



SCREENING LARYNGEAL DYSPLASIA USING GYNECOLOGICAL PRINCIPLES

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ABSTRACT

OBJECTIVE: Devise a pilot study to develop a non-invasive laryngeal screening technique that could be used to detect lesions not readily visible to the unaided eye via a laryngoscope. Secondly, extend the validity of the screening technique that is already accepted by the FDA for gynecological and oral cavity dysplasia.

METHODS: Pilot study examining fresh laryngectomy specimens using chemiluminescent staining techniques was undertaken from July – December 2010 at our institution. Specimens were painted with an acetic acid solution and examined under lumenoscopic lighting. Normal appearing and aceto-white areas were biopsied. Results of HPV testing, cytology, and tissue analysis were compared.

RESULTS: Four (N=4) total laryngectomy specimens from patients aged 40 - 81 yrs. old (Mean = 57 yrs.) where examined with chemiluminescent staining techniques to detect dysplasia not readily visible. Preoperative cancer stages of these larynges ranged from T2 to T4. There were 3 males and 1 female, 75% were smokers, and 50% used alcohol. High Risk HPV serotype 16 or 18 were found in 75% of specimens. Dilute acetic acid solution was successful in identifying dysplasia or invasive cancer not visible. HPV 16 stained more intensely than HPV 18. Cytobrush alone was not adequate to detect cells with HPV effect.

CONCLUSION: There seems to be a role for a dilute acetic acid solution in the detection of laryngeal dysplasia. HPV-16 subtypes seem to produce a more dramatic staining pattern. High risk subtypes found in 75% of laryngectomy specimens. Future research is needed to optimize the described technique.

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INTRODUCTION

Laryngeal cancer is often a debilitating and fatal disease that is mostly diagnosed after a lesion can be visibly detected on endoscopic examination by an otolaryngologist. Classically, one associates smoking and chronic alcohol consumption with a higher incidence of squamous cell carcinoma (SCC) of the larynx. Increasingly, those who have no smoking or alcohol exposure are being diagnosed with SCC. Presumably, this is related to human papillomavirus (HPV) exposure. The association of HPV and cervical cancer is well known. The diagnosis of cervical cancer has been effectively prevented and treated with the advent of immunizations, Pap-smears, and colposcopy. Currently, no screening techniques exist to diagnose or monitor laryngeal dysplasia or carcinoma-in-situ prior to being visible on laryngoscopic examination. The purpose of this study is try to adapt techniques of screening for SCC from gynecology and dentistry, and apply it to the laryngeal cancer.

