DETERMINATION OF KI-67 EXPRESSION IN ORAL LEUKOPLAKIA IN SNUFF USERS AND NON-USERS IN KHYBER PAKHTUNKHWA PROVINCE OF PAKISTAN.

Tehmina Naushin1, Muhammad Mumtaz Khan2, Sajjad Ahmed3, Mahmood-ul-Hassan4, Fatima Iqbal5, Nasiha Bashir6, Abbas Saleem Khan7

ABSTRACT... Objectives: The aim of the article was to evaluate Ki-67 expression in oral leukoplakia in snuff users and non-users. Study Design: Descriptive Cross sectional study. Setting: At different hospitals of Khyber Pakhtunkhwa province. Period: From August 2016 – March 2017. Material & Methods: Clinically diagnosed and histopathologically confirmed cases of oral leukoplakia in snuff users and non-users 30 cases each were immunohistochemically examined and percentage expression of nuclear Ki-67 was evaluated by a semi quantitative method in basal, parabasal and suprabasal layers of the lining epithelium. Results: The relationship of expression of Ki-67 in parabasal and suprabasal layers of affected epithelium in snuff users was found to be statistically significant with a p value of 0.007 and 0.002 respectively. Conclusion: The present study concludes that the proliferative index is high in snuff users as compared to non-users which can be a factor for malignant transformation.

Key words: Dysplasia, Ki-67 Antigen, Leukoplakia Oral, Tobacco Smokeless.

INTRODUCTION

Oral leukoplakia is defined according to WHO as a “white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer”1. The worldwide prevalence of oral leukoplakia is approximately 0.2 - 11.7%.2 There is marked geographic variation in prevalence even in the different regions of the same country.3 In a Karachi based Pakistani study the estimated prevalence of oral leukoplakia in Pakistan was found to be 5% and another study conducted in Islamabad reported 7% cases of oral leukoplakia.4,5

It is a potentially malignant disorder of oral cavity which carries a high risk of conversion into frank malignancy.6,7 The global malignant transformation rate of oral leukoplakia ranges from 0.13% to 34% depending on the site.8

Although etiology of oral leukoplakia is unknown however most common associated factors are tobacco use9-11, mechanical trauma, electro galvanic stimulation produced by different restorative metals, Ultraviolet radiations12, and Human Papilloma Virus 16 and 18.10,13

There is strong evidence of carcinogenic effect of tobacco and its smokeless forms on oral mucosa.10 Of smokeless tobacco users 40% develop oral leukoplakia as compared to 1.5% of non-users.14 Tobacco use is very common in Pakistan. Smokeless form called Paan is common in Karachi and certain areas of Punjab whereas oral snuff or naswar is widely used in Khyber Pakhtunkhwa and Baluchistan.15,16

Ki-67 is a human nuclear protein. Its expression is strictly associated with proliferation of the
cell. Therefore it can be reliably used as a marker of proliferative activity. 17,18,19

Usually the epithelial dysplasia evaluated by microscopy determines the potential for malignant transformation. 20 However, there is probability of false negativity in this method. Ki-67 being a marker of proliferative activity can specifically predict the conversion of oral leukoplakia into malignancy. 21

The aim of this article is to evaluate and compare Ki-67 expression and proliferative activity in basal, parabasal and suprabasal layers of epithelium in oral leukoplakia of both snuff users and non-users in Khyber Pakhtunkhwa province of Pakistan.

MATERIALS AND METHODS

It was a multicenter study which was conducted at different hospitals of Khyber Pakhtunkhwa province from August 2016 –March 2017.

The study comprised of 60 clinically diagnosed cases of oral leukoplakia which were divided into two groups. Group A consisted of 30 cases with a history of snuff use and Group B consisted of 30 cases without any history of snuff use. Cases were independently confirmed by two histopathologists. The relevant data of all the cases was entered in a predesigned proforma.

Immunohistochemistry for Ki-67 was carried out with Dako Envision™ FLEX detection system (Monoclonal Ki-67 Antibody). Tonsillar tissue was taken as positive control for Ki-67. Epithelial cells stained with clear brown color irrespective of staining intensity were taken as positive.

Ki-67 positivity was assessed using a scoring system proposed by Kannan. 22,23,24 The details of scoring interpretation are as follows: Score 0 = 0 - 5%
Score 2 = 6 - 25%
Score 4 = 26 - 60%
Score 6 = 61 - 99%

Statistical analysis was carried out using Statistical Package for Social Sciences version 19. Frequency and percentages were calculated for categorical variables, i.e., level of expression of Ki-67 in basal, parabasal and suprabasal layers of epithelium. Mann Whitney test was used for comparing percentages of Ki-67 expression in snuff users and non-users. Probability value of less than or equal to 0.05 (P value ≤ 0.05) was considered statistically significant.

