Evidence based demonstration of the concept of ‘field cancerization’ by p53 expression in mirror image biopsies of patients with oral squamous cell carcinoma – an immunohistochemical study

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Abstract
Objective: The main rationale for treatment failure and death of the patients with oral squamous cell carcinoma (OSCC) is loco-regional recurrence, development of second primary tumor (SPT) and metastasis, which could be well explained by concept of field cancerization. Identification of patients at high risk for development of SPT is an important part of research for cancer management. This study was designed keeping this aspect in mind and utilizing the increased expression of p53 as an indicator of existence of altered fields in mirror image biopsies of OSCC patients. Design: Forty clinically diagnosed oral cancer patients were included in the study. Biopsy tissue samples from clinically diagnosed oral cancer patients (Group A) and the mirror image, clinically normal looking mucosa at corresponding contralateral anatomical site (Group B) were studied for histopathological evaluation and p53 immunoeexpression. Results: Tissue alterations were observed in Groups A and B. There was statistically significant (χ²-square value – 126.6, p=0.001) difference in grades of epithelial dysplasia and p53 immunoeexpression in Group B. Spearman’s Rank Correlation Coefficient shows non-significant positive correlation between epithelial dysplasia and p53 (r=0.28, p=0.05) in Group B. Conclusions: Evidence of presence of field cancerization, evaluated by histopathological alterations and enhanced p53 expression was observed in mirror image biopsies of OSCC patients. This could predict the altered state of oral mucosa secondary to carcinogen exposure. The realization of a genetically altered field as a cancer risk factor provides a new paradigm. It would be prudent to keep these patients under close observation and to advice them chemotherapeutic regimes.

Keywords: oral squamous cell carcinoma, mirror image biopsies, p53, field cancerization.

Introduction
Head and neck cancer includes cancers originating in the oral cavity, the oropharynx, the hypopharynx, and the larynx [1]. Oral squamous cell carcinoma (OSCC) is the sixth most common cancer for both sexes in the general population, and the third most common cancer in developing nations with high incidence of mortality and morbidity rate [2]. OSCC is a heterogenous disease with complex molecular abnormalities. It may arise from a precancerous progenitor followed by outgrowth of clonal populations associated with cumulative genetic alterations and phenotypic progression to invasive malignancy. During this process of carcinogenesis, principal etiological factors like tobacco, in its various forms (chewing, sniff, smoking), and alcohol are responsible for genetic alterations, which result in inactivation of multiple tumor suppressor genes and activation of proto-oncogenes [3].

Survival of OSCC patients is related to the site and pathological stage of the primary tumor as well as the histological grade and pattern of invasion [4]. Despite advances in treatment, the five-year relative survival rate remains less than 50% and has not improved much over the past three decades [5]. The prognosis of OSCC is adversely influenced by the development of second primary tumor (SPT). The incidence rate of SPT is 10–35%, depending on both the location of first primary and age of the patient [6]. The term SPT may be used in connotation with ‘field cancerization’, the term and concept first put forth by Slaughter et al. in 1953 [7]. The principle of field cancerization encompasses the carcinogen induced early genetic changes in the mucosa of oral cavity, which leads to development of precancereous lesions and further multifocal tumors. Further, it is frequently used to explain the persistence of abnormal tissue after surgical removal of tumor, which may result in local recurrence and or SPT. The concept of field cancerization in context to SPT is further elaborated by many researchers [8–12]. The phenotypic expression of field cancerization could be attributed to independent molecular events affecting multiple cells independently in the whole tissue region subsequent to carcinogen exposure or to molecular events occurring in a single progenitor with widespread expansion or lateral spread across the mucosa. Two types of migration may be involved that is migration of tumor cells via saliva – micrometastasis or intraepithelial migration of the progeny of the initially transformed cells throughout the mucosa. There is also a possibility that the tumor itself exercises a regional effect on the oral mucosa [13].
The realization of a genetically altered field as a cancer risk factor provides a new paradigm. The existence of field with genetically altered cells is a distinct biological stage with continued risk for SPT [5]. Identification of patients at high risk for development of SPT is an important part of management of cancer. Advances in molecular biology have made it possible to investigate tumor growth and field at molecular level with some degree of accuracy. The process of carcinogenesis might involve not only increased cell proliferation but also decreased cell apoptosis and failure to undergo apoptosis is a major contributor to the uncontrolled growth of tumors [14]. The carcinogens might induce a “fingerprint”-like pattern of mutations at the p53 gene. The p53 gene inactivation by means of carcinogen induced mutations, deletions, and binding to viral proteins has been demonstrated in oral cancers [15].

