Introduction: The presence of epithelial dysplasia is generally accepted as one of the most important predictors of malignant development in pre-malignant lesions. Hence, evaluating the degree of dysplasia is important to envisage their potential for malignant transformation. Thus challenging the available methods of prognosis assessment and encourages the search for new and better molecular markers that relate comprehensively with known alterations of tumour initiation and progression.

Aim: To assess the expression of podoplanin (D2–40) in histopathologically confirmed cases of oral epithelial dysplasia and clinically normal oral mucosa.

Materials and Method: 30 histopathologically confirmed cases of oral epithelial dysplasia and 30 cases of clinically normal oral mucosa were immunohistochemically analysed for expression of podoplanin (D2–40).

Results: High expression of podoplanin was noted in the basal and suprabasal layers of oral epithelial dysplasia whereas the expression in normal oral mucosa was absent. The results of the study were statistically significant on analysis using One – Way ANOVA test in individual groups and Tukey’s post hoc test for pairwise comparison.

Conclusion: Correct diagnosis and timely treatment of potentially malignant disorders may help prevent malignant transformation in oral lesions. The present study found statistically significant results in association with podoplanin expression in various grades of oral epithelial dysplasia (OED), thus providing insights into the onset and progression of the diseases.

KEYWORDS: podoplanin, oral epithelial dysplasia, OSCC, malignant transformation
Table 1: Distribution of oral epithelial dysplasia according to age group, gender and site.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
<th>Site</th>
<th>Buccal mucosa</th>
<th>Gingival sulcus</th>
<th>Labial mucosa</th>
<th>Alveolus</th>
<th>Tongue</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 - 40</td>
<td>Male</td>
<td>18(60.00)</td>
<td>5(16.66)</td>
<td>5(16.66)</td>
<td>2(6.66)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>20 - 40</td>
<td>Female</td>
<td>4(13.34)</td>
<td>2(6.66)</td>
<td>5(16.66)</td>
<td>2(6.66)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>41 - 60</td>
<td>Male</td>
<td>12(40.00)</td>
<td>2(6.66)</td>
<td>5(16.66)</td>
<td>2(6.66)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>41 - 60</td>
<td>Female</td>
<td>2(6.66)</td>
<td>5(16.66)</td>
<td>2(6.66)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>Male</td>
<td>26(86.66)</td>
<td>18(60.00)</td>
<td>5(16.66)</td>
<td>2(6.66)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>Female</td>
<td>4(13.34)</td>
<td>5(16.66)</td>
<td>2(6.66)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Male</td>
<td>30 (100)</td>
<td>30 (100)</td>
<td>30 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Comparison of Podoplanin expression with histologic grades of oral epithelial dysplasia using One - Way ANOVA and pairwise comparison by Tukey's Post Hoc Test.

<table>
<thead>
<tr>
<th>Grades of oral epithelial dysplasia</th>
<th>n</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>ANOVA f value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>15</td>
<td>5.980</td>
<td>1.5548</td>
<td>65.050</td>
<td>0.000</td>
</tr>
<tr>
<td>Moderate</td>
<td>9</td>
<td>23.911</td>
<td>12.7032</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>6</td>
<td>44.833</td>
<td>3.9707</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PAIR WISE COMPARISONS BY TUKEY'S POST HOC TEST:

- Mild Vs. Moderate: 0.000
- Mild Vs. Severe: 0.000
- Moderate Vs. Severe: 0.000

Podoplanin positive cells were counted manually per 1000 positive epithelial cells in oral epithelial dysplasia on randomly selected 10 microscopic fields. The slides were moved in a single direction to avoid repetition of already examined fields. Cell counting was performed by two examiners independently to avoid intraobserver bias.

The number of positively stained cells was quantified as a percentage of total number of positive cells counted to calculate the % positivity of cells in dysplasia.

For the quantitative analysis of Podoplanin positive cells, immunostained slides were examined under high power magnification (400x) of light microscope (Olympus CH 20).

Podoplanin positive cells in dysplasia.

% positivity of Podoplanin positive cells = Number of Podoplanin positive cells / Total number of cells observed (1000) X 100

Statistical analysis:

Percentage distribution was used for demographic data. Descriptive statistics was used for discrete variables and summarized as mean with standard deviation and as number with percentage. Kruskal Wallis ANOVA, pair wise Tukey's post hoc test were applied to evaluate the differences among the mean values in different groups.

Results and observation:

On computing the demographic details pertaining to the age group in oral epithelial dysplasia, majority of the patients with oral epithelial dysplasia 16(53.33%) were in the age group of 20-40 years with a mean age of 42.66 years. On analysing the demographic data pertaining to the gender in oral epithelial dysplasia a definite male predominance was noted accounting for 26(86.66%) cases. On studying the site distribution of the lesions, buccal mucosa 18(60.00%) was the predominant site for oral epithelial dysplasia followed by gingivobuccal sulcus 5(16.66%) labial mucosa 5(16.66%) and alveolus 2(6.66%). (table 1)

The immunopositivity of podoplanin was determined by presence of brown stained cells in the tissue sections with respect to cytoplasmic and membranous immunoreactivity suggested by Yaun P et al.2006. The recommended positive control tissue was normal skin tissue.

