Impact of surgical and non-surgical interventions on post-operative recurrence and malignancy transformation rate of Oral Leukoplakia

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INTRODUCTION

Oral Leukoplakia (OL) is a common pre-malignant lesion with a global point prevalence of 2.60%.[1, 2] Defined as a "predominantly white lesion of the oral mucosa that cannot be characterised as any other definable lesion".[2-9] The lesion is found worldwide with a statistically significant gender prevalence ratio of 3.22.[1, 10] 36% of dysplastic OL lesions will transform to oral squamous cell carcinoma (OSCC), and those without may still transform (15%).[8] Because of this markedly high transformation rate, it is imperative that we are able to diagnose this lesion effectively.

Given that leukoplakia is at an increased risk of transformation to OSCC, the associated risk factors for OL are therefore not too dissimilar to that of OSCC.[11, 12] OL is significantly more prevalent among those who smoke, including areca nut; a 6-fold increase reported over non-smokers.[7, 11, 13, 14]

Alcohol-related habits are known as a risk factor for OL and when combined with tobacco, it works synergistically in its development.[11] Alcohol causes the accumulation of carcinogens (N-nitrosamines) and increases epithelial permeability (15). An in-vitro model of porcine oral mucosa showed a significant increase in permeability to N-nitrosonornicotine (tobacco-specific N-nitrosamine) at ethanol concentrations ≥ 25%.[16]

Another cause of OL is from constant irritation producing a mimicking lesion classified as "frictional keratosis". As frictional keratosis is reversible, unlike OL, it will disappear <4 weeks from resolution.[9] Human papilloma virus (HPV) is reported to be a risk factor for cancerous oral lesions however there is limited literature surrounding HPV and OL.[2, 10, 13, 17, 18]

CLINICAL MANIFESTATION

OL is categorised as homogeneous, often flat and thin, or non-homogeneous, which has three different sub-types:

1. Speckled – the lesion has both a white and red appearance (erythroleukoplakia)
2. Nodular – the lesion has red or white polypoidal outgrowths
3. Verrucous – the lesion has rough surface appearance

Proliferative verrucous leukoplakia (PVL) is a form of verrucous leukoplakia which initiates as hyperkeratosis and presents as multifocal exophytic regions. It is untreatable has both high recurrence and malignant transformation rates, transforming into verrucous carcinoma or OSCC over a 10-15 year period.[10, 13, 19]

Pindborg et al describes a pre-malignant lesion as a "morphologically altered tissue", whose malignant transformation rate is higher compared to normal mucosa.[3] The annual malignant transformation rate of OL to OSCC is 1%, with this rate being higher in non-homogenous lesions over homogenous.[13]

Risk factors for malignant transformation of leukoplakia
Female
Long duration of leukoplakia
Leukoplakia in non-smokers (idiopathic leukoplakia)
Located on tongue and/or floor of the mouth
Size >200 mm^2
Non-homogenous variant
Presence of *C. albican*
Presence of oral epithelial dysplasia (OED)

Table 1: Risk factors for the malignant transformation of leukoplakia to oral squamous cell carcinoma or verrucous carcinoma (PVL only). Data reproduced from Table 6 in van der Waal (2009)[13]

The presence of oral epithelial dysplasia (OED), frequently found in erythroleukoplastic lesions, and the extent of dysplasia is the current best marker of a pre-cancerous lesion (Table 1); not all dysplastic lesions progress and some may reverse.[7]

The aim of the structured review is to appraise current and future diagnostic methods. Most importantly, the intention is to determine if recurrence and malignancy transformation rates differ between the interventions available.