Although a visible lesion precedes the development of majority of OSCC, sometimes it may disguise itself and precancerous or cancerous changes are not always be discernible clinically or histologically. However, several studies support the fact that clinically normal appearing mucosa of OSCC patients may be genetically altered due to disordered epithelial maturation and differentiation resultant to carcinogen exposure [5, 16, 17]. The genetic changes present in normal appearing cells can be used for identification and recruitment of individuals at increased risk of developing SPT for primary chemoprevention.

Keeping this in mind, this study was aimed at demonstration of the concept of field cancerization by expression of structural and molecular alterations in mirror image biopsies of patients with OSCC.

Materials and Methods

Study subjects

The present study was carried out at the Department of Oral Pathology and Microbiology, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences (DU), Sawangi (Meghe), Wardha, Maharashtra, India. The duration of the study was six months, from 01/05/2012 to 30/10/12. The outpatients who reported to the Department of Oral Pathology and Microbiology presenting with unilateral, clinically diagnosed oral cancer, irrespective of their age, gender and race were enrolled in the study. Other criteria set for inclusion were that: all patients presenting with oral cancer of less than 12 months duration and with the habit of betel nut chewing, tobacco chewing, smoking, and alcohol consumption. The exclusion criteria were: patients with extensive midline tumors, bilateral tumors, and metastatic disease in the oral cavity, previously treated OSCC, clinical evidence of widespread precancerous lesions including contralateral site, history of another prior cancerous disease in the body.

Study protocol

The study was approved by the Ethical Committee of the Datta Meghe Institute of Medical Sciences (DU). A pre-enrolment screening questionnaire was used to record the history regarding the patients complaints associated with the lesion, duration of its presence, information regarding habits, and patient’s general health. After obtaining written informed consent, 40 patients were enrolled in the study. Detailed intraoral examination was done including both the pathological lesion and the contralateral mirror image biopsy site from clinically normal looking mucosa. The pathological lesions were designated as Group A lesions, whereas contralateral mirror image biopsy site from clinically normal looking mucosa were designated as Group B lesions. Under all aseptic precautions and surgical preparation, a clean and defined elliptical incision was given producing a “V”-shaped wedge that includes both the lesion and healthy margin and the soft tissue specimen was excised. Biopsy from mirror image site was performed from clinically normal looking mucosa at corresponding contralateral anatomical site. Biopsy samples were fixed in 10% formalin and forwarded for conventional tissue processing in the histopathology laboratory. Two sections of 4-μm thickness were obtained from both the groups (Groups A and B) tissue. One section of each was stained with routine Hematoxylin–Eosin (HE) stain for evaluation of the histopathological changes. The other section from both the groups (Groups A and B) tissue was subjected to immunohistochemistry (IHC), which was carried out by using monoclonal mouse anti-human antibody against p53 antibody.

