“Comparative assessment of cellular proliferative potential in oral lichen planus and oral lichenoid lesions by quantitative analysis of nucleolar organizer regions”

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Abstract

Background: The potential for malignant change in oral lichen planus (OLP) is still a matter of controversy. Adding to the persisting controversy, recently there is evidence that oral lichenoid lesions (OLL) may also possess a malignant potential. Increase in cellular proliferation rate enhances the chances that cells may undergo mutations during mitosis which in turn could result in a malignant phenotype.

Aims: The present study is aimed at comparing the proliferative rate of OLP and OLL based on quantitative argyrophilic nucleolar organizer region (AgNOR) analysis.

Materials and Methods: The study consisted of the use of 25 archival paraffin-embedded blocks of histopathologically diagnosed cases of OLP (n = 14) and OLL (n = 11). Sections were then stained using an AgNOR technique. Group mean AgNOR values were derived and independent student t-test was applied for statistical analysis.

Results: The mean AgNOR value in OLP group was 2.49 ± 0.91 and the same in OLL group was 2.31±0.43. There was no statistically significant difference noted between the two groups with a probability value of 0.531 (P ≥ 0.05).

Conclusion: On AgNOR analysis, OLP and OLL behave in a similar manner in terms of cellular proliferation. Accordingly, either OLP or OLL may have a similar predisposition to undergo malignant transformation.

Introduction

Oral lichen planus (OLP), a fairly common chronic mucocutaneous disease, can be attributed to a cell mediated immune response.[1] It may occur in either of the six clinical forms: Reticular, papular, plaque, erosive, atrophic, and bullous. Histologically, it is characterized by hyperkeratosis, basal cell layer liquefaction, and an intense subepithelial lymphocytic infiltrate causing effacement of the epithelial connective tissue interface.[2] Though OLP has been considered to have a potential for malignant change, the rate of malignant transformation is low and ranges from 0.04% to 1.74% with the highest rate noted in the erosive and erythematous forms.[3]

Clinicians may also be confronted with a group of lesions that may to some extent resemble OLP both clinically and histopathologically but which do not fulfill all the characteristic criteria used to identify an OLP lesion. These are referred to as oral lichenoid lesions (OLL).[3] Sometimes, these lesions may be related to a specific etiology as against OLP which does not have a definite cause.[3] Recently, there is evidence that few OLL lesions may also have potency for malignant transformation such as OLP.[4]

Assessment of the rate of cell proliferation can aid in gaining important information regarding the diagnosis and prognosis of a pathology since increase in proliferation capacity could signal the first indications of malignant transformation.[5] Studies on cell proliferation in OLP have reported an enhancement in the proliferation rate of epithelial cells in the basal layers and it has been speculated that this may be an important occurrence in the development of this lesion into cancer.[5]

The nucleolar organizer regions (NORs), which were first described by Heitz and McClintock, are loops of ribosomal DNA (rDNA) which encode for ribosomal RNA.[7] These regions can be visualized on staining with colloidal silver and the reaction products represent the argyrophilic nucleolar organizing regions called argyrophilic nucleolar organizer region (AgNORs).[8] Quantitative analysis of AgNORs provides information regarding the cellular activity and thus serves as a proliferation marker.[7] Increase in AgNOR count denotes a reduction in the duration of cell cycle and increase in the velocity of cell proliferation.[5]
Thus, the aim of the present study is to elucidate the information regarding the rate of cellular proliferation in OLP and OLL based on AgNOR analysis which can be helpful in identifying the potentiality for malignant transformation in these pathologies.

Materials and Methods

Materials obtained for the study consisted of 25 archival paraffin-embedded blocks which included 14 diagnosed cases of OLP (Group I) and 11 cases of OLL (Group II), all were accessioned by the Department of Oral and Maxillofacial Pathology, D.A. Pandu Memorial RV Dental College and Hospital, Bangalore. Categorization of OLP and OLL lesions was done using the modified WHO diagnostic criteria.[4] The study was approved by the Institutional Ethics Committee (IEC/IRBNo. 012/VO11/2013).

