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RESEARCH ARTICLE

EVALUATING THE EXPRESSION OF SYNDECAN-1 IN LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Background: The development of malignant epithelial neoplasm is associated with the disruption of cell to-cell and cell-to-matrix adhesion. A decrease in cell membrane syndecan-1 levels can be observed as early as in precancerous lesions, most of which are likely to develop into invasive cancers with time. Syndecan-1 down regulation is therefore regarded as an initial step towards malignant transformation. Therefore the aim and objectives of this study were to evaluate the expression of syndecan-1 in leukoplakia and oral squamous cell carcinoma and identify its role as a reliable marker for predicting malignant changes. Also, compare syndecan-1 expression in leukoplakia with different grades of dysplasia and in different grades of oral squamous cell carcinoma.

Materials and Method: The expression of syndecan-1 was evaluated in total of 52 cases which comprised of 10 cases of normal oral mucosa, 21 cases each of leukoplakia with dysplasia and oral squamous cell carcinoma. Mann-Whiteny '***U***' test and Tukey's test were used for statistical analysis and the level of significance was fixed at $P < 0.05$

Result: The expression of syndecan-1 decreased with increasing grades of dysplasia in leukoplakia and with increasing histological grades in oral squamous cell carcinoma

Conclusion: syndecan-1 can be considered as a useful biomarker for assessing dysplastic changes and as a reliable marker in predicting malignant changes. Also, it can be used as a prognostic indicator in oral squamous cell carcinoma and as a reliable marker in predicting malignant changes.

INTRODUCTION

Disease follows its own rules, neither those of kings nor those of slaves. Disease is essentially an individual problem and affliction is probably a result of genetic makeup and environmental influences. Most conditions that affect the oral mucosal health are acquired through environment and lifestyle factors. In India and Southeast Asia, the use of smokeless tobacco in various forms is very popular. This habit which usually involves chewing of a betel quid (Combined arecanut, betel leaf, tobacco and slaked lime) has lead to the development in a large proportion of users of a white lesion of the oral mucosa called leukoplakia. Leukoplakia is the most common potentially malignant disorder (PMD) of the oral cavity which is defined as "A predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion. [1] Many pathologists and clinicians have used the term leukoplakia synonymously with microscopic alterations in the epithelium, chiefly that of epithelial dysplasia or carcinoma.[2] The term dysplasia is very commonly associated with PMDs and can be expressed as abnormal growth or development of cells leading to abnormal size, shape or abnormal organization of adult cells, finally, leading to malignant condition.

Oral cancer term tends to be used interchangeably with oral squamous cell carcinoma (OSCC), which represents the most frequent of all oral malignancies with more than 90% of all oral malignancies are OSCC.[3]

Correct treatment begins with correct diagnosis. Arriving at a correct diagnosis requires knowledge, skill and art. Prevention and early detection of PMDs have the potential of not only decreasing the incidence but also in improving the survival of those who develop oral cancer.[4] Therefore, recent studies in this field have focused on the development of biomarkers for early detection, determining the prognosis of patients with oral malignancies.[4] Among currently available histochemical markers for oral squamous cell carcinoma, syndecan-1 is a new entity. Syndecans, cell-surface heparin sulphate proteoglycans (HSPGs), participate in cell–cell and cell–extracellular matrix (ECM) interactions.Syndecan-1 is the most studied member of the syndecan family. Syndecan-1 is

abundant in normal epithelial cells and tissues, localizing to both basal and suprabasal cell layers. Decreased expression of syndecan-1 has been reported to correlate with tumorigenicity, tumor invasion and progression.[5] Earlier studies noted that syndecan-1 levels correlate with malignancy in various tissue. The down regulation of syndecan-1 may offer the cell a possibility to detach and to invade. Immunohistochemistry (IHC) has been successfully used to study the expression of syndecan-1 in PMDs and OSCC.

This present study was carried out to evaluate the expression of syndecan-1 in leukoplakia and oral squamous cell carcinoma and to identify the role of syndecan -1 as a reliable marker for predicting malignant changes.

MATERIALS AND METHOD

Sample selection: A total of 52 paraffin embedded tissue specimens of the desired lesions were obtained from the archives of our institute. Samples were selected based on microscopic examination of slides stained with Haematoxylin and Eosin stain. The tissues were further grouped as follows:

Group I (n=10) comprised of normal oral mucosal tissue.

Group II (n=21) included leukoplakia with dysplasia which were further subdivided into leukoplakia with mild dysplasia (n= 7), moderate dysplasia (n=7) and severe dysplasia (n=7) according to World Health Organization (WHO)

Group III (n=21) comprised of oral squamous cell carcinoma cases (n=21) which were further sub grouped into well differentiated (n=7), moderately differentiated (n=7) and poorly differentiated OSCC (n=7). The study design was approved by the Institutional Ethics Committee and informed consent was obtained from the study population.