Histopathological evaluation

Histopathological evaluation of the clinically diagnosed oral cancer and mirror image tissue sections (Groups A and B) was done after staining with HE, by two oral pathologists in double-blinded manner. Cases with discordant results underwent a consensus review with a third oral pathologist. The inclusion criteria used for the clinically diagnosed oral cancer tissue sections was in accordance with the histological typing of cancer and precancer of oral mucosa [18]. The cases, which fulfill the definition of squamous cell carcinoma that is, “A malignant epithelial neoplasm exhibiting squamous differentiation characterized by the formation of keratin and the presence of intercellular bridges”, were included in the study. The histopathological grading of oral cancer is generally based on the subjective assessment of the degree of keratinization, cellular and nuclear pleomorphism, mitotic activity and lymphocytic infiltration. Thus, OSCC was graded as well, moderately, and poorly differentiated OSCC (WDOSCC, MDOSCC, and PDOSCC, respectively) and verrucous carcinoma [18]. The mirror image tissue sections, which is clinically normal looking, were analyzed histopathologically for abnormal features of hyperkeratosis with or without, mild, moderate, and severe epithelial dysplasia (dysplastic changes affecting the lower third of the epithelium, up to two-thirds of the epithelium, or greater than two-thirds of the epithelium, respectively) or carcinoma in situ (CIS), which shows dysplastic changes affecting entire thickness of the epithelium [19]. Subsequently, the patients were referred to the oncology clinic for further clinical management.

Immunohistochemical method for the detection of p53 antigen

For immunohistochemistry, universal immunoenzyme polymer method was employed. The tissue sections from Groups A and B were deparaffinized with xylene and hydrated. Antigen retrieval for p53 was carried out by
heating tissue sections in microwave oven for 10 minutes in 0.01 M sodium citrate buffer (pH 6.0) and bench cooled for 20 minutes, and again the same cycle was repeated once. Endogenous peroxidase activity was blocked by incubating the section with 3% H2O2 in methanol for 20 minutes. To prevent non-specific reactions, sections were incubated with 10% serum for 10 minutes. p53 antibody (concentrated (1:100 dilution) anti-human p53 protein clone – DO-7, code M7001, Dako, Denmark) was incubated at room temperature in humidifying chamber for 90 minutes. From archives of Department of Oral Pathology, sections of normal oral mucosa samples showing p53 expression positivity were used as a positive control. One section from each positive control was used as the negative control by omitting the primary antibody and by incubating with serum. After the primary antibody and antigen reaction, the sections were rinsed in Tris Buffer Saline (TBS) three times for 5 minutes each. The HRP Labeled Polymer Anti-mouse (Dako EnVision+ System HRP Labeled Polymer Anti-mouse, product code SM 802, Dako North America Inc.) was incubated at room temperature in humidifying chamber for 30 minutes. After the TBS washing three times for 5 minutes each, freshly prepared substrate/chromogen solution of 3,3’-Diaminobenzidine (DAB) in provided buffer (mixing 25 μL of concentrated DAB in 1 mL of substrate buffer) was used to visualize the antigen–antibody reaction. Finally, the sections were counterstained in Mayer’s Hematoxylin.

**Immunohistochemical evaluation**

Two oral pathologists evaluated immunohistochemically-stained tissues independently in double-blinded manner. Differences in interpretation were resolved by consensus review with a third oral pathologist. Cells were considered positive for the p53 when they showed nuclear staining (brown color), regardless of intensity of staining, in basal, parabasal and above cell layers, in clusters or discrete manner (Figure 1).

A semi-quantitative analysis was performed on immunohistochemically-stained sections. Scores 1–4 (1: 1–10%, 2: 11–30%, 3: 31–50%, 4: 51–100% of positive cells) were calculated after inspection of multiple neoplastic fields at higher magnification [2] (Figure 2).

**Statistical analysis**

Histopathological evaluation and p53 immunoreactivity were analyzed. Differences in p53 immunoreactivity and histopathological evaluation between and within Groups A and B were done by using descriptive and inferential statistics using chi-square test and Spearman’s Rank Correlation Coefficient. Statistical significance was set at p<0.05. All statistical analyses were performed using SPSS 17.0 ver. 8 software package.

