Head & Neck Oncology
Open Access

Methodology

Microendoscopy: a clinical reality in intra-operative margin analysis of head & neck lesions

Upile T[1,2], Jerjes W[3,4,5,6], Mahil J[2], Hamdoon Z[2,3,4,7], Radhi H[3,4*]

Abstract

Introduction

The tumour margin is an important surgical concept that significantly affects morbidity and mortality. We describe the clinical application of intraoperative microendoscopy in defining the surgical margins during head and neck surgery.

Method

A clinical margin is first marked by the operator followed by microendoscopic assessment of this margin. Biopsies are taken from areas suggestive of close or positive margins on microendoscopy. The histological margins are further analysed formally and the resection revised accordingly if necessary.

Discussion

The advantages of this technique is that a large area of mucosa can be sampled whose histomorphological changes can be visualised in real time allowing the operator to make important informed decisions regarding the resection margins at the time of surgery. Shrinkage, thermal and orientation artefacts are also avoided.

Conclusion

We have developed the use of the microendoscope to aid intra-operative decision making during surgery.

* Corresponding author
Email: Hani_hadi79@hotmail.com

[1] Head & Neck Unit, University College London Hospital, London, UK
[2] Department of Head and Neck Surgery, Chase Farm & Barnet NHS Trust, Enfield, UK
[3] Department of Surgery, School of Dentistry, Al-Yarmouk University College, Baghdad, Iraq
[4] Oral and Maxillofacial Surgery Unit, AL-Mustansirya University's, Baghdad, Iraq
[5] UCL Department of Surgery, University College London Medical School, London, UK
[6] Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK
[7] Department of Surgery, University of Mosul, Mosul, Iraq

Background

The mucosal surface of the upper aero-digestive tract is bathed in a 'milieu' of toxins which can give rise to disease when the host repair processes are overcome. These disease processes may be discrete or multi-focal and can occur anywhere within the aerodigestive mucosal blanket. The usual epithelial response to chronic injury is metaplasia and hyperplasia, which can be manifest as keratosis and leukoplakia. Further disruption of this already unstable mucosa can entrench carcinogenic changes leading to the development of squamous cell carcinoma. This may be multi-focal and difficult to differentiate from the surrounding unstable mucosa by simple observation. Several areas of mucosa can co-exist along this pathway to malignancy; hence any examination of the mucosa must be detailed and comprehensive in-order not to miss subtle lesions[1-3].

The diagnosis of dysplastic lesions cannot solely be based on clinical findings. Therefore histological evaluation of a representative specimen is necessary. Dysplasia and carcinoma *in situ* may herald invasive oral cancer[1,2], but carcinomas can occur in areas with no previous signs of dysplasia. This may be because of the rapid emergence of invasive cancer, or it may be that earlier biopsies were taken from unrepresentative sites of the lesion or before morphological changes could be detected. Furthermore, the grading of dysplasia also suffers from inter-observer variability[3].

There is no reliable method applicable to the upper aero-digestive tract that can replace biopsy for more definitive diagnosis of malignancy but some may be used as a supplement. Exfoliative cytology carries the risk of false positive or false negative results; a biopsy is still necessary for final diagnosis. Vital dyes have been used to identify a suitable site for biopsy, but literature has shown that false positive staining may be as high as 30%[4]. This is mainly caused by the enhanced staining of the hyperplastic edges of ulceration and filliform papillae of the tongue.

Histological assessment of a tissue sample is regarded as the most reliable criteria for correct diagnosis; accordingly, the specimen must be taken from the most representative area. In cases involving the uterine cervix (which can also undergo squamous metaplastic changes), microcolpohysteroscopy and colposcopy are used to examine the mucosa. This consists mainly of assessing the vascular pattern, inter-capillary distance, surface contour, colour, tone, and clarity of demarcation. However the accuracy of colposcopy for the detection of mucosal change is between 70-98%. Furthermore by using colposcopic techniques, direct oral microscopy of pre-stained mucosal lesions (with magnifications of up to x8, x12 and x20) has been shown to offer an advantage in selecting more representative sites for biopsy than routine clinical examination alone[5-7].