Immunohistochemistry

The 4 um section was placed on poly l-lysine coated slides.The sections were deparaffinized in xylene 3 times and rehydrated through 100%, 90%, 80%, 70% ethanol and Tris-buffered saline (TBS, pH 7.4). For antigen retrieval, the tissues were immersed in 10 mM sodium citrate buffer (pH 6.0) and boiled in a microwave for 20 minutes. After treating the tissues with 3% H_2O_2 in phosphate buffered saline (PBS), the tissues were incubated with diluted (1:50) mouse monoclonal antibody to syndecan-at 4^oC overnight. After incubating the tissue with biotinylated anti-mouse antibody,TSA HRP System was used to amplify signal intensity. For visualization, liquid DAB+substrate chromogen system (Dako, Glostrup, Denmark) was used. Immunore activity of syndecan-1 was evaluated quantitatively. Five high power fields (40X) were selected randomly and the cells were counted with a grid using binocular microscope (Motic attached to a computer with Motic Advanced Images 3.2 software.) The average percentage of positive cells from these 5 high power fields was considered for each slide.

Statistical analysis

The statistical analysis was carried out using Mann-Whiteny '***U***' test between three groups studied and also between three subgroups of leukoplakia. ANOVA and Turkey's test was used between individual groups of OSCC. The level of significance was fixed at 5%.

RESULTS

A quantitative estimation of syndecan-1 was done in the 52 cases. All the cases studied exhibited a uniform pattern of diffuse cytoplasmic membrane positivity for syndecan-1. The expression of syndecan-1 was modest in basal layer, strong in spinous layer and almost absent in the uppermost layer. In Group I, the staining for syndecan-1 was mostly along the cytoplasmic membrane, with little or no cytoplasmic staining, whereas in Group II the increased grades of dysplasia showed increased cytoplasmic staining. In Group III, well differentiated oral squamous cell carcinoma cells maintained membranous staining, concomitant with cytoplasmic staining. Tumor cells of moderately and poorly differentiated cases showed increased cytoplasmic staining with very little membrane staining

The mean of syndecan-1 positive cells for Group I was 97.20; for Group II was 61.24 and for Group III it was 41.81. (Table I) Mann-Whitney U test revealed that the difference was significant statistically between all the three groups. (Table II) **When syndecan-1 expression was studied among subgroups of leukoplakia**, there was a statistically significant difference between leukoplakia with mild dysplasia when compared to leukoplakia with moderate and severe dysplasia. Similar significant result was also observed between moderate and severe dysplasia cases.($p<0.05$)(Table III)

Table 1 Mean of syndecan-1 positive cells and Kruskal-Wallis test result for quantitative analysis in 3 Groups and subgroups of leukoplakia

		N	Mean	Standard Deviation	p value	Significant
Main 3 Groups	**Group I**	10	97.20	2.07	0.000	Highly significant
	Group II	21	61.24	16.97		
	Group III	21	41.81	22.97		
Subgroups of leukoplakia	Mild dysplasia	7	78.57	9.02		
	Moderate dysplasia	7	59.57	14.94	0.001	Significant
	Severe dysplasia	7	45.57	3.91		

Table 2 Mann-Whitney U test result for pair-wise comparison in main 3 groups and three subgroups of leukoplakia

Groups	Groups	p-value	Significant
Group I	**Group II**	.00	Highly significant
	Group III	.000	Highly significant
Group II	**Group III**	0.019	Significant
Mild dysplasia	**Moderate dysplasia**	0.025	Significant
	Severe dysplasia	0.002	Significant
Moderate dysplasia	**Severe dysplasia**	0.003	Significant

Table 3 Mean of syndecan-1 positive cells and ANOVA test result for quantitative analysis in three subgroups of OSCC.

Sub groups of Group OSCC	n	Mean	Standard Deviation	p value	Significant
Well differentiated OSCC	7	59.28	12.29	0.000	Highly significant
Moderately differentiated OSCC	7	52.57	13.12		
Poorly differentiated OSCC	7	13.57	3.69		

Syndecan-1 expression in subgroups of OSCC showed highly significant association between the subgroups of OSCC ($p<0.05$) except between well differentiated and moderately differentiated OSCCs where the result was not statistically significant.($p>0.05$) (Table IV, V)

Table 4 Tukey's multiple comparison test result for three subgroups of OSCC.

