Original Article

Assessment of extra cellular changes in Oral Squamous Cell Carcinoma : An Histochemical Study

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ABSTRACT:
Background: Head and neck cancer is one of the 10 most common types of cancer worldwide, afflicting >500,000 individuals each year. Oral cancer is considered to be a preventable condition, due to the possibility of early detection and treatment. **Aim of the study:** To assess extra cellular changes in oral squamous cell carcinoma. **Materials and methods:** The study was conducted in the department of oral pathology of the dental institution. For the study, we selected patients with a biopsy-proven malignant oral mass treated with wide excision of tumor. A total of 50 patients were selected for the study. The age of the patients ranged from 21-70 years. For the demonstration of elastic fibers, we studied the sections using Verhoeff's-Van Gieson (VVG) method. To study identify collagen, we used Masson's trichrome stain and to identify calcifications, we used Von Kossa stain. For the identification of metastasis, lymph nodes were studied. **Results:** A total of 50 patients with OSCC were selected for the study. 18 patients had grade 1 OSCC, 19 patients had grade II OSCC. Tables 2 shows the distribution of ECM changes among different grades of OSCC. 21 patients belonged to grade I, 7 patients belonged to grade II and 3 patients belonged to grade III. The results were compared and found to be statistically non-significant. **Conclusion**: From the results of present study, we conclude that routine histochemical techniques, which are easily accessible, can be used to identify these changes in oral biopsies so as to advise the surgeon regarding the propensity for invasion and metastasis, thus allowing him to plan the surgery accordingly.
Keywords: Oral cell squamous carcinoma, metastasis, malignancy.

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INTRODUCTION:
Head and neck cancer is one of the 10 most common types of cancer worldwide, afflicting >500,000 individuals each year. Oral cancer is considered to be a preventable condition, due to the possibility of early detection and treatment.[1] Oral squamous cell carcinoma (OSCC) represents 95% of all forms of head and neck cancer, and during the past decade its incidence has increased by 50%.[2,2] Snuff and alcohol consumption are associated with 90% of patients that exhibit oral cancer (1) and the two factors appear to have a synergistic effect.[4] Most OSCCs develop within fields of precancerized epithelium which contain keratinocytes at different stages of transformation. The persistence of such precancerized fields where there has previously been OSCC is the reason for the high-rate of occurrence of new tumours. Squamous cell carcinoma (SCC) consists of two interdependent components – the tumor epithelial cells and the stroma.[5] These components interact on a regular basis and to an extent that discrepancy in one could affect the other. Hence, for a better prediction of course and outcome of SCC, both should be given equal weightage in the evaluation/grading system.[6] Hence, the present study was conductedto assess extra cellular changes in oral squamous cell carcinoma.

MATERIALS AND METHODS:
The study was conducted in the department of oral pathology of the dental institution. For the study, we

selected patients with a biopsy-proven malignant oral mass treated with wide excision of tumor. A total of 50 patients were selected for the study. The age of the patients ranged from 21-70 years. The specimens of the biopsy were received in formalin solution. For the selection of section, we obtained sections from tumor areas, adjacent normal appearing areas, resection margins and lymph nodes. The sections were fixed with 10% neutral buffered formalin and embedded in paraffin. The sections ere stained with H and E stain. The diagnosis of OSCC was confirmed with histopathological evaluation. For the demonstration of elastic fibers, we studied the sections using Verhoeff's-Van Gieson (VVG) method. To study identify collagen, we used Masson's trichrome stain and to identify calcifications, we used Von Kossa stain. For the identification of metastasis, lymph nodes were studied. The presence of ECM changes was compared with the grade of tumor and lymph node metastasis.

The ethical clearance for study protocol was obtained from ethical committee of the institution. The statistical analysis of the data was done using SPSS version 11.0 for windows. Chi-square and Student's t-test were used for checking the significance of the data. A p-value of 0.05 and lesser was defined to be statistical significant.

RESULTS:
Table 1 shows the grading of OSCC patients. A total of 50 patients with OSCC were selected for the study. 18 patients had grade 1 OSCC, 19 patients had grade II OSCC. Tables 2 shows the distribution of ECM changes among different grades of OSCC. No change was observed in 31 patients. 21 patients belonged to grade I, 7 patients belonged to grade II and 3 patients belonged to grade III. The results were compared and found to be statistically non-significant.