DIAGNOSIS

Histological Typing

Histological typing is performed from an incisional biopsy of the lesion and then examined under light-microscopy for evidence of dysplasia.[8] Dysplasia is scored based on the characteristics of the lesion and the level infiltration as mild, moderate or severe.[3, 8, 9] Assuming that more dysplasia increases the likelihood of malignancy, a relationship between OED and malignant transformation rate is difficult to show due to subjectivity between lesions.[8]

Upon examination, homogenous leukoplakias' are hyperkeratotic lesions, no evidence of dysplasia but may exhibit some inflammation of the lamina propria. In contrast, non-homogenous lesions are dysplastic.[3]

Iodine-Staining Method

The iodine-staining method (IOM) can detect OED over white lesions. Iodine is applied and normal mucosa should turn a light brown colour (Table 2). Visualisation of OED is due to the formation of an iodine-glycogen complex. The Warburg effect, states that a malignant tissue will

have depleted glucose levels due to the increased rate of glycolysis.[20] Therefore in the presence of OED, there would be reduced glycogen concentrations in the mucosa thus explaining why it remains unstained; highly-keratinised tissues (gingivae) are also unstained.[21]

Auto-Fluorescence Visualisation Method

Yamamoto et al describes a new method of recognising dysplastic lesions known as auto-fluorescence visualisation method (AVM) that takes advantage of fluorophores contained within the mucosa such as flavin adenine dinucleotide (FAD) and collagen. Blue light is directed onto the area, and through their auto-fluorescent properties emit a green light (Table 2).[3, 21, 22]

Farah et al conducted a study using the commercially available AVM machine, Visually Enhanced Lesion Scope (VELscope). VELscope is able to achieve similar results without irritating the oral mucosa seen in IOM.[21, 22] It produces a blue wavelength of 400-460 nm and detects green light at 515 nm. OED causes degradation of collagen and a fluorophores, leading to an absence of a detectable emitted frequency (FVL(-) in Figure 1). The use of VELscope and other AVM alike serve as a non-invasive judgement of a lesion's margin.[22]

This field has been previously researched by detecting increases in red light emitted in areas of dysplasia.[23] Van Staveren et al reported the use of AVM with artificial neural networks (automated computer algorithm) to group OL based on its sub-type and OED and found 100% accuracy and 86% sensitivity towards the lesion.[6]

INTERVENTIONS

Management of OL can be split into conservative, medical and surgical intervention. Conservative management involves the cessation of tobacco and alcohol habits. Medical management involves the use of retinoids, which are vitamin A derivatives and lead to the down-regulation
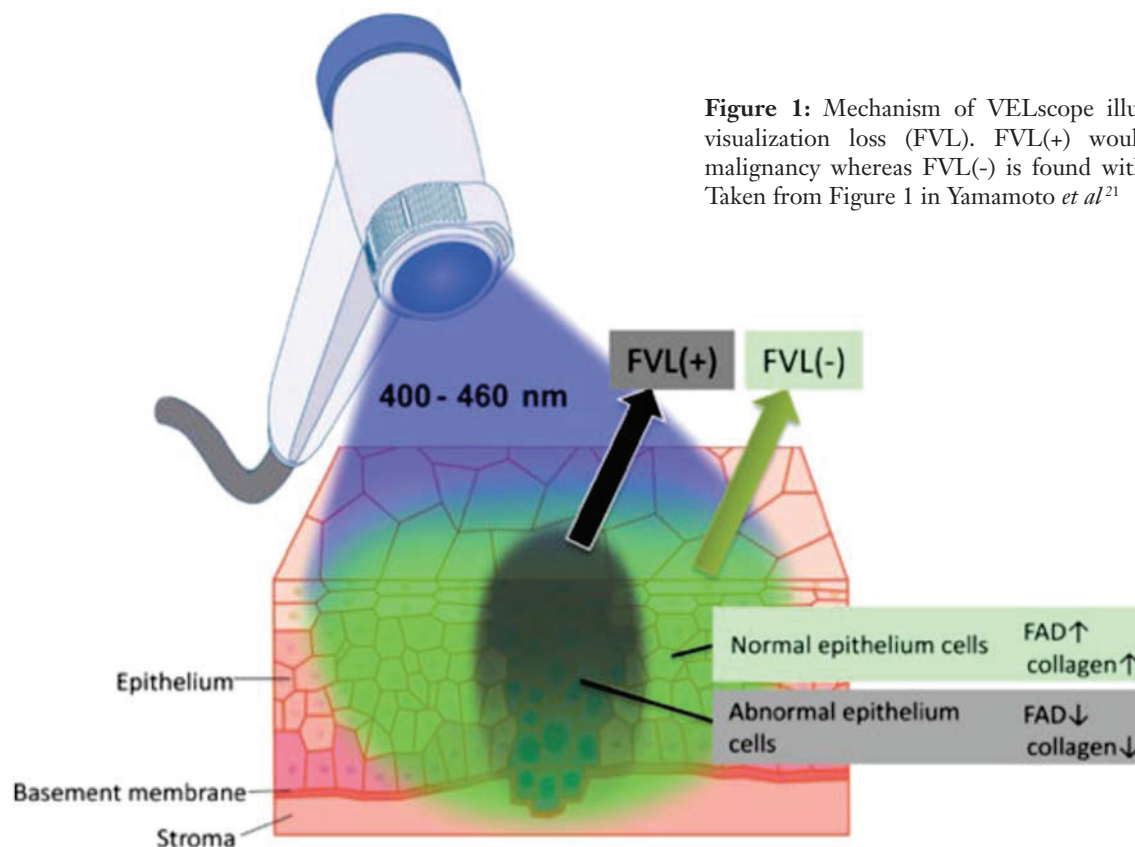

