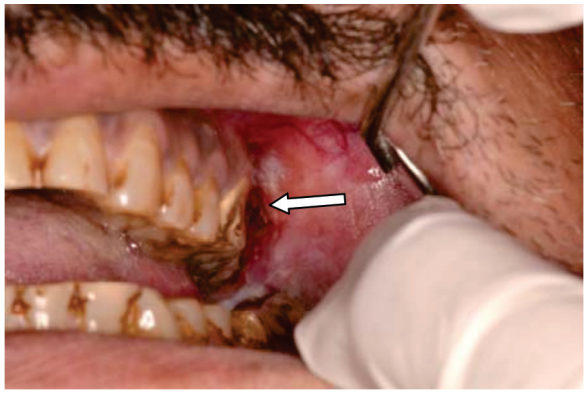

Fig 7: Speckling (neoplastic changes) in the left upper vestibular region of a patient enrolled in the study on the background of oral submucous fibrosis

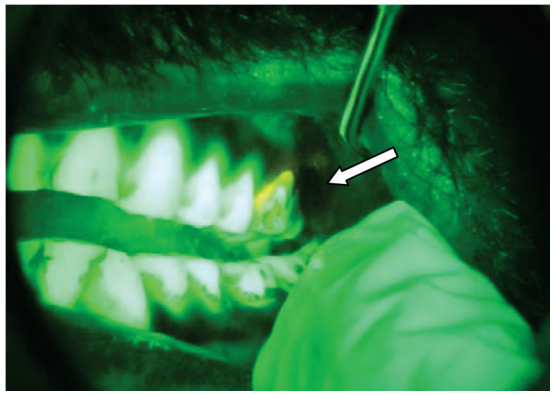

Fig 8: Loss of fluorescence as depicted by VELscope of the patient shown in fig 7

The Aga Khan University Hospital, Karachi.

SURGICAL PATHOLOGY Report

Medical Record # : LLGO[redacted] UR2813366-1
Patient Name : [redacted]
SpecimenID : 2013:BS19563R: Age : 47Y Sex : Male
Location : LHR Recieved Dt/Tm : 22/05/2013 - 09:41
Attending Doctor : [redacted] Rept Dt/Tm : 28/05/2013 - 16:34

Clinical Information: Limited mouth opening, burning sensation on spicy food. Ulcer against upper left molar left submandibular nodule enlarged 1 cm. D/D squamous cell carcinoma. Dysplastic lesion.

GROSS DESCRIPTION:

The specimen is received in formalin coded as "Left buccal vestibule/retromolar region". It consists of single tan brown mucosa covered soft tissue piece measuring 0.5 x 0.3 cm along with blood clots. Entirely submitted in a single cassette (FR).

MICROSCOPIC DESCRIPTION:

Sections examined reveal tissue fragment partially lined by squamous mucosa showing ulceration with neoplastic proliferation of squamous epithelium characterized by keratin pearl formation and individual cell keratinization. The underlying intervening stroma is desmoplastic, edematous and contains dense inflammation.

DIAGNOSIS: Left buccal vestibule/retromolar region:
Features favor well differentiated keratinizing Squamous cell carcinoma.

DR: [redacted]
M.B.B.S., M.D.

Fig 9: Biopsy (histopathology) report of the above patient mentioning the lesion as a well differentiated OSCC

Fluorescence visualization loss (FVL) was observed in majority of the patients especially for Group 1 patients in which FVL was observed in all the 22 patients though 3 of them came out to be false positive, hence winding up the sensitivity of VELscope to be 86%.