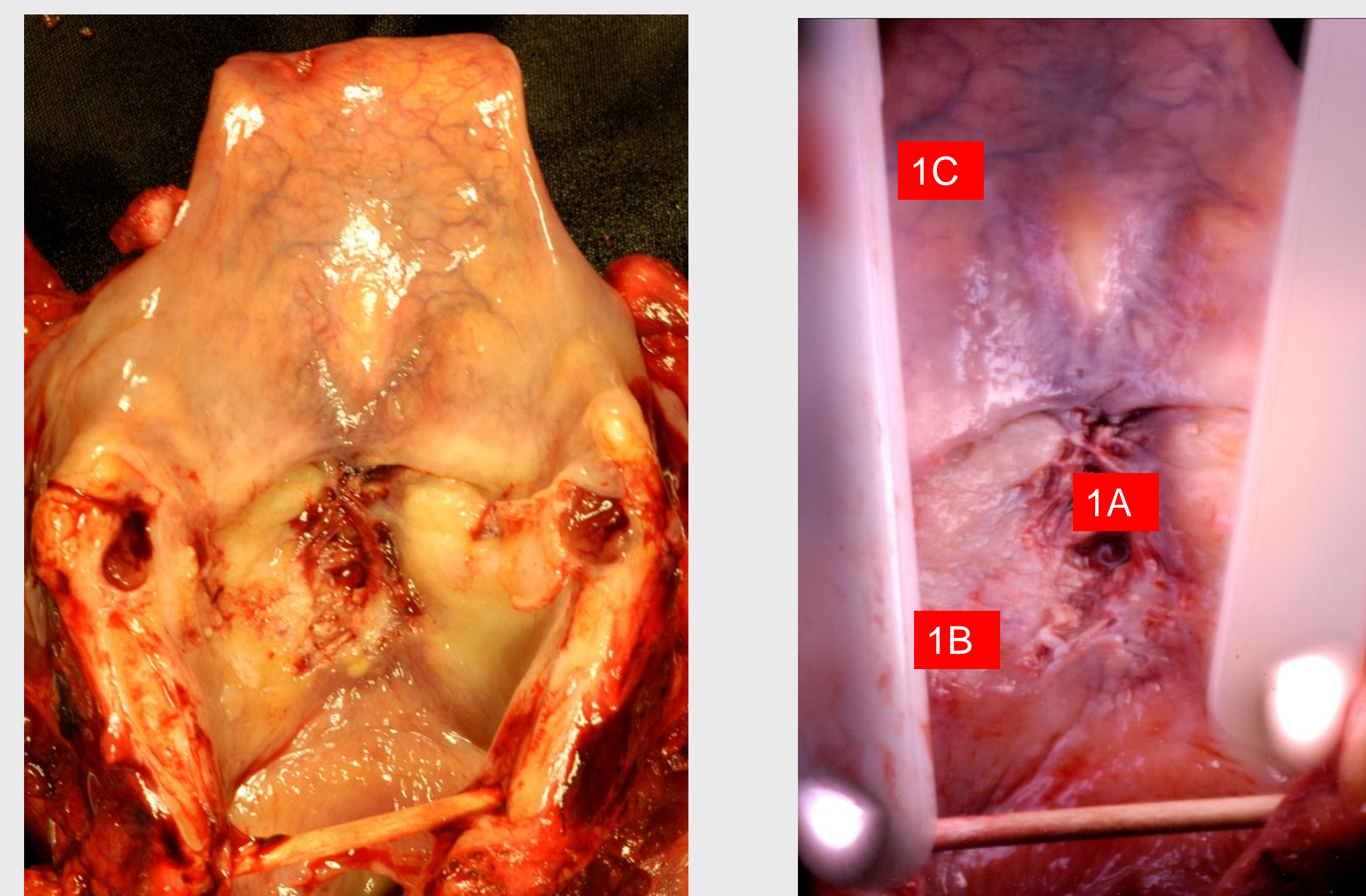
METHODS AND MATERIALS

The scope of this pilot study was to test the feasibility of a screening technique that could potentially be employed in the clinical setting. This study consisted of using a cytobrush and chemiluminescent staining techniques on fresh total laryngectomy specimens. Initially, the cytobrush was brushed around the mucosal surface of the larynx and sent for cytology. Any obvious areas of disease was avoided. The specimen was then painted with a dilute 1% acetic acid solution and examined under lumenoscopic lighting. Any areas that stained white were biopsied and sent for tissue analysis. Areas that did not stain white were also be biopsied for comparison. The visible tumor was biopsied for HPV PCR analysis and compared with final histology.

RESULTS

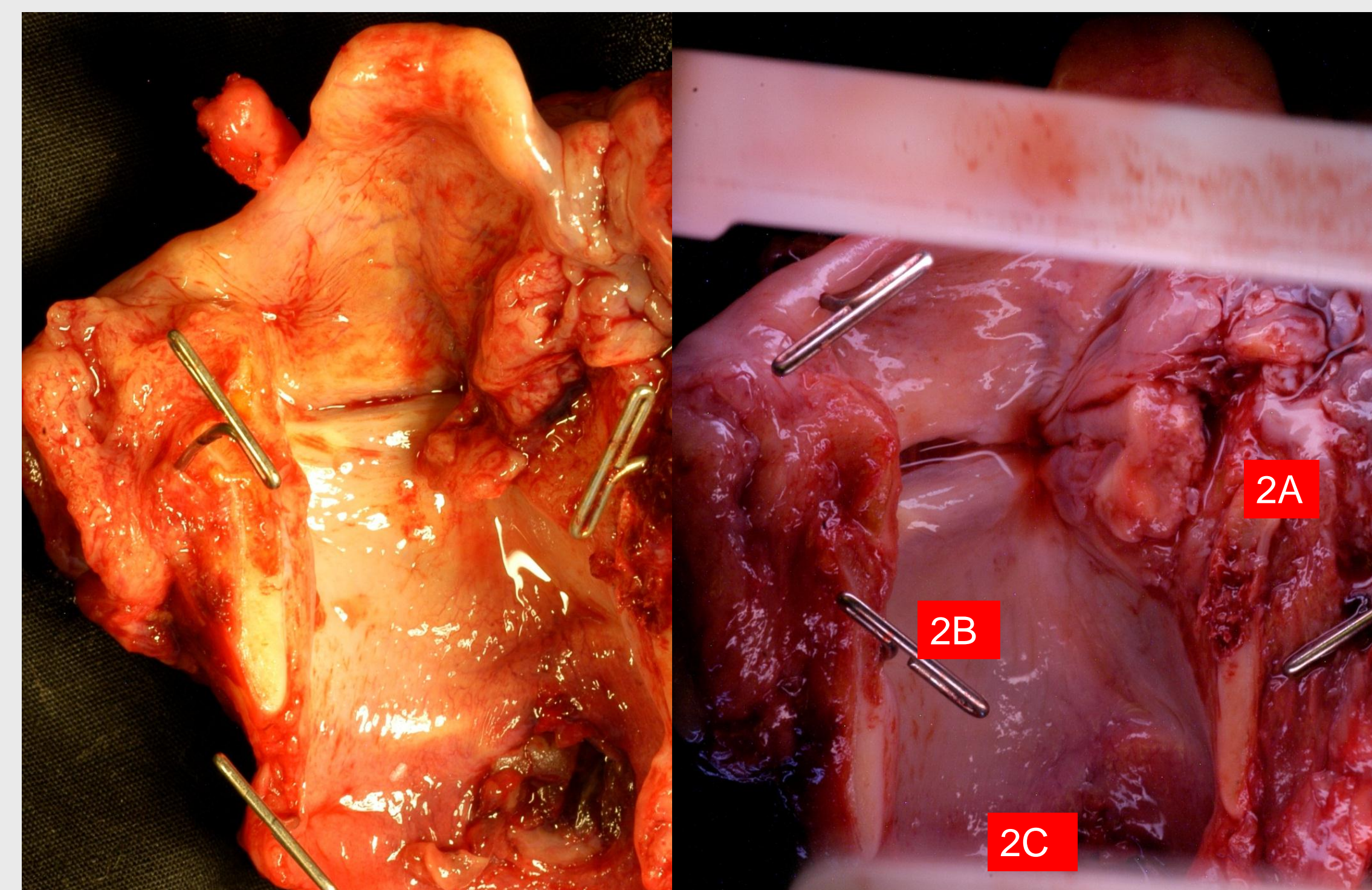
Pilot study examining fresh laryngectomy specimens using chemiluminescent staining techniques was undertaken from July – December 2010, Four (N=4) total laryngectomy specimens from patients aged 40 - 81 yrs. old (Mean = 57 yrs.) where examined with chemiluminescent staining techniques to detect dysplasia not readily visible. Preoperative cancer stages of these larynges ranged from T2 to T4. There were 3 males and 1 female, 75% were smokers, and 50% used alcohol. High Risk HPV serotype 16 or 18 were found in 75% of specimens. Dilute acetic acid solution was successful in identifying dysplasia or invasive cancer not visible. HPV 16 stained more intensely than HPV 18. Cytobrush alone was not adequate to detect cells with HPV effect