**Results**

We evaluated 80 tissue samples of 40 OSCC patients, to assess the possible correlation of p53 immunohistochemical expression with histopathological observations amongst Groups A and B. The demographic and clinicopathological characteristics of the patients are described in Table 1.

Regarding histopathological evaluation, in Groups A and B, there was presence of tissue alterations. These were in the form of verrucous carcinoma, WDOSCC and MDOSCC in Group A. There were no cases of PDOSCC included in the study. In Group B, the tissue alterations were in the form of hyperkeratosis with or without mild epithelial dysplasia (Table 2).

We observed correlation in histopathological alterations between Groups A and B. On correlation in histopathological evaluation between Groups A and B, 12.5%, 35% and 5% patients of MDOSCC, WDOSCC and verrucous carcinoma, respectively, shows hyperkeratosis with mild epithelial dysplasia in the mirror image biopsies. Whereas only 7.5%, and 2.5% patients of WDOSCC and verrucous carcinoma, respectively, shows hyperkeratosis without epithelial dysplasia (Table 3).

There was enhanced p53 immunoexpression in Group A as well as in Group B (Table 4).
Table 1 – Demographic and clinicopathological characteristics of the patients

<table>
<thead>
<tr>
<th>Demographic and clinicopathological characteristics</th>
<th>No. of patients</th>
<th>Percentage [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>34</td>
<td>85</td>
</tr>
<tr>
<td>Females</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age – male [years]</td>
<td>51.81±10.31</td>
<td></td>
</tr>
<tr>
<td>Mean age – females [years]</td>
<td>53±10.58</td>
<td></td>
</tr>
<tr>
<td>Mean age – total patients [years]</td>
<td>52±10.21</td>
<td></td>
</tr>
<tr>
<td>TNM staging of pathological lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td>Stage II</td>
<td>17</td>
<td>42.5</td>
</tr>
<tr>
<td>Stage III</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Stage IV</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Site of pathological lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left buccal mucosa</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>Right buccal mucosa</td>
<td>14</td>
<td>35</td>
</tr>
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</table>

Table 2 – Comparison of histopathological diagnosis amongst Groups A and B

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>Group A</th>
<th>Group B</th>
<th>χ²-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>9</td>
<td>22.5</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Hyperkeratosis with mild epithelial dysplasia</td>
<td>13</td>
<td>32.5</td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td>VC</td>
<td>7</td>
<td>17.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WDOSCC</td>
<td>26</td>
<td>65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MDOSCC</td>
<td>7</td>
<td>17.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VC: Verrucous carcinoma; WDOSCC: Well-differentiated oral squamous cell carcinoma; MDOSCC: Moderately differentiated oral squamous cell carcinoma; sig.: Significant.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 – Correlation in histopathological evaluation amongst Groups A and B

<table>
<thead>
<tr>
<th>Correlation in histopathological alterations</th>
<th>Group B / No. of cases (percentage)</th>
<th>Total / No. of cases (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Hyperkeratosis</td>
</tr>
<tr>
<td>VC</td>
<td>4 (10%)</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>MDOSCC</td>
<td>2 (5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>WDOSCC</td>
<td>9 (22.5%)</td>
<td>3 (7.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (37.5%)</td>
<td>4 (10%)</td>
</tr>
</tbody>
</table>

VC: Verrucous carcinoma; MDOSCC: Moderately differentiated oral squamous cell carcinoma; WDOSCC: Well-differentiated oral squamous cell carcinoma.

Table 4 – Comparison of p53 immunoexpression amongst Groups A and B

<table>
<thead>
<tr>
<th>p53</th>
<th>Group A</th>
<th>Group B</th>
<th>χ²-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Score 1</td>
<td>9</td>
<td>22.5</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>Score 2</td>
<td>13</td>
<td>32.5</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td>Score 3</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Score 4</td>
<td>8</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

χ²-value = 18.25, p-value = 0.0004 (sig.)