Subgroups	Subgroups	p value	Significant
Well differentiated OSCC	Moderately differentiated OSCC	.477	Not significant
	Poorly differentiated OSCC	.000	Highly significant
Moderately differentiated OSCC	Poorly differentiated OSCC	.000	Highly significant

DISCUSSION

Leukoplakia is a potentially malignant disorder that may develop into oral squamous cell carcinoma. The cellular changes in the epithelium known as dysplasia are often observed in oral pre-malignancies. Thus epithelial dysplasia points toward the possible subsequent development of malignancy.[4] The risk of malignant transformation of dysplasia to invasive carcinoma increases with the degree of dysplasia. The progression of a lesion from a premalignant state to invasive squamous cell carcinoma is believed to result from a series of intracellular events. Thus, identification of specific intracellular events in carcinogenesis is a necessary prerequisite to the identification of agents that target and interrupt specific steps in the progression of cancer.[6]

Syndecans are a family of heparan sulfate proteoglycan receptors that participate in cell to cell and in cell to matrix adhesion. [7] Under physiological conditions, syndecan-1 cell surface heparan sulfate proteoglycan is induced during normal differentiation of keratinocytes and contributes to maintain the normal architecture of the epithelium.[7] Down regulation of syndecan‑1 may offer the cell, a possibility to detach and invade.[4] Syndecan-1 is down-regulated in intra-oral pre-malignant lesions and oral squamous cell carcinoma.[8] In line with these findings, Inki *et al*. (1994)[9]found a statistically significant correlation between syndecan-l expression and histological differentiation of head and neck squamous cell carcinoma. This provides further support for the yet unidentified role of syndecan-l in keratinocyte differentiation, which is lost from anaplastic cells.

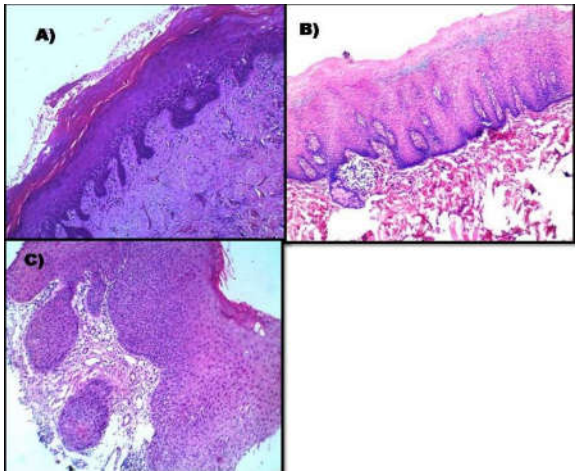

Figure no.1 photomicrograph showing A)mild leukoplakia (B) moderate leukoplakia, C)severe leukoplakia (H & E 10x)

Quantitatively, the mean of syndecan-1 positive cells in the three groups was computed and subjected to statistical analysis using Kruskal-Wallis and Mann Whitney U test. A statistically significant ($p < 0.05$) association was found between the 3 groups (Group I, Group II and Group III) compared.

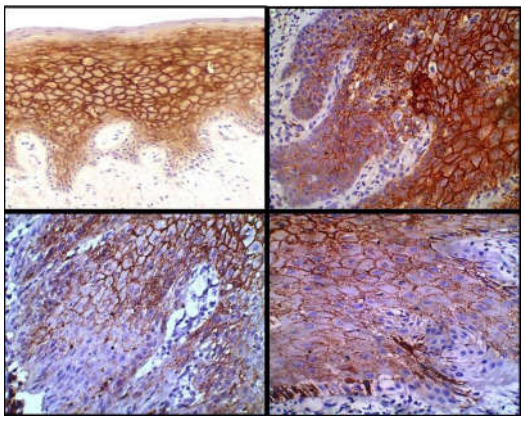

Figure no.2 Photomicrograph showing Syndecan-1 expression in A) normal mucosa (B) mild leukoplakia C) moderate leukoplakia, D)severe leukoplakia (10x)

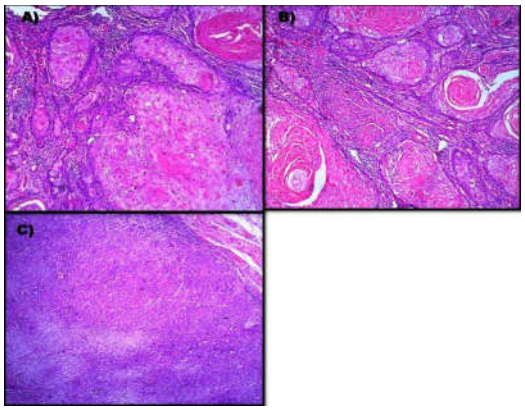

Figure no.3 photomicrograph showing A) WDSCC(B), MDSCCC and C)PDSCC(H AND E-10x)

