Expression of survivin and p53 in oral lichen planus, lichenoid reaction and lichenoid dysplasia: An immunohistochemical study

Shaini Basheer, PM Shameena1, S Sudha2, Sujatha Varma2, S Vidyanath3, Aniruddha Varekar4

Private Practitioner, Mysore, Karnataka, 1Department of Oral and Maxillofacial Pathology, Government Dental College, Thrissur, Kerala, 2Department of Oral and Maxillofacial Pathology, Government Dental College, Kozhikode, Kerala, 3Century International Institute of Dental Science And Research Centre, Kasargod, Kerala, 4Bharati Vidyapeeth Deemed University Dental College and Hospital, Sangli, Maharashtra, India

INTRODUCTION

Oral lichen planus (OLP), lichenoid reaction and lichenoid dysplasia are three lesions with close clinical and histological resemblances, yet differ in their etiology, pathogenesis and biological behavior.

Lichen planus (leichen; Greek, meaning “tree moss,” Planus; Latin meaning “flat”), first described by Erasmus in 1869 is a lesion characterized by the presence of flat-topped, shiny, violaceous papules on flexor surfaces of skin. It also affects mucosal surfaces and most frequently the oral mucosa.[1]
Lesions not compatible with Van der Meij’s modified WHO criteria (2003) for lichen planus are considered to be lichenoid lesions. These include mainly lichenoid reaction, lichenoid dysplasia and graft versus host reaction. The lichenoid lesions when induced by drugs, flavors or metallic restorations are called oral lichenoid reactions (OLR). These lesions tend to resolve once the inciting factor is removed (although this resolution is not universal). Epithelial maturational disturbance with cellular aberrations ranging from mild atypia to severe dysplasia in an otherwise lichen planus like lesion is recognized as a separate entity termed as oral lichenoid dysplasia (OLD).

The possible malignant transformation of OLP and related lesions has been the subject of controversy in ongoing research and existing scientific literature. The first reported case of carcinoma arising from OLP was by Hallopeau, in 1910, following which numerous cases have been reported. However, recent retrospective studies have shown that at least a part of the reported cases of OLP turning malignant was in fact, from other lichenoid lesions. Hence, the large difference in the malignant potentiality of OLP − 0%–12.5% may be due to the inclusion of OLD and OLR in their study groups. There are very few studies where the malignant potential of these three different entities has been studied together.

Apoptosis, also termed programmed cell death, is a key component in embryogenesis and tissue homeostasis, as well as in tumorigenesis. Besides the well-known pro- and anti-apoptotic Bcl-2 family proteins, another family of inhibitors of apoptosis proteins (IAPs) has recently been identified. Survivin protein (MW: 16.5 kDa) is the smallest of the known IAPs. It is a unique bi-functional protein that inhibits apoptosis by suppressing caspase-3 and caspase-7 and modulates the G2/M phase of the cell cycle through association with mitotic spindle microtubules. Mutation of survivin leads to loss of the antiapoptotic function, thus favoring the development of neoplastic clones. The role of survivin expression in the early steps of the carcinogenetic process has been studied in the uterine cervix, colon, skin and oral mucosa. Survivin is rarely expressed in normal epithelium, making it a reliable marker of dysplastic changes. Here, we aimed to evaluate the immunohistochemical expression of p53 and survivin in OLP and related lesions and hence predict the relative risk of malignant potential of these lesions.

MATERIALS AND METHODS

The study sample included 40 formalin-fixed paraffin-embedded tissue blocks retrieved from the archives of the Department of Oral Pathology of our Dental College. The study group comprised of 10 cases each of OLP, OLR and OLD. Strict criteria were followed in selecting the three lesions since they have considerable overlap in their clinical and histopathological features. Only those OLP cases from the archives which fulfilled the modified the WHO criteria (2003) were included in the study. For this clinical information was obtained from case history records of patients from the Department of Oral Medicine. Hematoxylin-eosin stained slides were reviewed by two pathologists to confirm the histologic diagnosis [Figure 1a]. The cases included as OLR in our study included only those cases which both clinically and histopathologically were consistent with a diagnosis of OLR. Clinically, either there was a positive local cause like