Podoplanin positive cells quantification:

For the quantitative analysis of Podoplanin positive cells, immunostained with wash buffer. Sections were counterstained with haematoxylin and supra basal layers in moderate dysplasia

Fig 1: Expression of podoplanin at 100X (A,C,E) and 400X (B,D,F). A,B – strong membranous immunopositivity with podoplanin in basal layer in mild dysplasia C,D – strong membranous immunopositivity with podoplanin in basal and supra basal layers in moderate dysplasia E,F - strong membranous immunopositivity with podoplanin in basal and supra basal layers in severe dysplasia.

Podoplanin expression increased from mild dysplasia (5.980), through moderate (23.911), to severe (44.83). The differences were found to be
statistically significant (p<0.05). Pair wise comparisons of Podoplanin expression and the histologic grade was found to be statistically significant (p<0.05) among groups under study. (table 2, fig 1)

Immunohistochemical positivity was absent in the normal oral mucosa tissue.

Discussion:
Podoplanin is a 38-KDa mucin type 1 transmembrane glycoprotein consisting of 162 amino acids, nine of which form the intracellular domain. The extracellular domain is highly O-glycosylated with sialic acid, α-2, 3 linked to galactose, forming the main part of the protein carbohydrate moieties.13

The concept of a step-wise transition from oral potentially malignant disorders (OPMD) to oral squamous cell carcinoma (OSCC) is well established, but it can be difficult to predict if and when an OPMD will undergo full transformation and result in a tumour. The presence of epithelial dysplasia in OPMD is generally accepted as one of the most reliable predictors of development of malignancy. However, histopathologic diagnosis is subjective and lacks sensitivity. Thus, the challenge in the field of oral precancer is to predict which lesions will eventually develop into carcinoma.14

Hence, recent research is focused on identifying various markers for early cancer detection and prognostication.15

In the present study, podoplanin expression was assessed in normal oral mucosa and histological grades of oral epithelial dysplasia. Expression was absent in normal oral mucosa. The difference in podoplanin expression was found to be statistically significant among histopathologic grades of oral epithelial dysplasia (p<0.05). Podoplanin expression progressively increased with the severity of dysplasia with the highest expression in severe oral epithelial dysplasia (fig 1 E, F). Pairwise comparison of podoplanin expression and the histopathologic grade of oral epithelial dysplasia was found to be statistically significant (p<0.05) among the various groups in our study. Our results are in concordance to a study performed by Li YY et al (2015)16 where the podoplanin expression was enhanced as the severity of dysplasia increased.

Funayama et al (2011)17 found statistically significant relationship between the histologic grade and podoplanin expression in oral epithelial dysplasia. It was related to the severity of oral premalignant lesions.

The expression of podoplanin has been found to be upregulated in a number of different human cancers, including squamous cell carcinoma of the lung, the cervix, the oesophagus, and the skin and oral cavity, in dysgerminomas of the ovary and granulosa cell tumour, in mesothelioma, and in many tumour of the central nervous system (CNS).18 The high expression of podoplanin in severe dysplasia may reflect potential clonal expansion and a high risk of malignant potential. In fact, podoplanin has been identified as a marker of tumour-initiating cells (TICs) in squamous cell carcinomas. Tumourigenic and capability of recapitulating human Squamous cell carcinoma are by definition properties of TICs. Pre-malignant lesions with podoplanin expression beyond the basal cell layer may represent truly early neoplastic lesions, enriched in TICs and with a higher risk of progression to invasive cancer.19

Limitations and Future perspective:
Some variances exist in our study which could result from the differences in the sample size, lack of uniformity in selection of grades of oral epithelial dysplasia Also, our study had a relatively small sample size and was unicentric. For clinical application, prospective studies involving larger numbers of patients are needed to further evaluate the clinical utility of podoplanin as a biomarker for oral cancer risk assessment providing additional value beyond the clinical and histological marker. On establishment of the role of podoplanin in invasion and tumourigeness, podoplanin can serve as an effective therapeutic target.

Conclusion:
Assessment of podoplanin expression in oral epithelial dysplasia may aid in identifying the patients with higher risk of tumour initiation, progression and developing regional metastasis to determine whether overexpression of podoplanin in oral premalignant lesions may serve as a biomarker to predict the development of invasive cancer. Unlike all the previous studies done, the present study is novel as we have analysed intragroup podoplanin expression histological grades of oral epithelial dysplasia

References

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