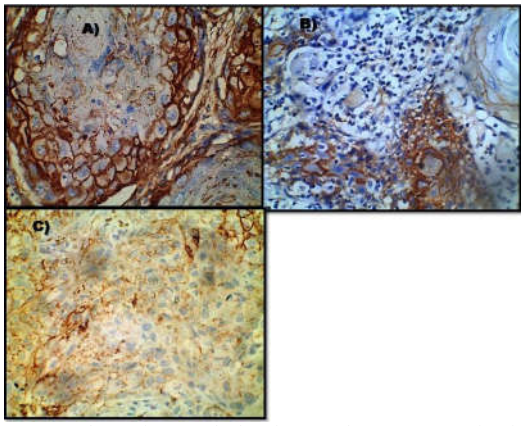

Figure no.4 Photomicrograph showing Syndecan-1 expression in A) WDSCC(B), MDSCCC and C)PDSCC(10x)

In leukoplakia with dysplasia, the expression of syndecan-1was found to decrease significantly with increased grades of dysplasia. Similar observations were also made by other workers such as Soukka *et al*. (2000)[4,10] and H. Kurokawa *et al*.,(2003)[8]when they studied syndecan-1 expression in dysplastic epithelium of potentially malignant lesions of the oral cavity. A study carried out by Sushant Kamat *et al*.(2013) [4] and Lakkam *et al*.(2010) [11] in dysplastic epithelium found

that syndecan-1 immunopositivity was lost gradually as the extent of epithelial dysplasia increased. Therefore, the down-regulation of syndecan-1 expression may be the most important reliable marker for dysplastic changes.

When we studied the expression of syndecan-1 in the three histological grades of OSCC, we found that the percentage of positive cells decreased from well differentiated to poorly differentiated OSCC. Tukey's test result showed highly significant association between the subgroups of OSCC with p value of less than 0.05except between well and moderately differentiated OSCC where the result was not statistically significant.($p>0.05$) This may be attributed to the small sample size. Similar observations were also made by Martinez *et al*. (2009) [12] who found significantly decreased expression of syndecan-1in the dysplastic areas and infiltrating neoplastic islands of lip carcinoma and concluded that reduction in syndecan-1 expression in premalignant and malignant lesions may be an early genetic event contributing to tumor progression.Thus,syndecan-1 expression may be one of the most important prognostic factors in oral cancers and can be used as cancer biomarker.

In our study we found that normal membranous pattern of syndecan-1 is disrupted in dysplastic cells and increased expression of syndecan-1 is found in the cytoplasm of epithelial cells. The translocation of syndecan-1 from the cell membrane to the cytoplasm of tumor cells is anticipated to result in low level of the functional syndecan-1on the cell surface. This may result in the tumor cells having fewer extracellular matrix interactions, and thus allows them to move more freely, leading to invasion and distant metastasis.[13]

The development of malignant epithelial tumors is associated with reduced intercellular adhesion, disturbed differentiation and cellular changes suggesting that the expression and function of cell adhesion molecules could also change during malignant transformation. It has been shown that suppression of endogenous syndecan-1expression in epithelial cells by transfection with syndecan-1 antisera c DNA causes loss of epithelial characteristics of their parental cells and acquisition of an elongated fusiform morphology, which has the ability to invade and migrate within collagen and anchorage independent growth.[14] Thus, low expression levels of syndecan-1 with increased dysplasia can be attributed to its role in cell–cell and cell–matrix interactions. Cell surface syndecan-1 is thought to enhance cell-extracellular matrix (ECM) cohesion and restrict cell migration. Thus, the loss of epithelial syndecan-1 increases the migratory capacity of tumor cells. Loss of syndecan-1 from malignantly transformed cells could be one mechanism by which tumor cells loosen their attachment to each other and to the extracellular matrix and become non-responsive to the signals coming from their microenvironment.[4]

CONCLUSION

A leukoplakia cases shows a definite down regulation of syndecan-1 with increased grades of dysplasia. Therefore syndecan-1 can be considered as a useful biomarker for assessing dysplastic changes. Results of this study demonstrated an inverse relationship between syndecan-1 expression and degree of OSCC differentiation. Thus, the expression of syndecan-1 decreased from well differentiated OSCC to poorly differentiated OSCC. Hence we can conclude that syndecan-1 can be used as a prognostic indicator in OSCC and as a reliable marker in predicting malignant changes. In literature, very little information is available on comparative account of expression of syndecan-1 in case of leukoplakia and oral squamous cell carcinoma. Further studies directed towards comparative expression of syndecan-1 in a larger sample size with clinico-pathologic correlation and long term patient follow-up will definitely aid to elucidate the role of syndecan-1 as an important prognostic indicator in leukoplakia and oral squamous cell carcinoma.

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