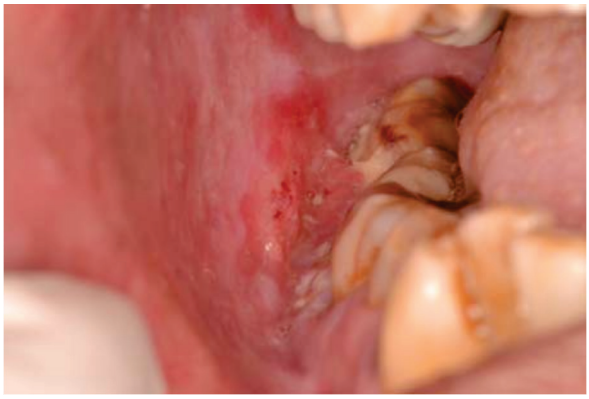

Fig 10: A red lesion (atrophy) in a Stage V oral submucous fibrosis patient

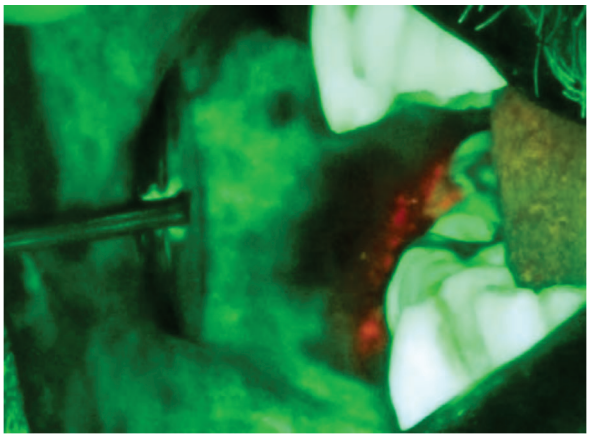

Fig 11: Area of a black shadow showing fluorescence loss in a patient shown in fig 10

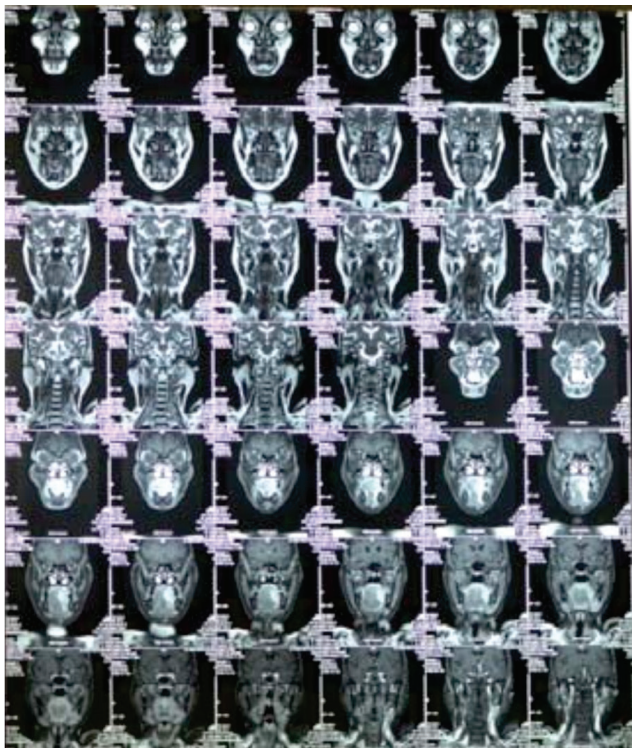

Fig 12: MRI of the patient in figs 10 & 11

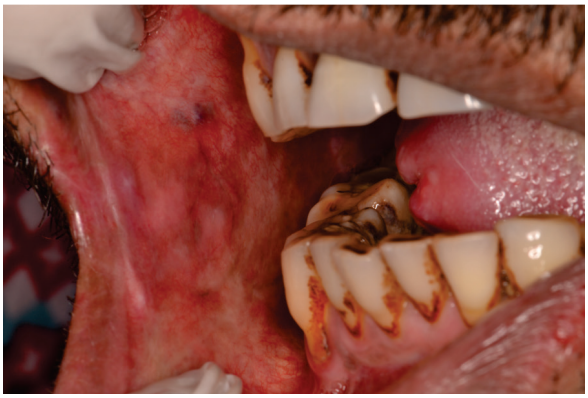

Fig 13: White fibrotic bands, microstomia, and cervical betel nut staining in a patient with oral submucous fibrosis

