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Original Article

Histologic and immunohistochemical evaluation of mirror image biopsies in oral squamous cell carcinoma

Vineet Gupta [a,*], Pratibha Ramani [b]

[a] Department Oral Pathology and Microbiology, Shree Bankey Bihari Dental College, NH 24, GZB, U.P., India
[b] Department Oral Pathology and Microbiology, Saveetha Dental College, Chennai, Tamil Nadu, India

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ABSTRACT

Background and objective: The concept of field cancerization has frequently been quoted to explain the occurrence of multiple primary cancers in the head and neck region and recurrence following complete excision of the original tumor. The main objective is to study the occurrence of field changes in mirror image biopsy in relation to histopathological changes in the oral mucosa among oral cancer patients using hematoxylin and eosin and to study the expression pattern of cytokeratin, Ki-67 and p53 in oral squamous cell carcinoma and mirror image biopsy.
Materials and methods: A pilot study of 15 patients clinically diagnosed with carcinoma lesion and their corresponding mirror image sites were taken and stained using immunohistochemistry method for the expression of cytokeratin, Ki-67 and p53.
Results: Primary tumors showed strong positive staining for cytokeratin throughout both the epithelium and malignant epithelial islands but absence of staining for Ki-67 and p53.
Conclusion: CK may be useful in predicting epithelial differentiation and Ki-67 and p53 act as weak indicators of malignant disease progression in oral tissues.

1. Introduction

The concept of field cancerization, proposed by P.J.Thomson,[1] has frequently been quoted to explain the occurrence of multiple primary cancers in the head and neck region and recurrence following complete excision of the original tumor. A second primary tumor that is detected within 3–6 months of detection of index tumor is called as "synchronous lesion". Tumor that arise after 6 months are called as "metachronous tumors". 41–49% of secondary primary tumors (SPTs) are synchronous tumors.

Recent molecular genetic studies provide evidence that the majority of, if not all, head and neck squamous cell carcinomas (HNSCCs) develop within a contiguous field of preneoplastic cells.[2] According to Brakhuis et al.,[3] cells of a field show genetic alterations associated with the process of carcinogenesis. A subclone in a field gives rise to an invasive carcinoma. An important implication of this knowledge is that, after surgery of the initial carcinoma, part of the field may remain in the patient. A field with preneoplastic cells that share genetic alterations with cells of the excised tumor has been detected in the resection margins of at least 25% of patients, indicating that this frequently occurs. Fields can be much larger than the actual carcinoma, sometimes having a diameter >7 cm. When a field remains after resection of the tumor, the risk for another carcinoma, designated as a second field tumor (SFT), is considerably greater.

Boffeta et al.[4,5] found altered telomeres and unbalanced allelic loci present in both tumors and surrounding histologically normal tissues at distances at least 1 cm from the visible tumor margins. Although the extent of these genetic changes decreases as a function of the distance from the visible tumor margin, unbalanced loci are conserved between the surrounding tissues and the tumors, implying cellular clonal evolution. This is in accordance with the concept of "*field cancerization*".

As very few literature is available on mirror image biopsies in patients with oral squamous cell carcinoma, the present study, a pilot study was designed to examine the changes that are occurring in the apparently (clinical) normal appearing mucosa opposite to carcinoma by using hematoxylin and eosin and immunohistochemical markers namely cytokeratin, Ki-67 and p53 for predicting the risk and detecting any changes that is suggestive of a strong possibility of development of OSCC.

2. Materials and methods

After obtaining institutional approval and obtaining informed consent from the patient, incisional biopsies from 15 patients

* Corresponding author. Tel.: +91 9971549459.
E-mail address: ving.30@gmail.com (V. Gupta).

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clinically diagnosed with oral squamous cell carcinoma lesion and their corresponding mirror image site were taken in the Department of Oral Surgery, Saveetha Dental College and Hospitals, Chennai. Patients treated with chemotherapy or radiotherapy, with metastatic diseases and carcinoma occurring in the midline was excluded from the study. The blocks were sectioned and stained with hematoxylin and eosin. Subsequently, immunohistochemical staining for cytokeratin, Ki-67 and p53 (Biogenex, San Ramon and DAKO Corporation, Carpenterica, CA) was done.

The selected cases were further graded as well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma for the primary lesion of oral squamous cell carcinoma.

The mirror image biopsied tissue was analyzed histologically and any abnormal features including reactive change to chronic irritation, cellular atypia, dysplasia or malignancy recorded using standardized pathological criteria. Immunohistochemical staining of cytokeratin, Ki-67 and p53 was determined by three observers to eliminate inter observer bias. The grading was based on the intensity of the stain and the area of positive staining as follows:

−: Absence of staining.

+: Weak positive staining (cytokeratin)/average of 1–50 positive cells (p53 and Ki-67).

++: Moderate positive staining (cytokeratin)/average of 51–150 positive cells (p53 and Ki-67).

+++: Strong positive staining (cytokeratin)/average of >150 positive cells (p53 and Ki-67).

Location of immunohistochemical staining was observed as follows:

BL: Positive cell staining within basal layer of oral epithelium.

1/3: Positive cell staining with in lower third of oral epithelium.

2/3: Positive cell staining with in middle third of oral epithelium.

3/3: Positive cell staining with in upper third of oral epithelium.

Statistical analysis has been done by using Statistical Package for Social Science (SPSS package). Mean, median, standard deviation, chi-square test and Kruskal–Wallis test were used to test objectives in the study.