AgNOR staining procedure

Four-micron thick sections were made for each lesion. The prepared sections were deparaffinized in xylene and passed through decreasing grades of ethanol and then to double-distilled deionized water. The deparaffinized sections were then hydrated. After hydration, the sections were treated with silver colloidal solution (freshly prepared) for 55 min at room temperature. The solution consists of one part of 2% gelatin in 1% formic acid (by volume) and two parts of 50% (by volume) aqueous silver nitrate solution. A thoroughly dark environment was maintained throughout the reaction time. After which the sections were washed in running double distilled deionized water. Dehydration of the stained sections was done with increasing grades of ethanol. The sections were cleared and mounted.

AgNOR count

About 100 keratinocytes in the epithelium per case were examined using the ×100 oil immersion objective of the light microscope. AgNOR dots were counted using Image ProExpress software (Ver 6.0., Media Cybernetics). Intra-nucleolar and extra-nucleolar dots were counted. Wherever clusters of AgNOR were encountered, they were counted as a single AgNOR dot. The mean AgNOR values were obtained for each case. Mean AgNOR value for each group was also derived. Student’s independent t-test was applied for statistical analysis.

Results

After silver staining, nucleus exhibited a yellow-brown staining and AgNORs appeared as discrete round or oblong black dots in three patterns-intra-nucleolar, extra-nucleolar, and scattered in the nucleoplasm [Figure 1]. Mean AgNOR values of Groups I and II are tabulated in Tables 1 and 2, Figures 2 and 3 show AgNORs in OLP and OLL cases, respectively.

The mean AgNOR value in OLP group was 2.49 ± 0.91 and the same in OLL group was 2.31 ± 0.43 [Table 3]. Student t-test was applied for the statistical analysis and the P = 0.531 implying that there is no statistically significant difference between the mean AgNOR values in the studied groups. Thus, it can be inferred that OLP and OLL behave in a similar manner in terms of cellular proliferation. AgNORs were comparatively more in number in the lower third of the epithelium as against the upper layers in the OLP group while this differentiation was not seen in OLL group [Figure 4a and b].

Discussion

Over the years, OLP has been considered to be a disorder with a potential for malignant change (1); however, this potential in
Table 2: AgNOR value in OLL cases (Group-II)

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>Site</th>
<th>AgNOR value</th>
</tr>
</thead>
<tbody>
<tr>
<td>79/male</td>
<td>Gingiva 14,15</td>
<td>1.8</td>
</tr>
<tr>
<td>28/male</td>
<td>Buccal mucosa</td>
<td>2.85</td>
</tr>
<tr>
<td>29/male</td>
<td>Buccal mucosa</td>
<td>3.17</td>
</tr>
<tr>
<td>58/male</td>
<td>Buccal mucosa</td>
<td>2.19</td>
</tr>
<tr>
<td>38/male</td>
<td>Gingiva</td>
<td>1.96</td>
</tr>
<tr>
<td>43/female</td>
<td>Buccal mucosa</td>
<td>2.45</td>
</tr>
<tr>
<td>53/female</td>
<td>Buccal mucosa</td>
<td>2.63</td>
</tr>
<tr>
<td>43/male</td>
<td>Buccal mucosa</td>
<td>1.92</td>
</tr>
<tr>
<td>56/male</td>
<td>Gingiva</td>
<td>2.32</td>
</tr>
<tr>
<td>36/female</td>
<td>Buccal mucosa</td>
<td>2.14</td>
</tr>
<tr>
<td>32/male</td>
<td>Buccal mucosa</td>
<td>1.94</td>
</tr>
</tbody>
</table>

OLL: Oral lichenoid lesions, AgNOR: Argyrophilic nucleolar organizer region

Table 3: Mean AgNOR values and statistical analysis

<table>
<thead>
<tr>
<th>AgNOR values</th>
<th>OLP</th>
<th>OLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (%)</td>
<td>Number of cases (%)</td>
<td></td>
</tr>
<tr>
<td>1-2.0</td>
<td>3 (21.4)</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>2.1-3.0</td>
<td>8 (57.1)</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td>3.0-4.0</td>
<td>2 (14.3)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>1 (7.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>14 (100.0)</td>
<td>11 (100.0)</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>2.49±0.91</td>
<td>2.31±0.43</td>
</tr>
</tbody>
</table>