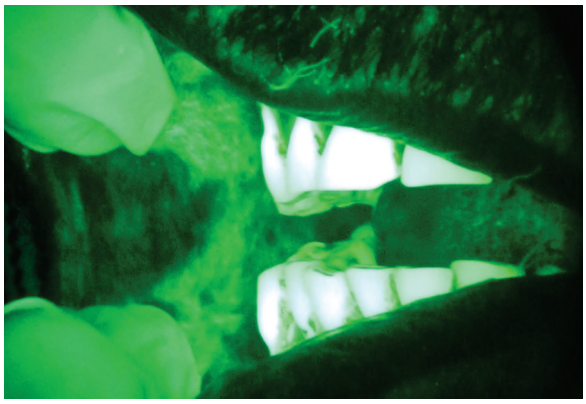

Fig 14: Fibrotic bands in this picture appear as bright greenish white specifying the location of the bands that may appear difficult to locate clinically as shown in fig 13

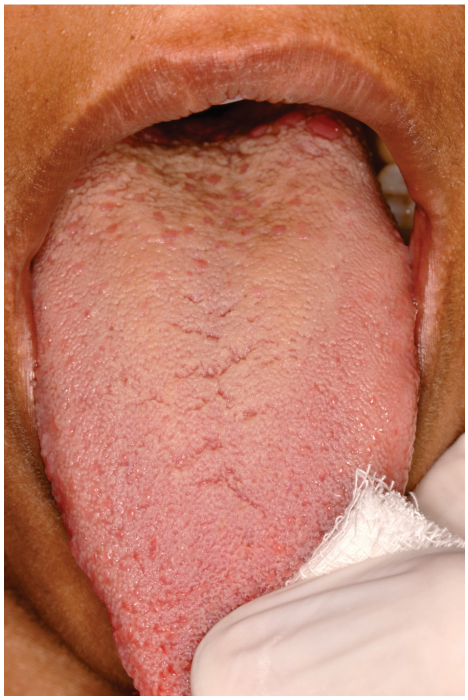

Fig 15: Coated tongue

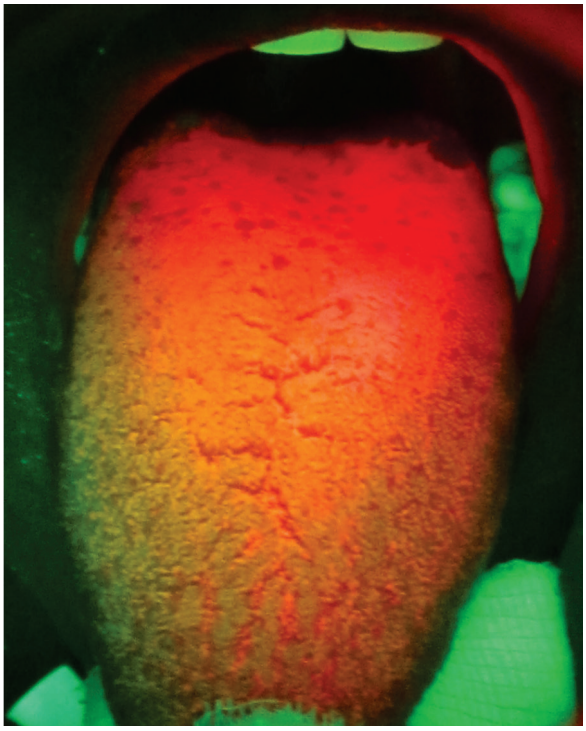

Fig 16: Red hue on the posterior part of the tongue depicts the presence of bacteria and orange hue shows mixed presence of bacteria and candida