RESULTS



Specimen	Type 16	Type 18	Type 6	Type 11	Type 33	Type 45	Type 31	Type 39
1A - Tumor	Yes	Yes	-	-	-	-	-	-
1B - Acetowhite	Yes	-	-	-	-	-	-	-
1C - Normal	-	-	-	-	-	-	-	-

Figure 1: HPV(+) Specimen with Dramatic Staining Pattern, Histology, and PCR Results



Specimen	Type 16	Type 18	Type 6	Type 11	Type 33	Type 45	Type 31	Type 39
2A - Tumor	-	-	-	-	-	-	-	-
2B - Acetowhite	-	-	-	-	-	-	-	-
2C - Normal	-	-	-	-	-	-	-	-

Figure 2: HPV(-) Specimen with Non-Specific Staining, Histology, and PCR Results

Patient	Description of Cytology
1	Mild to Moderate Dysplasia with HPV effect.
2	Atypical squamous metaplastic cell

Figure 3: Summary of Cytobrush Results

DISCUSSION

Although, Toluene staining of the larynx has been extensively studied, very little data has been reported on laryngeal staining with acetic acid. Soh et al. (1996) used a 4% acetic solution on 6 patients followed by direct laryngoscopy and biopsy. They concluded that the entire specimen turned white and that acetic acid staining of the larynx was not useful. Classically, gynecologists use a 4% solution on the vaginal walls and 3% on the cervix. In more recent years a 1% acetic acid solution has been approved by the FDA for screening of dysplasia in the oral cavity by dentists. This led this study to choose an 1% acetic acid solution in the staining of larynges.

HPV(+) larynges stain much more dramatically than those specimens which were HPV (-). Figure 1 and 2 represent the most and least dramatic staining examples of the 4 patients in the study. The letters in the respective figures designate biopsy locations. The cytobrush data in Figure 3 suggests that is may too, be a useful tool in detecting abnormal cells in the larynx. Interestingly, 3 out of 4 (75%) patients in this study were confirmed to HPV (+). Although, the sample is small with this pilot study, it raises the suspicion of a much higher prevalence of HPV (+) SCC of the larynx than the frequently reported 25% by multiple authors.

CONCLUSIONS

There seems to be a role for a dilute acetic acid solution in the detection of laryngeal dysplasia. HPV-16 subtypes seem to produce a more dramatic staining pattern. High risk subtypes found in 75% of laryngectomy specimens. Future research is needed to optimize the described technique

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