Table 1: Grading of OSCC patients

Distribution of cases	No. of patients	p-value
Grade I	18	0.21
Grade II	19	
Grade III	13	

Figure 1:

Grading of OSCC

■ Grade I ■ Grade II ■ Grade III
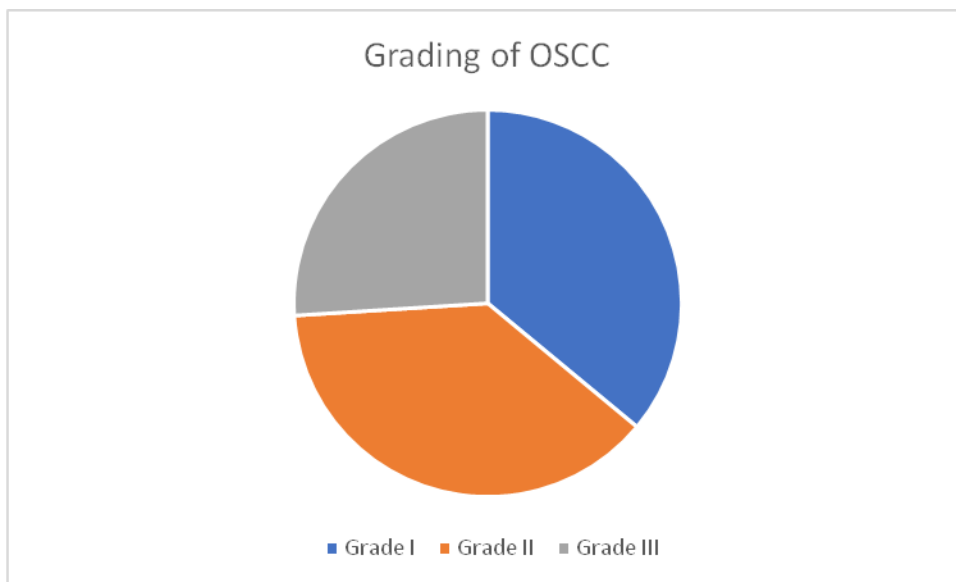
Tables 2: Distribution of ECM changes among different grades of OSCC

ECM change	No. of patients	p-value
No change	31	0.32
Grade I	21	
Grade II	7	
Grade III	3	

Figure 2: ECM changes among different grades of OSCC
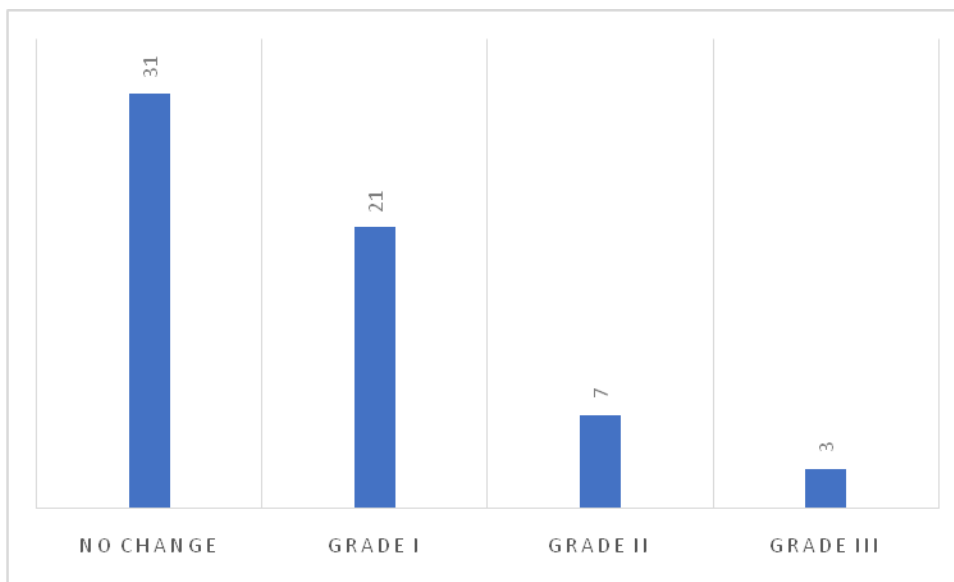
DISCUSSION:

In the present study, we observed that 31 patients had no ECM changes in the oral squamous cell carcinoma. The study results were statistically analyzed and were found to be statistically non-significant. The results were compared to previous similar studies. Kardam P et al analyzed the changes in collagen and elastic fibers in varying grades of (OSCC). Three sections each were cut from fifty samples of varying grades of OSCC and ten samples of control followed by staining with H and E, Picrosirius-Red and Verhoeff–Van Gieson. Qualitative and quantitative analysis of collagen and elastic fibers were accomplished using set criteria. A change in colors of collagen fibers was seen on progressing from well to poorly differentiated OSCC. Thin collagen fibers predominantly exhibited greenish yellow, but the thick fibers exhibited a variety of colors. As the grade of OSCC progressed, collagen fibers were loosely packed haphazardly arranged. Statistically insignificant results were obtained for quantitative analysis of collagen and qualitative analysis of elastic fibers. They concluded that the collagen fibers undergo a change in color, orientation and packing in the stroma of varying grades of OSCC. The uniqueness of this study lies in the exploration of elastic fibers in OSCC which has not been done so far. Li Y et al evaluated the correlation between the positive expression rate of mutant p53 and the clinical characteristics of patients with oral squamous cell carcinoma (OSCC), as well as the effectiveness of intra-arterial chemotherapy. Expression of mutant p53 in tumor tissues was determined by immunohistochemical analysis of 51 OSCC patients, prior to and following intra-arterial chemotherapy. Prior to intra-arterial chemotherapy, mutant p53 positive rates in patients with higher pathological grades were significantly higher than those of the patients with lower pathological grades. The mutant p53 positive rate in patients with lymph node metastasis was 73% (19/26), which was significantly higher than that of the patients without lymph node metastasis (20%, 5/25). Mutant p53 was expressed in 17% (3/18) of clinical phase II patients, while 64% (21/33) of clinical phase III and IV patients exhibited positive expression of mutant p53. The mutant p53 positive rate in chemotherapy non-responsive patients was 69% (11/16), which was significantly higher than that in the chemotherapy-responsive patients (37%, 13/35). It was concluded that the mutant p53 has a significant role in the differentiation, development and treatment guidance of OSCC. Intra-arterial chemotherapy with 5-fluorourcil and carboplatin potentially exerts a therapeutic effect by reducing the expression of mutant p53.[7, 8]

Yellapurkar S et al evaluated laminin expression in oral squamous cell carcinomas OSCC and to determine any associations with clinico-pathological parameters (surgical margin status, lymph node involvement, survival and recurrence). Laminin expression was evaluated in 31 cases of biopsy-proven OSCC by immunohistochemical staining and its association with prognosticators and the Brynes grading system was determined by appropriate statistical analysis. They observed a significant increase in linear staining pattern (p<0.001) at the tumour-host interface in well-differentiated OSCC cases, in contrast to poorly differentiated lesions which exhibited intense cytoplasmic expression within tumour cells. Higher cytoplasmic laminin expression was seen in 33.3% of cases with involved surgical margins and 69.2% of cases with lymph node metastasis (along with weak/absent staining of laminin around the tumour-host interface – Basement membrane around tumour islands). Similarly, in 60% of the cases who

died and in 81.8% of cases with tumour recurrence, moderately intense cytoplasmic laminin expression was seen within tumour cells. On comparing variables of the Brynes grading system, significant cytoplasmic expression of laminin was linked with mild inflammation and increased mitotic activity. Based on these observations, immunohistochemical expression of laminin might be useful to evaluate histological differentiation and aggressiveness of OSCCs. Anuradha A et al evaluated the number of mast cells in tissue sections of oral squamous cell carcinoma (OSCC) in comparison with normal mucosa. A total of 40 cases (20 OSCC and 20 normal mucosa) were stained with 1% toluidine blue and the quantitative analysis was done by using light microscope under , 400x magnification. A significant increase in the mast cell count was observed in the sections of OSCC when compared to normal mucosa suggesting their contributing role in tumor growth and progression.[9, 10]

CONCLUSION:

From the results of present study, we conclude that routine histochemical techniques, which are easily accessible, can be used to identify these changes in oral biopsies so as to advise the surgeon regarding the propensity for invasion and metastasis, thus allowing him to plan the surgery accordingly.

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