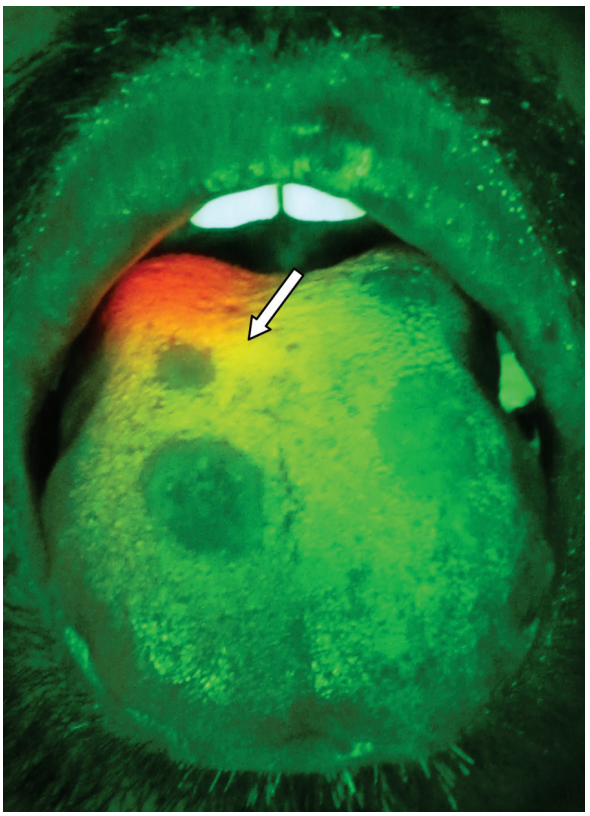

Fig 17: A patient of geographic tongue showing yellow and orange hue on VELscope depicting the presence of candida (marked by the white arrow) and the presence of mixed bacteria and candida respectively