Figure 1: Mechanism of VELscope illustrating fluorescence visualization loss (FVL). FVL(+) would be suggestive of malignancy whereas FVL(-) is found with normal epithelium. Taken from Figure 1 in Yamamoto *et al*[21]

Technique	Mechanism	Positive	Negative
Histological Typing	Biopsy taken for examination	Dysplasia/OSCC in situ	No evidence of dysplasia
Iodine-Staining Method	Formation of Iodine-Glycogen complex	Tissue stained yellow-brown thus normal epithelium	Unstained tissue may indicate presence of malignancy or a highly keratinised tissue
Auto-Fluorescence Visualisation Method	Fluorophores are auto-fluorescent when excited by blue photons at 400-460 nm	Green light emitted at 515 nm therefore normal epithelium	No light emitted due to breakdown of fluorophores, suggestive of malignancy

Table 2: Summary of diagnostic techniques for Oral Leukoplakia. Data taken from[3, 8, 21, 22]

of proto-oncogenes and cellular differentiation; the current retinoid for treatment is 13-cis Retinoic Acid (13-cRA).[24, 25]

Current best practice for the treatment of OL is surgical resection. Other modalities used include CO_2 laser excision, neodymium-doped yttrium aluminum garnet (Nd:YAG) laser evaporation and cryosurgery.

RESULTS

Reference	Intervention	Number of patients	Number of lesions	Recurrence rate (%)	Malignancy transformation rate (%)
Holmstrup et al [25]	Surgical excision	89	94	13.48 (12 patients)	12.77 (11 lesions)
Kuribayashi et al [26]	Surgical excision	52	53	15.09 (8 lesions)	1.89 (1 lesion)
Van der Hem et al [27]	CO_2 laser excision	200	282	9.93 (28 lesion)	1.06 (3 lesions)
Del Corso et al [28]	CO_2 laser excision	30	30	13.33 (4 lesions)	0
	Nd:YAG laser evaporation	47	47	38.30 (18 lesions)	6.38 (3 lesions)
Kawczyk-Krupa et al [29]	Cryotherapy	37	72	24.32 (9 patients)	5.41 (2 patients)
Hong et al [30]	13-cis retinoic acid	24	24	N/A	4.17% (1 patient)
	Placebo	20	20	N/A	10% (2 patients)