We believe that this can be achieved by the use of the microendoscope. Microendoscopy was first popularised by Hamou in 1979 as a technique to study the uterine epithelium. The scope was later modified for use in the upper aero-digestive tract by Andreas[8]. The

FOR CITATION PURPOSES: *Upile T, Jerjes W, Mahil J, Hamdoon Z, Radhi H.* Microendoscopy: a clinical reality in intra-operative margin analysis of head & neck lesions. Head Neck Oncol. 2012 Sep 9;4(2):43.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Methodology

Background (Cont.)

scope is used to obtain an accurate microscopic assessment at the time of clinical examination, to inform the surgical decision as to the nature of the cellular characteristics of the mucosal lesion and its edge aiding biopsy yield and excision margin[7].

Microendoscopy allows "*in vivo*" and "*in situ*" examination of the epithelium. The scope allows the monitoring of the whole mucosal surface both normal and pathological, and allows the detection of patterns specific for pathology e.g. inflammation, metaplasia, dysplasia, and neoplasia.

The advantages of the scope are that once the microendoscope is acquired at relatively low initial cost it is used like any autoclavable Hopkins rod, and maintenance costs are similarly low. The scope can be used to guide further surgery, biopsy or simple surveillance of large areas of suspect mucosa. The microendoscope enables on table analysis to inform one's choices of further surgery even when biopsy (frozen section) is not available.

We present the head & neck surgical oncologist with a workable protocol for the use of the microendoscope in the assessment of the margins of excised lesions.

Method

"Written informed consent was obtained from the patient for publication of this report and any accompanying images." Institutional ethical approval was obtained. The operating Storz Hopkins II auto-clavable microendoscope (Figure 1), is attached via an adapter to a camera system (3 chip Olympus\Sony) linked via a video recorder with outputs to a monitor and photo-printer. Video encoding was done, in the main by, using a Xenon-light source (Karl Storz 300 attached via a Storz fibre optic cable), a 3 CCD camera (Karl Storz Tricam) and a Sony DV Cam system (DSR-20P) that enable a high resolution playback. Static

Figure 1: Storz HopAkins II autoclavable microendoscope

documentation was performed in the form of simple photography (sony printer/dpi) as well as dynamic (in the form of a video-clip, stored in a DV magnetic tape, which required editing). High quality photographic images can also be extracted from the edited video. The scope is available in different sizes which can be used for different anatomical sites; the dimensions of the microendoscope were 5.5 mm diameter and 23 cm long and the vital stain used is Methylene blue.

The microendoscope scope has a fitted rotating screw which allows magnification to be changed from 0x to 60x to 150x, minor movements permit focussing and de-focussing at specific depths of field. The clinician would, having prepared the patient, mark out his proposed margin for excision (Figure 2). The generalised area would be surveyed at 0x magnification, until an area of abnormality is found; this would then be examined at 60x and then

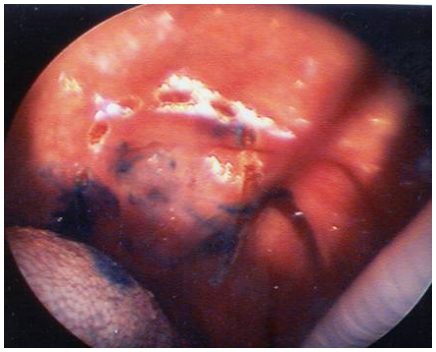

Figure 2: Clinical margin of oral lesion

150x. Examination would always proceed from an area of normality (Figure 3) to abnormality (Figure 4); the whole of the surface of the lesion would be reviewed to determine any heterogeneity. The microendoscopic margin of the lesion would then be delineated and any areas of discrepancy between the clinical and microendoscopic margin were photo-documented. The clinician would excise the lesion, which would be sent for frozen section analysis as would be the noted areas of discrepancy between the clinical and microendoscopic margin. A later analysis is made between the histological photomicrograph and microendoscopic image. When frozen section is not available a microendoscopic margin is taken and the biopsy sent for formal histological analysis.