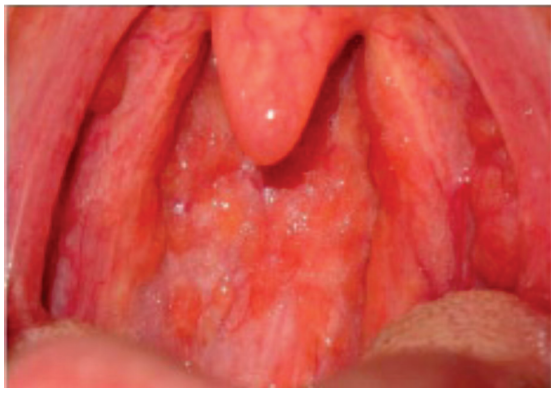

Fig 18: Tonsillar pillars and lymphoid tissues

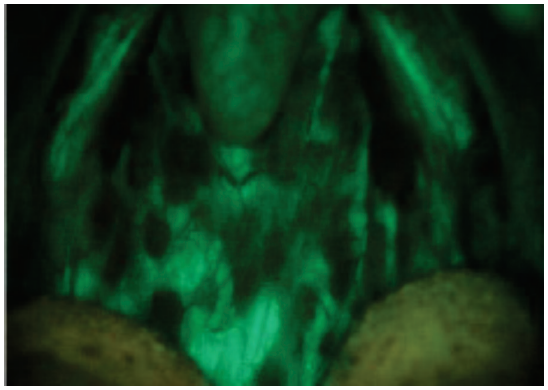

Fig 19: Tonsillar pillars and lymphoid tissues appearing black on VELscope

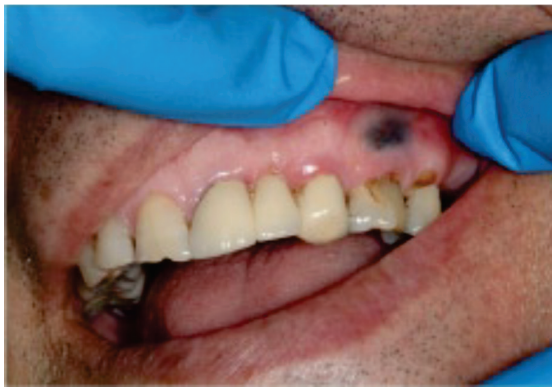

Fig 20: A pigmented lesion

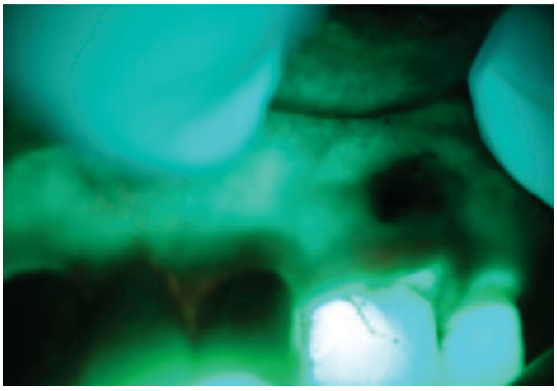

Fig 21: Pigmented lesion appearing black on VELscope showing false FVL

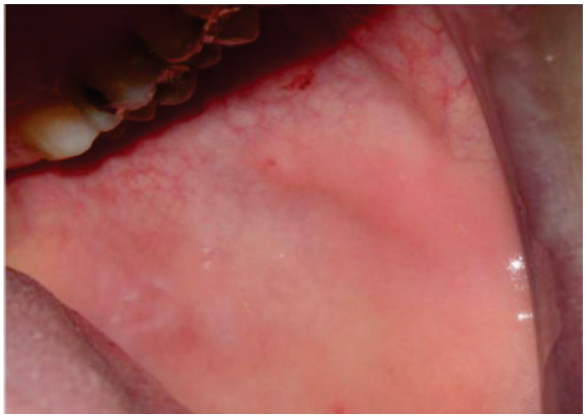

Fig 22: Clincially healthy looking oral mucosa (left buccal mucosa)

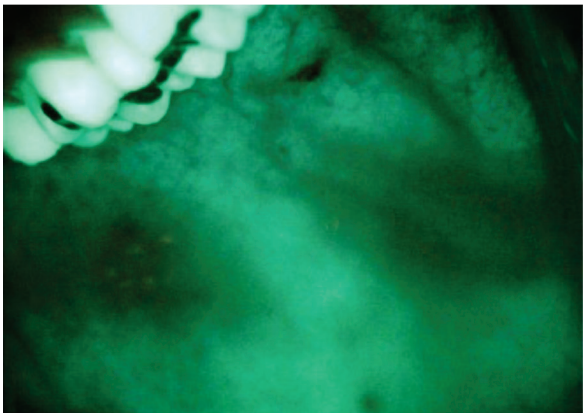

Fig 23: Chronic irritation and mild inflammation showing false FVL on VELscope in relation to its clinical appearance in fig 22

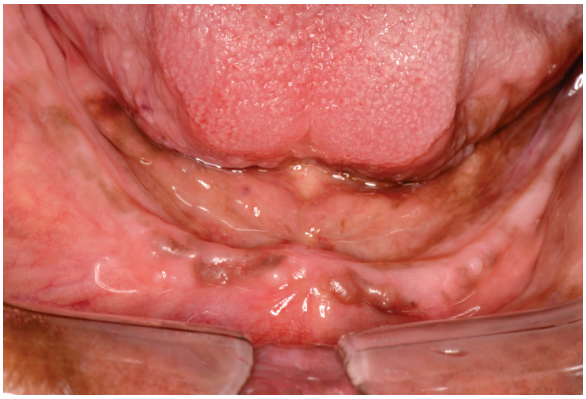

Figure 24: A patient with clinically normal looking residual ridge mucosa having no visible sign of hyperkeratosis

These results notably demonstrate the ability of the technique to detect high risk lesions. Sensitivity and specificity of autofluorescence as shown in Table 7 was calculated by using the formulas shown in Table 8.