RESULTS

Among these 60 cases of oral leukoplakia 43 (71.7%) were males and 17 (28.3%) were females. Male to female ratio was 2.5:1.

All 60 cases of oral leukoplakia were evaluated for expression of Ki-67 marker in epithelial layers with IHC. Nuclear expression in oral leukoplakia in snuff users and non-users is shown in Tables-III and IV respectively.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Histopathological Features</th>
<th>Snuff Users</th>
<th>Non-users</th>
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<tr>
<td></td>
<td></td>
<td>No of Cases (%)</td>
<td>No of Cases (%)</td>
</tr>
<tr>
<td>1</td>
<td>Dysplasia</td>
<td>6(20)</td>
<td>6(20)</td>
</tr>
<tr>
<td>2</td>
<td>Hyperplasia</td>
<td>3(10)</td>
<td>11(36.7)</td>
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<tr>
<td>3</td>
<td>Hyperkeratosis</td>
<td>5(16.7)</td>
<td>7(23.3)</td>
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Table-II. Distribution of most common histopathological features in oral leukoplakia in snuff users and non-users.
Relationship of expression of Ki-67 in snuff users and non-users in parabasal and suprabasal was significant with a p value 0.007 and 0.002 respectively.

**DISCUSSION**

In our study oral leukoplakia was more common in males (71.7%) than in females (28.3%). This finding is consistent with the international study conducted by Nair et al.\(^25\) In the present study 20% cases showed histopathological features of dysplasia in oral leukoplakia which is in contrast with an Indian study by Kumar et al. with 52% cases of dysplasia.\(^26\) However, work by Marne et al., showed no dysplasia in snuff induced oral leukoplakia. The marked difference in the results of these studies from different parts of the world may be due to the composition of snuff which varies from country to country.\(^27\)

Clinical identification of oral leukoplakia with risk of malignant transformation is a challenge for clinicians.\(^26\) On histological examination malignant transformation begins as a mildly dysplastic lesion which may or may not progress to full thickness dysplasia (carcinoma in situ).\(^28\) So early detection of abnormal changes is of utmost importance and helps to reduce morbidity and mortality by adopting the right approach to management of the patient.\(^29\) For this purpose different immunological markers are used for assessment of cellular proliferation which includes PCNA and Ki-67. Among these markers Ki-67 is more commonly used\(^23\) because its nuclear expression in specific period of cell cycle (G1 and M phases) makes it more reliable as an indicator of the level of mitotic activity. Moreover it has a short half-life so it produces less residual staining in cells which have passed through proliferative stage.\(^17\)
Most of the proliferation takes place in basal layer of epithelium while in rest of the layers maturation of cells takes place without proliferation. Therefore any proliferative activity above the basal layer carries an alarming sign. In snuff users the mean of percentages of Ki-67 expression in basal layer was 60.93±30.6 and in non-users it was found to be 57.10±35.7. Relationship of Ki-67 expression in basal layer in snuff users and non-users was statistically not significant (p value 0.79). It means that it was expressed in almost the same percentage in snuff users and non-users in basal layer with a cut off value ranging from 25-30%.

The mean of percentages of Ki-67 expression in parabasal layer in snuff users was 64.9±19.7 and in non-users it was 51.03±22.0. Relationship of expression of Ki-67 in snuff users and non-snuff users was significant (p value .007) which revealed that it is highly expressed in parabasal layer in snuff users than non-users.

The mean of percentages of Ki-67 expression in suprabasal layer in snuff users was 20.4±21.0 and in non-users it was 6.23±10.7. Relationship of Ki-67 expression in snuff users and non-users was statistically significant (p value 0.002), showing that there is a marked difference of its expression in suprabasal layer in snuff users.

In our study hyperkeratosis was found more in non-snuff users (23.3%) than snuff users (16.7%). Several authors tried to correlate hyperkeratosis with OL but they did not associate it with the use of snuff. Similarly, international literature regarding immunohistochemical expression of Ki-67 in snuff users in basal, parabasal and suprabasal layers for comparison with this study could not be found on extensive search. Though snuff is used worldwide but the methods of manufacturing and application of snuff in oral cavity is diverse in different parts of the world and even in various regions in the same country.

**CONCLUSION**

Ki-67 expression was specifically found in parabasal and suprabasal layers in snuff users indicating an increased proliferative activity, so chances of malignant transformation are more in snuff users than in non-users.

**REFERENCES**


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