Score 0: Absence of p53 expression; Score 1: <10% cells positive for p53 expression; Score 2: 1–30% cells positive for p53 expression; Score 3: 31–50% cells positive for p53 expression; Score 4: >51% cells positive for p53 expression; sig.: Significant.

We further assessed Group B for comparison in alterations at structural level by evaluating grades of epithelial dysplasia and at molecular level by evaluating p53 immunoexpression (Figure 3). Alterations at molecular level as evaluated by p53 immunoexpression in clinically normal looking mucosa showed alterations in 85% patients (Figure 3). At structural level, we observed 62.5% patients showed tissue alterations, which were in the form of hyperkeratosis and hyperkeratosis with mild epithelial dysplasia. 37.5% of patients showed no changes. Increase in p53 immunoexpression was reflected as increase in degree of epithelial dysplasia in 92% patients (out of 62.5% patients with epithelial dysplasia).

![Figure 3 – Comparison of epithelial dysplasia with p53 immunoexpression in Group B. Score 0: Absence of p53 expression; Score 1: <10% cells positive for p53 expression; Score 2: 1–30% cells positive for p53 expression; Score 3: 31–50% cells positive for p53 expression; Score 4: >51% cells positive for p53 expression.](image-url)
The other finding of interest in this study is that 73.33% (out of 37.5% patients without epithelial dysplasia) patients showed increase in p53 immunoexpression in clinically normal looking mucosa, without presence of epithelial dysplasia on histopathological evaluation (Figures 3 and 4).

Figure 4 – Correlation of epithelial dysplasia with p53 immunoexpression in Group B.

Discussion

The main rationale for treatment failure and death of the patients with OSCC is loco-regional recurrence, development of SPT and metastasis. The loco-regional recurrence and development of SPT could be well explained by the concept of field cancerization. The oral epithelium is inevitably exposed to environmental substances, including carcinogens. The accumulation of genetic alterations due to carcinogen exposure can create a large area of genetically altered precancerous fields, which forms the basis for the expanding fields in carcinogenesis. The need of the hour is to identify the specific biological markers, which will help identify an altered state of tissue that is field. The altered state of tissue is rarely clinically apparent and it is difficult for a clinician to identify the presence of field based on clinical examination only. This is supported by the finding that as many as 50% of OSCC arise from apparently clinically normal mucosa [20].

As suggested by current theories of carcinogenesis, the transition from normal oral epithelium to oral dysplasia and cancer results from accumulated genetic and epigenetic alterations due to exposure to carcinogens [21]. However, the precise nature of genetic or structural alterations preceding cancerous change is unclear. To support the concept of field cancerization in routine histological tissues various molecular techniques like microsatellite analysis [8–10, 22, 23], electron microscopy [24], exfoliative cytology [25, 26], PCR (polymerase chain reaction) [27], IHC (immunohistochemistry) [13, 28–31] and frozen IHC [32] have been used which also confirm the presence of cellular alterations.

The first molecular indicator of disturbance in cell division is enhanced expression of p53 protein. Mutations in the p53 gene frequently result in the synthesis of a mutant protein that has a longer half-life than that of the wild-type protein, causing an accumulation of altered p53 protein within the nuclei of affected cells and thereby increases its expression [33]. Loss of function of p53 tumor suppressor gene results in uncontrolled cell division and progressive genomic instability. Therefore, over-expression of p53 may be indicative of an increased risk of SPT in apparently unaffected mucosa of OSCC patients [34]. Focal p53 positivity was detected more often in normal mucosa adjacent to cancer than healthy control epithelium from HNSCC (head and neck squamous cell carcinoma) patients [21, 29, 35]. The frequency of p53 positive cells gradually increases as oral epithelium progresses from normal to hyperplasia to dysplasia to carcinoma [21]. This study was designed keeping this aspect in mind and utilizing the increased expression of p53 as an indicator of existence of altered fields in mirror image biopsies of patients with OSCC.

