

Ploidy Analysis on Brush Biopsy Samples. Peng W¹, Sirois DA², Sacks PG², Kerr AR².

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Introduction: Oral squamous cell carcinomas are the result of an accumulation of genetic alterations, many of which are present in premalignant lesions. DNA ploidy analysis of tissue samples from oral premalignant lesions can detect cells demonstrating a deviation from the normal diploid complement of chromosomes (“aneuploidy”) and which may be a predictive marker for malignant transformation. **Objective:** The objective of this pilot study was to assess the feasibility of DNA ploidy analysis of cytological specimens procured by oral brush biopsy in a research cohort of subjects with oral cancer or premalignant lesions, and to compare the performance of ploidy analysis versus the brush biopsy (CDX Labs, Suffern, NY), and toluidine blue staining (Zila, Phoenix AZ) against matched tissue histopathology (“gold standard”). **Methods:** As part of an ongoing oral cancer study, subjects recruited with oral epithelial lesions at risk for cancer underwent a number of tests including a brush biopsy, whereupon a cytological sample was plated and fixed on a glass slide. Instead of discarding the brushes, the tips were retained, and placed in a fixative (SedFix, Surgipath Medical Inc, Richmond, IL). 15 residual cell samples (5 squamous cell carcinomas, 5 oral dysplasias and selected sites from 5 healthy controls) were processed as follows: brushes were agitated to remove cells, samples were centrifuged, resuspended in SedFix, and shipped in deidentified vials to Perceptronix Medical Inc. Vancouver, Canada. Cell samples were then deposited on glass slides using a Thermo-Shandon Cytospin 4 centrifuge, underwent Feulgen-Thionin staining, and DNA ploidy analysis using an automated image cytometry system ClearCyte™. Galleries containing nuclei with a DNA index > 2.3 were reviewed by a cytopathologist, and a dichotomous outcome of DNA normal versus abnormal was rendered. **Results:** See Table 1. Compared to the “gold standard”, the accuracy of DNA ploidy analysis in detecting dysplasia or carcinoma in terms of sensitivity and specificity were 70 and 100% respectively. In this population of elevated risk individuals, the positive and negative predictive values were 100% and 62.5%. Combination of the 3 techniques provided 100% sensitivity and specificity. **Conclusion:** Despite small cell samples, this pilot suggests that DNA ploidy of cytological samples has utility as a diagnostic adjunct. Additional studies with larger numbers of subjects and larger cell samples are necessary to further ascertain the accuracy of DNA ploidy analysis and, longitudinal studies are needed to assess it as a marker to predict malignant transformation in premalignant lesions.

Table 1: Performance of DNA ploidy analysis compared to histopathology and other techniques

#	Histopath/control	Site	Ploidy	Brush Bx*	T. Blue**
1	Control	Lateral Tongue	-	-	n/a
2	Control	Ventral Tongue	-	-	n/a
3	Control	Floor of Mouth	-	-	n/a
4	Control	Retromolar Trigone	-	-	n/a
5	Control	Soft Palate	-	-	n/a
6	Dysplasia (Mild)	Floor of Mouth	-	+/-	-
7	Dysplasia (Mild)	Floor of Mouth	+	+/-	-
8	Dysplasia (Moderate)	Lip/Labial Mucosa	-	+/-	-
9	Dysplasia (Moderate)	Buccal Mucosa	+	+/-	+
10	Dysplasia (Moderate)	Ventral Tongue	+	inc	+
11	Squamous Cell Carcinoma	Hard Palate/Alv. Ridge	-	+/-	+
12	Squamous Cell Carcinoma	Floor of Mouth	+	+	+
13	Squamous Cell Carcinoma	Lateral Tongue	+	+/-	+
14	Squamous Cell Carcinoma	Floor of Mouth	+	+	+
15	Squamous Cell Carcinoma	Soft Palate	+	+/-	+

*+, -, +/-, inc = positive, benign, atypical, incomplete specimen; **n/a,+,- = not performed, intense blue, no stain.

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