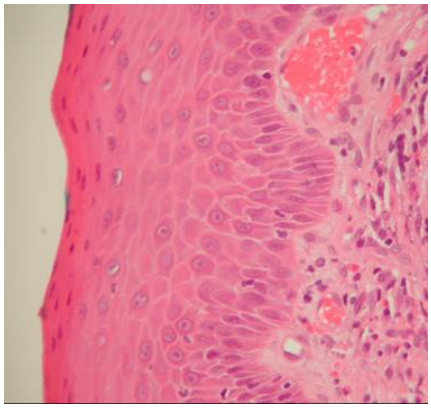

Figure 3: Normal mucosal histology of previous lower margin (200x)

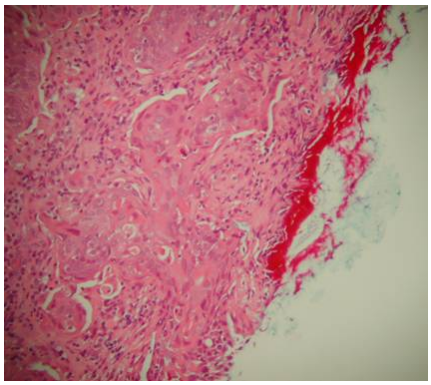

Figure 4: Abnormal histological features in right mucosal margin (150x)

FOR CITATION PURPOSES: *Upile T, Jerjes W, Mahil J, Hamdoon Z, Radhi H.* Microendoscopy: a clinical reality in intra-operative margin analysis of head & neck. Head Neck Oncol. 2012 Sep 9;4(2):43.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Method (Cont.)

After suction clearance, the tip of the microendoscope is firmly applied to the pre-stained (0.1% Methylene blue) area of interest to obtain an occlusive contact and then moved for dynamic assessment of the lesion (Figure 5), its margins, the local tissue and the underlying mucosal vasculature and blood flow. The examination is found to be reproducible between operators. Staining is repeated if further assessment is required (Figures 6 and 7).

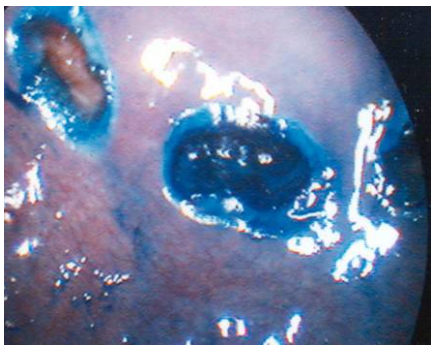

Figure 5: Stained margin

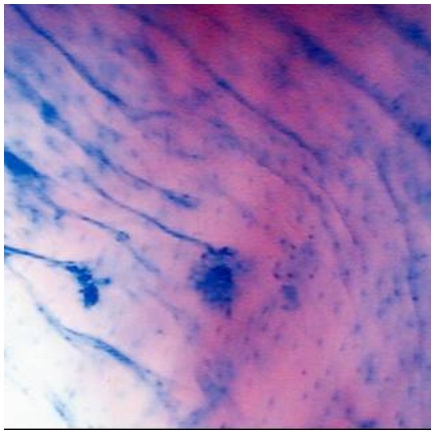

Figure 6: Normal mucosal microendoscopic image of previous lower margin, corresponding to histology (150x)

The examination can be performed at operation *in situ* and repeated on the excised *ex vivo* specimen (with the clinical margin) to assess involvement of the macroscopically clear tissue. A large area of mucosa can be quickly assessed and accurately sampled.

Table 1	Diagnostic criteria assessed to determine abnormality
Cellular level	Cell: seen in longitudinal tissue plane rather than transverse/depth
	Cellular morphology, expected histology
	Nuclear staining pattern- orientation, size, shape, limits
	Nucleolar staining morphological pattern, orientation
Tissue Level	Cell-cell regularity
	Extracellular matrix- homogenous, heterogeneous
	Margin- discrete, blurred
	Underlying cells, micro-vessel density

Adverse microendoscopic predictors of abnormality (Table 1) would include the presence of a large nuclear to cytoplasmic ratio in cells together with evident bizarre shaped nuclear staining and the presence of numerous mitotic figures per field, punctate staining of cells and the heaping up of bizarre sheets of stained cells suggesting rapid cell turnover. There may even be pseudo-tissue borders in unexpected sites e.g. not at traditional squamo-columnar junction sites. By using these criteria, it should be possible to obtain a high sensitivity and specificity in determining abnormal mucosa compared to formal histopathological examination.