AgNOR values are statistically similar in two groups with t=0.637; P=0.531 (Student t-test). OLL: Oral lichenoid lesions, AgNOR: Argyrophilic nucleolar organizer region, OLP: Oral lichen planus, SD: Standard deviation

OLP is still a target of much debate. Adding to the persisting controversy, Van der Meij et al. concluded from their 5 year follow up study of 192 cases that OLL also possesses an increased risk for malignant transformation with estimated percentage being 0.71% per year. Fitzpatrick in an extensive literature review concluded that the potential for malignant risk in these two lesions in unclear and that further studies are needed to be carried out to assess their behavior.

It has been proposed that as the cell proliferation rate increases, the risk of cells suffering mutations during mitosis also increases, which in turn could in a malignant phenotype. The use of proliferative markers can aid in determining the potential a lesion has for malignant conversion. However, labeling indices such as Ki 67 and PCNA only measure the percentage of proliferating cells by giving information regarding the cells entering the cell cycle. On the other hand, AgNOR count helps to determine the rapidity of cell proliferation by providing information regarding the duration of the cell cycle. Quantitative estimation of AgNOR can throw light on this aspect in routinely processed tissue samples.

After silver staining, the AgNORs are recognized as black dots throughout the nucleolar area. In quantitative terms, the total number of AgNORs per nucleus suggests it to be an indicator of the proliferative activity of the cell.

In the present study, the mean AgNOR value in OLP was 2.49±0.91 which is similar to other cumulative published results combining both studies exclusively on OLP and studies on potentially malignant disorders.

The mean AgNOR value in OLL was 2.31 ± 0.43. To the best of our knowledge, there are no published studies on evaluating AgNOR in OLL, thus making this the first such study.

On comparing the mean AgNOR value in OLL and OLP, there was no statistical difference observed between them. Though there are studies dealing with cell proliferation in OLP, there is a paucity of studies comparing the same in OLP and OLL with only Acay having evaluated the two lesions using p53 and Ki67. Our findings are similar to his results where no statistically significant difference was found in the proliferative capacity.
of the two lesions. Hence, it can be elucidated that the rate of cellular proliferation is the same in both OLP and OLL. Since cell proliferation can be related to malignant transformation, it can thus be assumed that both the lesions might have similar potential for such a change.

Clinically and histopathologically, there are many overlapping features between these two lesions imparting difficulty in the definitive diagnosis of these pathologies. In the present study, as an additional overlapping feature between OLP and OLL, that of a similar cellular proliferation potential was noted.

The increased cell proliferation seen in OLP could be secondary to the epithelial damage caused by infiltrating lymphocytes. The liquefaction of the epithelial cells makes them more susceptible to genetic damage as the mucosal cells enter an “inflammation-carcinogenesis loop.” A similar damage to the basal cells is seen in OLL as well. In the present study, it was noted that AgNORs in OLP were more in number in the lower third of the epithelium as compared to the upper layers, while this locational differentiation was not seen in OLL cases. It can be speculated that this observation may be attributed to the distinct dense band of subepithelial inflammatory cells seen in OLP, wherein, in most of the OLL cases, the inflammation is scattered.

Since the present study is an exploratory attempt, the authors suggest an increase in the sample size to further establish the results with confidence. Apart from preliminary comparison of OLP and OLL, the study of proliferation markers in OLP cases with dysplasia and without dysplasia can also be evaluated and compared with OLL to probe more into the persisting controversy. Additionally, qualitative assessment of AgNOR can provide more information on the malignant potential of these lesions.

**Conclusion and Clinical Significance**

Since the mean AgNOR value did not show any statistically significant difference between the two groups, it is evident that both OLP and OLL behave in a similar manner in terms of cellular proliferation. Accordingly, either OLP or OLL could present a potential for malignant transformation. However, since the risk of malignant potential in these two lesions is still unclear, a need for thorough follow-up of the patients with these lesions exists.

**References**