Table 3: Comparison of included studies illustrating number of patients, number of lesions, recurrence rate and malignancy transformation rate. Adapted from Table 5 in van der Hem et al.[27] Data taken from [25-30]

Reference	Median follow-up period (months)
Holmstrup et al [25]	90
Kuribayashi et al [26]	31
Van der Hem et al [27]	52
Del Corso et al [28]	60
Kawczyk-Krupa et al [29]	52.81
Hong et al [30]	9

Table 4: A comparison of follow-up periods in the compared studies. Data taken from [25-30]

SURGICAL INTERVENTION

All patients were given either local or general anaesthetic dependent on the lesion's size.[25-29]

Holmstrup et al conducted a retrospective study of 84 patients with 94 oral lesions. Lesions were divided based on classification (Table 5) and on location, with most occupying the base of mouth and buccal surface. Mean lesions size was 486 mm² and were surgically excised with a margin of 3-5 mm and depth of 4-5 mm. Lesions were then either closed with sutures or using a local mucosal flap if primary closure was not achievable.[25]

70% of non-homogenous leukoplakia, 64% of homogenous leukoplakia and 56% of erythroplakias resolved with no recurrence. A recurrence rate of 13.48% was found from 12 lesions: 8 of which were non-homogenous, 2 homogenous and 2 erythroplakias. Over 2.7-15.1 years (Table 4), 11 transformed into carcinoma, of which 4 were from the recurrent lesions; 37.5% (3 out of 8) non homogenous and 50% of the erythroplakias (1 out of 2).[25]

Kuribayashi et al investigated recurrence of OL after surgical excision through a retrospective study of 52 patients. Participants had an average age of 58.3

Reference	Homogenous lesions	Non-Homogenous	Erythroplakia
Holmstrup *et al* (2006) [25]	39	46	9
Kuribayashi *et al* (2012) [26]	21	23	0
van der Hem *et al* (2005) [27]	136	61	0
Del Corso *et al* (2015) [28]	33 (Group 1)	14 (Group 1)	0
	12 (Group 2)	9 (Group 2)	0

Table 5: Comparison of the sub-types of oral leukoplakia in the compared studies. Data taken from [25-28]

years with a mean lesion size of 334 mm². Lesions were grouped as non-homogenous or homogenous (Table 5). Before surgery, suitable resection margins free of OED were achieved using of Lugol's iodine. A positive result from Lugol's iodine was also noted for comparison in the follow-up. Closure was achieved through a split-skin graft or sutures where feasible.

Patients had a post-operative follow-up length between 6 and 89 months (Table 4). Out of the 53 lesions there were 9 reported recurrences. One transformed to OSCC, thus a malignancy transformation rate of 1.89%. Five out of the 9 lesions were female and 2/3rd of the lesions were homogenous. Lesions with a positive staining margin i.e. presence of OED were found to have a higher recurrence rate compared to a negative margin.[26]

Van de Hem *et al* reviewed the malignancy transformation rate of 200 patients between 1976 and 2001 who had undergone CO_2 laser excision. All 282 lesions were predominantly found at the buccal mucosa or the floor of mouth and were grouped into its sub-types (Table 5). The apparatus was moved so that a defocused laser spot of 1mm was produced, with beam strengths of 15-20 W.[27]

Over a mean follow-up period of 52 months (Table 4), 89% of lesions did not recur. Out of the 282 lesions, 28 recurrences were noted (9.93%). In 4 of these lesions they showed an increase in OED compared to their original respective lesions. Recurrences were also found to be 1.8 times more likely in males (18:10). Only 3 cases of OSCC were found presenting between months 7-19 of the follow-up duration.[27]