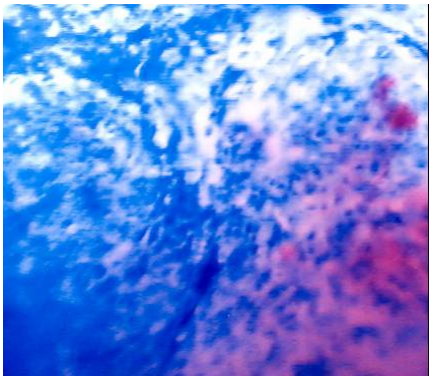

Figure 7: Abnormal microendoscopic features in right mucosal margin, corresponding to histology (150x)

Discussion

Microendoscopic assessment of mucosal lesions can act to inform the surgical decision regarding the nature of the cellular characteristics of a lesion and its edge, aiding biopsy yield and the adequacy of the laser excision margin.

Forty patients undergoing resection of oral squamous cell carcinoma were recruited by Upile et al. The surgical margin was first marked by the operator followed by microendoscopic assessment. Biopsies were taken from areas suggestive of close or positive margins after microendoscopic examination. These histological samples were later scrutinized formally and the resection margins revisited accordingly when necessary. Using the microendoscope, Upile et al. reported their experience in the determination of surgical margins at operation and later comparison with frozen section and paraffin section margins "gold standard". They were able to obtain a sensitivity of 95% and a specificity of 90%. Inter-observer Kappa scores comparing the microendoscope with formal histological analysis of normal and abnormal mucosa were 0.85[9].

The same group used microendoscopic technique for microvascular monitoring of free autologous jejunal flap by the direct visualisation of the flow of erythrocytes through the capillary vasculature on both the mucosal and serosal surfaces. Blood flow was seen to be pulsatile, with individual erythrocytes visible in the capillaries. The best view was obtained when the scope was focussed directly on the capillary rather than the graft surface. The view of the unstained mucosal surface was bland apart from the fine capillary loops which were seen to fill with each pulsatile event. The microendoscopic examination of the

FOR CITATION PURPOSES: *Upile T, Jerjes W, Mahil J, Hamdoon Z, Radhi H.* Microendoscopy: a clinical reality in intra-operative margin analysis of head & neck lesions. Head Neck Oncol. 2012 Sep 9;4(2):43.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Head & Neck Oncology
Open Access

Methodology

Discussion (Cont.)

serosal surface revealed much larger calibre vessels with obvious blood flow[10].

A fundamental knowledge of regional histology and pathology is obviously assumed on part of the clinician. We feel there must be a high degree of cooperation between the surgeon and histopathologist. Perhaps initially correlating microendoscopic still or video footage with formal histology slides until confidence is gained. This correlative exercise should continue until a measure of proficiency is gained in topographical histopathologic interpretation. Local specificity and sensitivity in detecting areas of abnormality should be ascertained to develop skills of having a raised index of suspicion of when to biopsy.

The potential pitfalls of the technique are that it is a new skill and the equipment is still relatively expensive (although costs could be offset against the saving of potentially residual disease and failed grafts). Costs can be reduced by the local manufacture of the various adaptors necessary to connect the microendoscope to existing camera systems (Laparoscopic, Cystoscopic, Otolaryngological).

The disadvantages of this technique are that the microendoscope does not provide three dimensional information to provide depth of invasion however this may be ascertained by a series of 'MOHR'S'-like 2 dimensional analyses using the microendoscope. A degree of depth field information may be obtained by varying the focus to allow visualisation of underlying structures cells or blood vessels. Despite this, cellular details can be determined up to tens of cell layers deep depending on illumination. Intra-wound interpretation using the microendoscope for assessing deep margins is difficult and requires a thorough understanding of the topographical histopathological appearance of the area especially when observing the oblique cuts made with excision. It also requires the surgeon to have an intimate knowledge of the histology of the area on which they are operating.