The concept of field cancerization in oral cavity is based on the fact that carcinogens via the saliva affect the entire mucosa. As entire mucosa is affected by exposure to carcinogens, it can be assumed that genetic aberrations would not be limited to one particular area but an entire exposed mucosa. The proof to this concept was sought by evaluating mirror image biopsies from clinically normal appearing oral mucosa from contralateral sites of established OSCC patients. This evaluation was carried out both by histopathology and p53 immunoexpression. The present study results demonstrated histological evidence of high incidence of tissue instability in the mirror image biopsies of clinically normal looking oral mucosa. The immunohistochemical expression of p53 protein could be because of altered p53 protein metabolism, which is either caused by mutation or altered turnover of the wild p53 protein. This indicates that the molecular alteration is an early event in the process of field cancerization and it occurs even before there is histological evidence in the form of epithelial dysplasia. All these alterations are suggestive of the concept of field cancerization that, there is comprehensive change in entire mucosa exposed to carcinogen. The proof lies in architectural, cellular as well as molecular level changes seen in the mirror image biopsies.

Our results are in agreement with Thomson [35], who demonstrated histologically abnormal tissue, varied from reactive change/cellular atypia, dysplasia, CIS, or microinvasive SCC from mirror image biopsies of OSCC patients. Partridge et al. reported that two-third of diagnosed cases of oral precancer subsequently shown development of invasive cancer at sites distinct from their original dysplasia [9].

To predict the recurrence and development of second primary tumors in HNSCC, Shin et al. [31] examined p53 protein expression in primary HNSCC. They believed that p53 protein has the prognostic significance for identifying individuals at high risk of developing a recurrence of primary disease and also SPT. Nees et al. [34] noted the expression of mutated p53 protein in histologically normal appearing tumor distant epithelial cells in HNSCC patients. These findings were confirmed by Waridel et al. [29], who observed the presence of p53 mutations in histologically normal mucosa of aero digestive cancer patients. They believed that this observation coincides with the likelihood of development of SPT. To check the predictive power of p53, Homann et al. [32] investigated p53 expression in tumor distant epithelia and in the corresponding primary tumors by frozen IHC. They observed that p53 over expression was specifically associated with SPT but not with recurrences. Our findings seem to find agreement with all of these studies. However, our observations were
not in agreement with that of Bongers et al. [30] and Ogden et al. [36] who found that p53 overexpression in normal mucosa of oral cancer patients, could not predict the likelihood of SPT.

The field cancerization concept has always emphasized the fact that, carcinogens do not act in isolation on limited fields but conversely act over a large generalized area. Due to interaction with additional factors, certain fields get converted into malignancy. This, however, does not rule out the fact that a SPT could develop in a geographically independent location later. The increased expression of p53 in mirror image tissue is seen in geographically altered cells supporting the concept of field cancerization. Thompson evaluated mirror image biopsies of OSCC patients, to quantify the incidence and type of field change in oral mucosa. This is to our knowledge is the first study providing evidence of p53 overexpression in clinically normal looking mucosa (mirror image) of OSCC patients, which could be predictable to an altered state of oral mucosa secondary to carcinogen exposure. The realization of a genetically altered field as a cancer risk factor provides a new paradigm. We are in agreement with van Oijen & Slootweg [6] that the field changes appear to be induced by continuous carcinogenic influence rather than field cancerization due to migrated transformed cells. The development of field at contralateral site of OSCC may be due to carcinogen exposure rather than migrated transformed cells.

\section*{Conclusions}

Presence of field cancerization, in mirror image biopsies of OSCC patients could predict the altered state of oral mucosa secondary to carcinogenesis exposure and shows increased predisposition or susceptibility of the mucosa to progression to second primary tumor.

\section*{Conflict of interests}

The authors declare that they have no conflict of interests.

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\section*{References}


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