Del Corso *et al* evaluated the outcomes of patients who had undergone Nd:YAG laser evaporation or CO_2 laser excision between 2004-13. Two groups were established (Table 5): group 1 consisting of 47 lesions for treatment with Nd:YAG laser evaporation and group 2 with 30 lesions for CO_2 laser excision. Treatment with Nd:YAG used a laser of 1064 nm with a spot of 600 µm. A beam strength of 9 W is used on the labia, tongue, and gingival margin and 12 W elsewhere. Contrasting with Van der Hem *et al* a different CO_2 laser was used for group 2 with a spot of 200 µm set to pulse for 0.5 seconds with a rest of 0.1 seconds at 8-15 W.[28]

Group 1 consisted of 35 lesions with no dysplasia, 11 with mild, and 1 with moderate dysplasia. Group 2 contained 18 leukoplakias with no dysplasia, 5 with mild, and 7 with moderate dysplasia. Those treated with Nd:YAG laser evaporation had a greater recurrence rate of 38.30% compared to 13.30% with CO_2 laser excision. All 3 of the transformed lesions were treated with Nd:YAG laser evaporation.[28]

Kawczyk-Krupa *et al* studied the outcomes of cryotherapy on 72 white lesions occupying the buccal, gingival and labial mucosas. Lesions were identified either as homogenous and non-homogenous leukoplakia (Table 5). Using a scapular probe and N_2O: lesions ≤4 cm underwent a single session of 2-4, 40-second applications, larger lesions had multiple sessions with a 3-week rest period. They were followed up twice monthly until no recurrence. A recurrence rate of 24.32% was reported from 9 lesions, and 2 patients developed OSCC both in different locations to their original lesions.[29]

MEDICAL INTERVENTION

Hong et al carried out a double-blinded randomised control trial comparing 13-cRA against a placebo. Patients were randomly assigned either 13-cRA or a placebo. Those given 13-cRA took 1-2mg per kg of body weight per day for 3 months and then reviewed 3 months later.

As measure of the effectiveness of 13-cRA, the degree of dysplasia present in the epithelium was used for comparison. In 13 out of 24 of patients assigned 13-cRA, a significant reduction in OED (P=0.01) was shown compared to only 2 out of the 20 lesions following the placebo. Clinical improvement was also used as a subjective measure of effectiveness. In those treated with 13-cRA, 16 cases showed clinical improvement, and in 8 cases no clinical improvement. Histopathological findings report a positive result in 9 and 4 cases respectively. Most astonishingly, the 3 lesions that entered the trial with severe dysplasia or OSCC had reduced moderate, mild and normal epithelium. Transformation to OSCC occurred in 1 patient with 13-cRA and 2 with placebo.[30]

DISCUSSION

Unfortunately, there was not consistent distinction of whether OSCC was from recurred lesions or from new lesion. If the recurrent lesion was at or adjacent to the site of treatment, it would be a better measure of how that particular modality was affecting the recurrence and malignancy transformation rates.

Van der Waal states that OL has an annual transformation rate estimated at 1%, worryingly, only CO_2 laser excision complies with this figure with malignancy transformation rates of 1.06% and 0% for van der Hem et al and Del Corso et al respectively.[13, 27, 28]

Interestingly, in the study by Holmstrup *et al*, out of the 36% of unresolved lesions, 31% were homogenous leukoplakia compared to 5% from non-homogenous lesions. They were not able to show that surgical excision deferred malignant transformation, but it did show us that out of the lesions treated, homogenous leukoplakia was less likely to resolve.[25]

Lodi and Porter report that the use of CO_2 laser excision achieves better aesthetics and functionality of the lesion from a reduction in contractures.[31] It is reported that the better aesthetic outcomes are linked to an increased chance of spotting OSCC and so an increased chance of resolution of the lesion. However, the aspect of detection is still subjective and may be based on the experience of the clinician.[13]

The use of cryotherapy has a five-fold increase on the malignancy transformation rate (5.41%) compared to CO_2 laser excision (1.09%).[27, 29] The high recurrence and malignancy transformation rates may be explained by a study investigating the link between cryotherapy and carcinogenesis in hamster cheek pouches. The results showed an increase in the permeability of carcinogens following cryotherapy and this could be one justification for the high rates.[32]