As discussed earlier, tissue fluorescence imaging or Autofluorescence does not only aid in detecting the

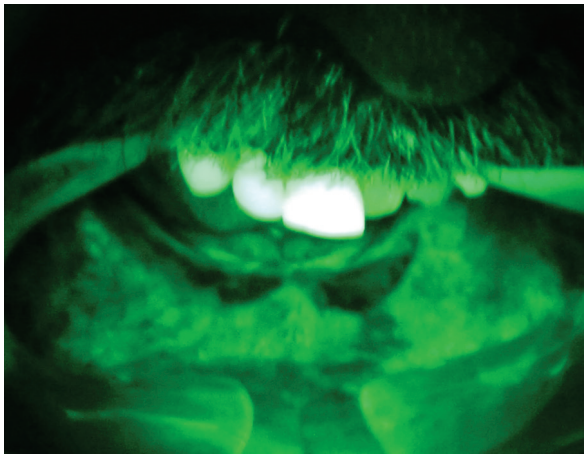

Fig 25: Hyperkeratosis becomes visible on VELscope examination producing a bright hue because of strong keratin fluorescence. Also black shadows on the ridge are due to pigmentation visible in its clinical counter part (fig 24)

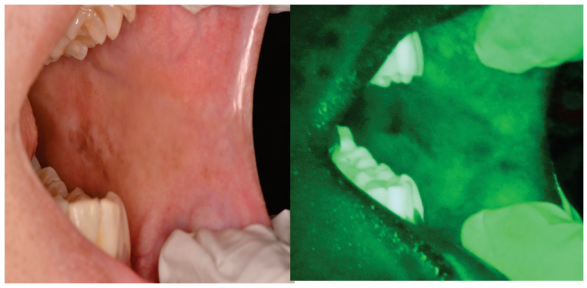

Fig 26: A patient that was enroled in the control group

presence of dysplasia but also can help Oral Surgeons to identify diseased tissue around a clinically apparent lesion and thus aid in determining the appropriate margins for surgical excision.[12,13] Such an example is shown in the photographs of one of the patients enrolled in the study (Fig 3 & Fig 4).

Comparison of the results of the recent study with published data proved to be difficult due to limited number of studies in the literature reporting sensitivity and specificity of the device. Only two previous studies appear to have employed autofluorescence in a systemic examination on a cohort of patients. One of the studies was conducted at the British Columbia Cancer Agency (BCCA) where a prototype of the VELscope was investigated by the group.[13] Using the blue-excitation light, 50 lesions were examined which included 33 oral cancers, 11 severe dysplasia and carcinoma-in-situ and 6 with no oral mucosal lesions. The authors reported a sensitivity of 98% and specificity of 100% against the gold standard (histology). However the data of the current clinical trial/research shows a moderately low specificity (74%) for the technique. It became possible to demonstrate this by the inclusion of several benign disorders, thus reducing the 'spectrum bias' encountered in published studies – a desirable feature of this study/clinical research. In this clinical trial it was also found that certain manifestations of particular diseases behave in a strange fashion when viewed by the VELscope. For instance the reticular pattern/variant of oral lichen planus even when clinically very prominent and extensive somehow disappears completely when viewed by VELscope, the phenomena of which is hard to explain. Similarly, the fibrotic lesions, such as in oral submucous fibrosis, mucositis and others appear bright white in auto-fluorescence (Fig 13 & 14). The bacteria and candida in a coated tongue appear red (Fig 15 & 16) and yellow (Fig 17) respectively in VELscope and orange (Fig 15 & 16) in the presence of both at the same site. Other spectroscopic features of VELscope that should be taken into account are shown in Table 9. Two studies on VELscope reported contrasting results on its utility. Huber et al.[24] reported that VELscope failed to detect any additional suspicious lesions not identified by conventional oral examination, and Huff et al.[25] reported an increase in prevalence of mucosal disorders in a second cohort subjected to VELscope, compared with an earlier cohort examined visually only. Their research was seriously flawed as they did not consider alternative possible reasons for a true increased prevalence of disorders in the later cohort. As sufficient studies had not examined sensitivity and specificity of the VELscope system, this clinical trial data need to be discussed against the backdrop of sensitivity and specificity reported for clinical visual screening. This study should not be seen as a screening study as the data are specific to a hospital population referred following the detection of a range of mucosal abnormalities by primary care practitioners/dentists. So far no studies have been reported for evaluating VELscope for screening the population.