Figure 1: Photomicrographs of haematoxylin and eosin stained histological sections of (a) oral lichen planus, (b) oral lichenoid dysplasia, (c) oral lichenoid reaction (a,b,c, x100)
amalgam restoration or positive drug history associated with the appearance of the lesions. Histopathologically, poorly differentiated lower border to subepithelial inflammatory zone; the presence of plasma cells among the lymphocytes and perivascular infiltration were taken into consideration to be per McCartan and Lamey’s criteria [Figure 1c]. As for OLD, all diagnosis was on the purely histological basis. Lesions which clinically resembled OLP or leukoplakia, if on histopathological examination showed features of dysplasia in the epithelium, along with characteristic lichenoid features like a subepithelial band of inflammatory cells was diagnosed as OLD [Figure 1b].

Details of all patients’ age, gender, location, clinical form of lesions, and number of sites involved were recorded from previous medical and dental charts. Ten normal controls were biopsy specimens of oral mucosa of healthy individuals undergoing impacted tooth removal or preprosthetic surgery.

Immunohistochemistry
The immunohistochemical kit consisted of primary rabbit monoclonal anti-survivin (clone: EP2880Y, Biogenex Laboratories, USA) and primary mouse monoclonal anti-p53 antibody (clone-D07, Biogenex Laboratories, USA) and the Super Sensitive Polymer-HRP/DAB system (Biogenex Laboratories, USA) as the secondary antibody detection kit. OSCC tissues known to express p53 and survivin were used as an external positive control and immunostaining of OSCC sections by substituting primary antibodies with TBS were used as negative controls for each group.

Scoring and statistical analysis
For evaluation of p53 and survivin expression in the epithelium of OLP, OLR and OLD, the slides were examined under a compound microscope with eyepiece graticule at x400 magnification. Nuclei with fine to coarse brown staining, irrespective of staining intensity, were considered as positive for both p53 and survivin. At least 1000 epithelial cells in areas showing maximum reactivity for p53 and survivin were counted in OLP, OLR, OLD and normal mucosa, and the number of positive cells was expressed as a percentage of counted cells. Staining of <5% of counted cells were considered as negative. Positivity of lymphocytes was not evaluated. Two investigators performed the counting-first independently and where consensus was required, the specimens were reassessed using a double-headed light microscope.

According to the statistical characteristics of our investigated data, Chi-square and one-way ANOVA were used to compare the immunostaining results of p53 and survivin in OLP, OLR, OLD and normal mucosa. The correlation between p53 and survivin expression in the three lesions was further analyzed by Pearson’s correlation coefficient. The values of \( P < 0.05 \) were considered to be statistically significant. For all statistical analysis, Statistical Package for Social Science version 12 (SPSS Inc., Chicago, IL, USA) was used.

RESULTS
The expressions of p53 and survivin in OLP and related lesions are listed in Table 1. The expressions of p53 and survivin were both observed as nuclear staining of varying intensity [Figures 2 and 3, respectively]. Immunoreactivity for p53 was seen in 7 of 10 cases (70%) of OLD, 4 of 10 cases of OLP (40%) and 2 of 10 cases of OLR (20%). This difference in positive staining rate of p53 between the different entities was found to be statistically significant (\( P = 0.026 \)). The difference in mean p53 expression between the different entities was also statistically significant (\( P = 0.01 \)). In OLD and normal mucosal sections, p53 staining was confined to the basal layer of epithelium. On the other hand, OLP and OLR Table 1: Expression of p53 and survivin in oral lichen planus, oral lichenoid dysplasia, oral lichenoid reaction and normal oral mucosa

|                  | p53 expression, n (%) | Mean p53 score | Survivin expression, n (%) | Mean survivin score |
|------------------|-----------------------|----------------|---------------------------|--------------------|
| OLD              | 7 (70)                | 30.650         | 5 (50)                    | 19.60              |
| OLP              | 4 (40)                | 14.250         | 1 (10)                    | 2.75               |
| OLR              | 2 (20)                | 7.100          | 0                         | -                  |
| NOR              | 1 (10)                | 0.850          | 0                         | -                  |