Kuribayashi et al states that the location of the lesion affects the recurrence rate. The gigival region is thin and highly keratinised hence resection margins of 1.25mm can only be achieved; reports suggest at least 3 mm margin.[25-28] Lugol's iodine is used not only to identify positive margins but also to identify a safe margin as a positive result indicates presence of OED.[26] Surgical intervention is only preventative when margins clear of OED are applied.[28] Lesions that recurred with mainly homogenous fitting with the above evidence that homogenous leukoplakias are more resistant to treatment.[27]

The greatest recurrence rate of 38.30%; was recorded with Nd:YAG laser evaporation; much higher than the other modalities. Given that this is the only paper profiling this treatment, findings may not be representative of others papers.[28]

Hong et al was the only study in this comparison using 13-cRA as treatment; although conducted in 1986, the findings are still relevant. Cheilitis and facial erythema developed in 19 cases and hypertryglycerideaemia in 17. Table 3 illustrates the ability of 13-cRA to regress lesions, which was reported in no other treatment: those with severe dysplasia were able to be reversed to moderate, mild and normal epithelium. With the follow-up time being only 9 months, the long-term effects of 13-cRA have not been well established and so further investigation is needed.[30]

Although all of our studies grouped OL by its subtypes, the number of homogenous lesions to non-homogenous lesions was not consistent in all studies. Because non-homogenous lesions, in particular PVL, have an increased rate of malignant transformation compared to other lesions, the rates calculated are not truly comparable (Table 5).[25]

Limitations on this comparison (Table 3) are that there are huge discrepancies in median follow-up period (Table 4). Because of this, and the nature of OL transforming into OSCC, it is inevitable that we see differences in the recurrence and malignancy transformation rates treatments.[13]

All of the results compared in Table 3, excluding Hong et al, are gathered from retrospective case report studies. Case report studies compare the effects of treatment, in our case surgical or non-surgical interventions, against a desired outcome. Although they are considered to be at the bottom of the hierarchy of study designs, they are excellent at eliciting the effects of a treatment. For the purpose of this comparison, they are a good way to indicate recurrence and malignancy transformation rate. On the other hand, Hong et al conducted a double-blinded randomised controlled trial; this is considered to be one of the best studies to do in order to eliminate bias from your results.[33]

CONCLUSIONS

The main premise of this review was to consider the treatment modalities available for OL and come to a conclusion on which offers the lowest recurrence and malignant transformation rates.

It can be seen in Table 3 that the highest malignancy transformation rate (12.77%) was achieved using surgical excision and the lowest rate (1.06%) seen with CO_2 laser excision.[25, 27] From the comparison, the lowest recurrence rate was also found with CO_2 laser excision thus illustrating that this is the best treatment for OL. As CO_2 laser excision had the largest cohort with 282 lesions, it is arguably the most representative data.[27]

Unfortunately due to the toxicities of 13-cRA as a treatment for OL, it does not have the widespread usage that the surgical interventions do. Given that the paper was published in 1986, newer drugs may provide similar outcomes without the toxicity. It would be interesting to see in the future if a dual-therapy combining both medical (13-cRA) and surgical management for OL changed the recurrence and malignant transformation rate; and to see if it works synergistically with any modality.

My other aim in this study was to consider the current and future diagnostic tools available for OL. One of the major pitfalls of histological typing is the degree of subjectivity between lesions; future methods such as AVM and IOM are also not free from this. The one large difference between current and future methods is that there is now a shift into non-invasive techniques that can be carried out when the patient presents. In my opinion, the future of AVM and in the detection of pre-malignant lesions such as OL is with artificial intelligence. As mentioned earlier, AVM has already been coupled with an artificial neural network in the detection of OL with 86% sensitivity and 100% accuracy.[6]

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