3. Results

Total of 30 biopsies, 15 each from oral squamous cell carcinoma and its corresponding mirror image site were selected. Mirror image biopsies (Figs. 1 and 2) for each case were assessed on the basis of hematoxylin and eosin staining and immunohistochemical staining. Primary tumors showed strong positive staining for cytokeratin throughout both the epithelium and malignant epithelial islands but absence of staining for Ki-67 and p53. For cytokeratin, out of 15 mirror image biopsies corresponding to OSCC, 7 cases showed strong positive staining and 8 cases showed moderate positive staining (Figs. 3 and 4). For Ki-67, out of

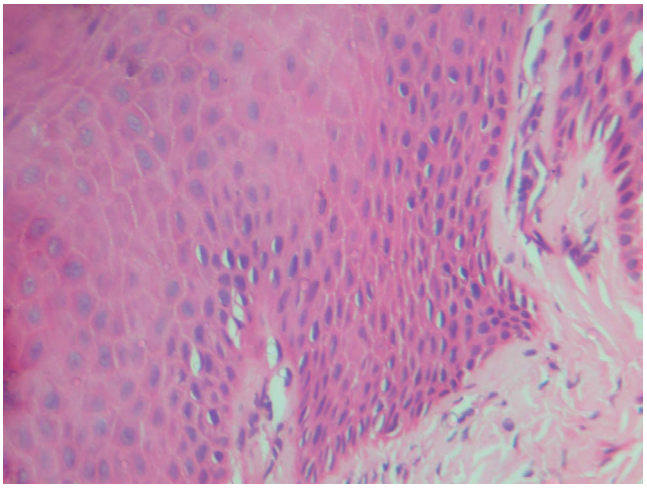

Fig. 1. Mild dysplasia (H&E) 40× mirror image biopsy.

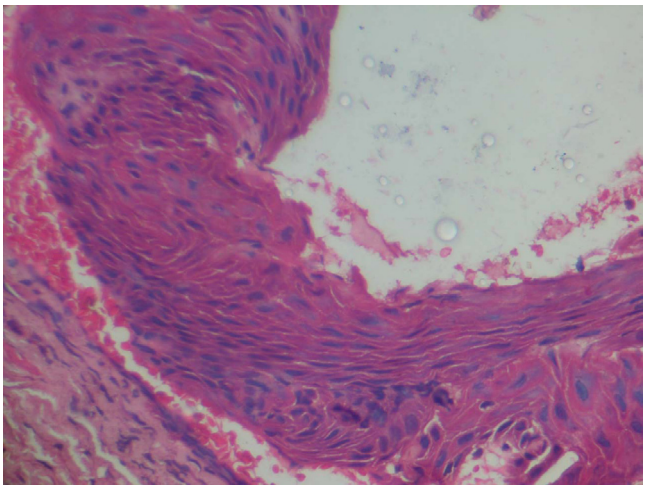

Fig. 2. Moderate dysplasia (H&E) 40× mirror image biopsy.

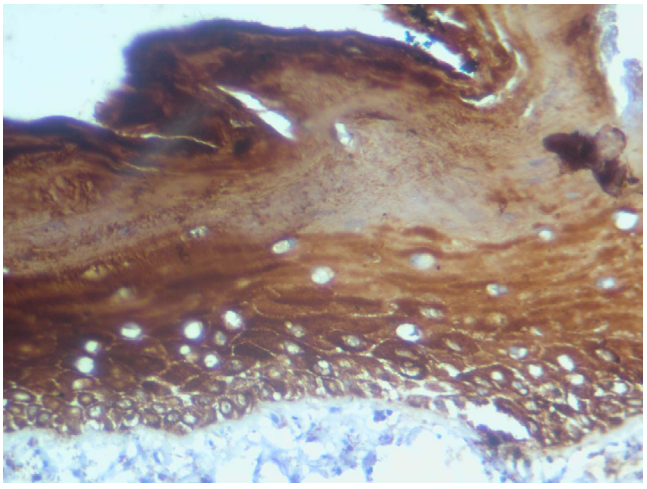

Fig. 3. Strong positivity for cytokeratin throughout the parakeratinized stratified squamous epithelium (mirror image biopsy) 40×.

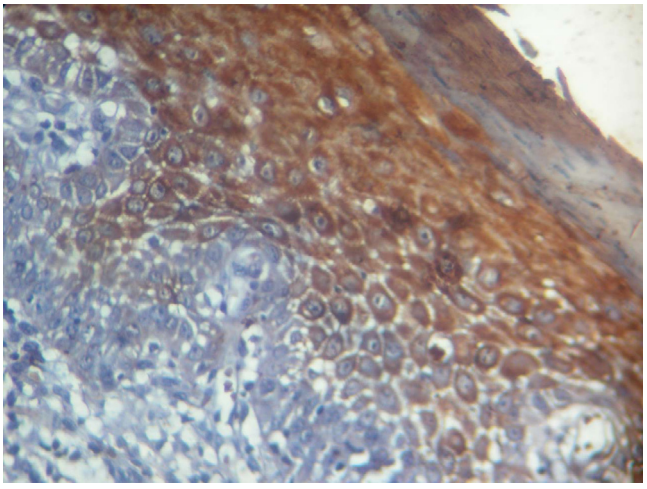

Fig. 4. Moderate positivity for cytokeratin with negative basal cell layer and presence of chronic inflammatory cells (mirror image biopsy) 40×.

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