The technique of microendoscopy will no doubt be improved by advances in optical systems, illumination, recording and image processing. The microendoscope has a range of exciting applications in Oral Medicine, Otolaryngology, Oral & Maxillofacial Surgery and Plastic Surgery; however the main advance with the scope is that we have more informed choice of the state of the *in situ* epithelial margin taken when excising squamous cell carcinoma. We found that the microendoscopic examination of lesions had great utility in the excision of mucosal lesions. This combined with the recent advancement of vital or immunologically tagged antibody targeted staining will advance the type of surgical margin we take from the standard clinically visible margin to that of a histopathological margin during surgery[9,11].

Conclusion

We describe the clinical application of this once orphan technology and provided a practical guide to its everyday use by the surgeon both in the clinical and surgical fields. The advantages of this technique is that a large area of mucosa can be sampled whose histomorphological changes can be visualised in real time allowing the operator to make important informed decisions with regards the intra-operative resection margin at the time of the operation. Shrinkage, thermal and orientation artifacts are also avoided. For the technique to be successful the surgeon, pathologist and cytopathologist will need to be familiar with the subtleties of microendoscopy and this will require close collaboration between specialties.

References

1. Upile T, Fisher C, Jerjes W, El Maaytah M, Searle A, Archer D, Michael's L, Rhys-Evans P, Hopper C, Howard D, Wright A. The Uncertainty of the Surgical Margin in the treatment of Head & Neck Cancer. Oral Oncol. 2007 Apr;43(4):321-6.
2. Silverman S Jr, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow up study of 257 patients. Cancer 1984, 53:563–568.
3. Karabutul A, Reibel J, Therkildsen MH, Praetorius F, Nielsen HW, Dabelsteen E. Observer variability in the histological assessment of oral pre-malignant lesions. J Oral Pathol 1989, 67:282–285.
4. Silverman S Jr, Dillon WP. Diagnosis. In Edited by Siverman S Jr. Churchill Livingstone: Oral cancer; 1982:21–31.
5. Clinel A, Oselladore M, Insacco E, Minucci D. The accuracy of colposcopically directed biopsy in the diagnosis of cervical intraepithelial neoplasia. Eur J Gynaec Oncol 1990, 6:433–437.
6. Gynther G, Rozell B, Heimdahl A. Direct oral microscopy and its value in diagnosing mucosal lesions. Oral Surg Oral Med Oral Pathol 2000, 90(2):164–170.
7. Tsai MR, Shieh DB, Lou PJ, Lin CF, Sun CK. Characterization of oral squamous cell carcinoma based on higher-harmonic generation microscopy. J Biophotonics. 2012 May;5(5-6):415-24.
8. Andrea M, Dias O, Santos. Contact endoscopy during micro-laryngeal surgeryA new technique for the endoscopic examination of the larynx. Ann Otol Rhinol Laryngol 1995, 104:333–339.
9. Upile T, Fisher C, Jerjes W, El Maaytah M, Singh S, Sudhoff H, Searle A, Archer D, Michaels L, Hopper C, Rhys-Evans P, Howard D, Wright A. Recent technological developments: in situ histopathological interrogation of surgical tissues and resection margins. Head & Face Medicine 2007, 3(1):13.
10. Upile T, Jerjes W, El Maaytah M, Hopper C, Searle A, Wright A. Direct microvascular monitoring of a free autologous jejunal flap using microendoscopy: a case report. BMC Ear, Nose and Throat Disorders 2006, 6:14.
11. Ann Otol Rhinol Laryngol. 1995; 104:333–9. Andrea M, Dias O. Contact endoscopy of the Upper Aerodigestive Tract. In Endo-press, Tuttlingen; 2001. pp. 10–16.

FOR CITATION PURPOSES: *Upile T, Jerjes W, Mahil J, Hamdoon Z, Radhi H.* Microendoscopy: a clinical reality in intra-operative margin analysis of head & neck lesions. Head Neck Oncol. 2012 Sep 9;4(2):43.

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.