OLD: Oral lichenoid dysplasia, OLP: Oral lichen planus, OLR: Oral lichenoid reaction, NOR: Normal oral mucosa

Figure 2: Immunohistochemical staining for p53. (a) oral lichen planus showing nuclear p53 expression in basal and parabasal layers of epithelium. (b) oral lichenoid dysplasia demonstrating strong nuclear p53 staining in the basal and suprabasal epithelial cells. (c) oral lichenoid reaction showing p53 staining limited to the basal layer alone (a,b,c, x100)
demonstrated positive staining in the basal layer and lower part of the spinous layer.

The positive staining rate of survivin was found to be significantly different between OLP, OLR, OLD and normal mucosa \( (P = 0.004) \). Furthermore, there was a significant increase \( (P = 0.003) \) in mean survivin expression in OLD (19.6\%) when compared to OLP, OLR and normal mucosa according to Bonferroni statistical analysis. Both OLD and OLP demonstrated positive survivin staining in the basal layer and lower half of spinous layer of the epithelium.

Of the ten cases of OLD, eight cases were clinically diagnosed with OLP and two as leukoplakia. Of the 8 cases of OLP, five and four cases showed positivity for p53 and survivin, respectively. Of the two cases where clinical diagnosis was given as leukoplakia, both cases were positive for p53 while survivin exhibited positivity in one of the cases.

The positive correlation was obtained between p53 and survivin expression in OLP and OLD using Pearson’s correlation coefficient [Table 4]. In all cases of OLP or OLD where survivin was positive, we observed that p53 was also positive.

**DISCUSSION**

Oral lichen planus, lichenoid reaction and lichenoid dysplasia are three lesions with close clinical and histological resemblances [Figure 1], yet differ in their etiology, pathogenesis and biological behavior. The studies pertaining to potentially malignant nature of OLP have been largely inconclusive or give contradictory results. A recent study of OLP patients by Bombeccari et al. (following the modified WHO criteria), reported an annual malignant transformation rate of 0.36\%.\[18\] As for OLR and OLD, there is a definite paucity of studies regarding their biologic behavior.

Resistance to apoptotic stimuli is frequently involved in cancer development and IAPs like survivin are believed to...
enable dysplastic cells to acquire this property. Recent studies have found survivin expression to be increased from epithelial dysplasia to OSCC with no expression in normal mucosa. The study may be considered the first study where survivin expression was evaluated in the three closely related lesions OLP, OLR and OLD. We found a significant difference in the expression of survivin in OLP, OLR, OLD and normal mucosa, with OLD exhibiting maximum expression compared to normal, OLP and OLR. The expression of survivin in OLD is in the range of the expression seen in other dysplastic lesions such as leukoplakia, OSMF and esophageal dysplasia. However, OLR did not show any survivin expression at all. There was no survivin expression in normal mucosa which was in agreement with previous studies. In contrast, Chaiyarit et al., have found an increase in survivin expression in normal mucosa compared to OLP, which they have attributed to confounding variables.

In our attempt to elucidate the difference in p53 expression between OLP, OLR and OLD, we found a statistically significant increase in expression of p53 in OLD compared to normal mucosa, OLP and OLR. Similar to survivin, this p53 expression in OLD is in agreement with the expression in other dysplastic lesions such as leukoplakia, OSMF and esophageal dysplasia. p53 protein has fundamental importance in maintaining the genome integrity because it allows the action of DNA repair mechanisms and the removal of damaged cells through the apoptosis process. In fact, several studies suggested that p53 mutations are essential for the initial steps of oral carcinogenesis.