To have an impact on the incidence of oral cancer, a broad range of stakeholders must be involved, including (but not limited to) professional societies, educational institutions, health care facilities, government and the public. A combined effort will guide the evolution of oral cancer screening toward population-based coverage (Fig 27).

Dentists	Obstacles	Detection/Risk assessment/Management	New technologies
	a) Time consuming oral mucous membrane thorough examination of all patients regardless of the dental chief complaint. b) Differentiating inflammation from infection. c) Differentiating sinister changes/speckling from homogenous pathological changes of oral mucous membrane. d) Audacity to refer patients having non-homogenous oral & maxillofacial pathological lesions/disorders to oral & maxillofacial physicians for proper diagnosis of the presence of speckling and of the disease as a first and most important step.	Detection	i. Fluorescence visualization (FV, VELscope®) ii. Computer Imaging System iii. Saliva markers
Oral physicians	a) Clinical differentiation of presence or absence of dysplastic changes via clinical cancer surveillance methods. b) Identification of degree of dysplasia. c) Clinical demarcation between dysplasia and well differentiated invasive squamous cell carcinoma. d) Deciding when and where to Biopsy. e) Managing patients pre-adequate therapy of dysplastic lesions (surgery alone, radiotherapy alone, photodynamic therapy alone OR combination of two or more of the above mentioned treatments.) f) Managing patients refractory to above mentioned therapeutic modalities.	Detection/Risk assessment/Management	i. Fluorescence visualization (FV, VELscope®) ii. Microsatellite analysis iii. Computer Imaging System iv. Saliva markers v. Genomic analysis
Oral pathologists	a) Histopathological differentiation of the degree of dysplasia. b) Correct diagnosis regarding the histopathological presence of dysplastic changes in case of low grade neoplastic changes.	Detection/Risk assessment	i. Microsatellite analysis ii. Computer Imaging System iii. Saliva markers iv. Genomic analysis
Radiologists	a) Identification of presence or absence of bony involvement and also radiological assessment of deep invasive soft tissue involvement. b) Combating the chances/risks of development of mucositis post radiation therapy via I/V keratinocytes growth factors (KGF) e.g. Palifermin (Kepivance).	Detection/Risk assessment	i. Computer Imaging System
Oral surgeons	a) Establishing surgical margins. b) Managing patients post-treatment (Complication/ Recurrences/ Second primary tumours/ Field cancerization).	Risk assessment/Management	i. Fluorescence visualization (FV, VELscope®) ii. Microsatellite analysis iii. Computer Imaging System iv. Saliva markers v. Genomic analysis
Clinical oncologists	a) Managing patients refractory to treatment in conjugation with Oral surgeons & Oral physicians and handling complications.	Management	i. Fluorescence visualization (FV, VELscope®) ii. Computer Imaging System iii. Saliva markers iv. Genomic analysis

Fig 27: Tools to overcome barriers to screening for oral disease at detection, risk assessment and management levels

TABLE 9: FLUORESCENCE VISUALIZATION IN THE NORMAL MOUTH

- Understand what a normal oral cavity looks like under VELscope to best appreciate what may be abnormal.
  - (i) The attached gingiva and anterior tonsillar pillars, for example, often have a naturally darker appearance (Figures 18 & 19)
  - (ii) Pigmented tissue appearing dark under white light usually also looks dark under VELscope Vx (Figures 20 & 21)
- Inflammation typically appears darker under VELscope due to the excess blood content.
- The oral cavity is naturally exposed to varying degrees of chronic irritation and mild inflammation (Figures 22 & 23)
  - (i) Due to inflammation, the buccal mucosa, lateral surfaces of the tongue and hard palate may sometimes show darker areas typically characterized by poorly-defined borders.
- Hyperkeratosis may often appear bright under VELscope because of strong keratin fluorescence (Figures 24 & 25)

CONCLUSION

In conclusion, this study demonstrated a relatively high sensitivity (86%) and a moderately low specificity (74%) in discriminating high-risk (dysplasias) from benign lesions. Further well designed multi-institutional national- based studies are needed to examine the role of VELscope as an oral examination system in primary care.

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