The expression of p53 protein in 40% of OLP cases in our study is in accordance with the previous monograph. Although not statistically significant this p53 protein expression in OLP was observed to be more than OLR and a significantly less expression compared to OLD. There was a positive correlation between p53 and survivin expression in our study. Survivin is a downstream gene in the p53 pathway, and we observed that in all cases where

| Gender | OLP Total | OLP Positive, n (%) | OLR Total | OLR Positive, n (%) | OLD Total | OLD Positive, n (%) | Normal Total | Normal Positive, n (%) |
|--------|-----------|---------------------|-----------|---------------------|-----------|---------------------|-------------|-----------------------|
| Male   | 3         | 0                   | 4         | 0                   | 5         | 3 (60)              | 4           | 0                     |
| Female | 7         | 1 (14.3)            | 6         | 0                   | 5         | 2 (40)              | 6           | 0                     |
| P      | 0.7       | NA                  | NA        | 0.5                 | NA        | NA                  | NA          | NA                    |
| Age (years) |  |          |           |                     |           |                     |             |                       |
| <40    | 3         | 0                   | 5         | 0                   | 3         | 2 (66.7)            | 4           | 0                     |
| ≥40    | 7         | 1 (14.3)            | 5         | 0                   | 7         | 3 (42.9)            | 6           | 0                     |
| P      | 0.7       | NA                  | NA        | 0.5                 | NA        | NA                  | NA          | NA                    |
| Site   | Single    | 0                   | 4         | 0                   | 5         | 3 (60)              | NA          | NA                    |
| Multiple | 10       | 1 (10)              | 6         | 0                   | 5         | 2 (40)              | NA          | NA                    |
| P      | NA        | NA                  | NA        | 0.5                 | NA        | NA                  | NA          | NA                    |
| Clinical form |  |          |           |                     |           |                     |             |                       |
| Hypertrophic | 6       | 1 (16.7)           | 7         | 0                   | 5         | 3 (60)              | NA          | NA                    |
| Erosive | 4         | 0                   | 3         | 0                   | 5         | 2 (40)              | NA          | NA                    |
| P      | 0.6       | NA                  | NA        | 0.5                 | NA        | NA                  | NA          | NA                    |

OLD: Oral lichenoid dysplasia, OLP: Oral lichen planus, OLR: Oral lichenoid reaction, NA: Not available
survivin was expressed, p53 was also expressed. Mirza et al. and Hoffman et al. demonstrated that the cellular accumulation of wild-type p53 results in down-regulation of survivin, thus suggesting a negative feedback loop between survivin expression and wild-type p53. p53 mutations in dysplasia and carcinomas could be responsible for the failure of this negative feedback loop between wild-type p53 and survivin and thereby result in survivin accumulation with mutant p53.[18,21] Although D07 clone of p53 does not differentiate between wild-type and mutant p53[21] this positive correlation seen in OLP and OLD between p53 and survivin suggest that the p53 is indeed mutant p53 in these cases.[7]

Another important finding we noted was that in OLD and OLP there was suprabasal p53 staining, whereas in normal and OLR the staining was restricted to the basal layer alone. The expression of p53 protein in the basal layer in oral mucosa may be due to the wild-type protein induced by environmental factors that occur commonly in the oral cavity. This phenomenon might be protective; allowing keratinocytes to repair damaged DNA.[25] On the other hand, p53 expression in suprabasal cells is more suggestive of a mutant p53 showing the presence of cells with DNA damage in more superficial layers of the epithelium, rather than a cross-reactivity with wild-type.

In summary, lichenoid dysplasia has shown p53 and survivin expression in the range of leukoplakia and not OLP. Therefore, it requires an increased frequency of follow-up and management similar to leukoplakia. On the other hand, the negligible expression of p53 and survivin in OLR suggests that it is probably an innocuous lesion and is best managed, where possible, by removal of the causative agent. Our results with OLP are inconclusive but points toward a small but important malignant potential in OLP. The expression of p53 protein in OLP in our study was similar to other studies in literature, and this was determined to be mutant p53 as suggested by the positive correlation between p53 and survivin and the suprabasal location of p53. These may be indicative of a potentially increased malignancy risk in OLP. This kind of comparative study highlights the importance of biopsying OLP and related lesions for proper diagnosis and appropriate management.

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