INTRODUCTION

Despite improvement in the treatment schemes, including novel therapeutic regimes, surgeries, radiotherapy and chemotherapy; the prognosis of patients of oral squamous cell carcinoma (OSCC) remains prominently inadequate, due to loco regional recurrences. The five-year survival rate is less than 50% and the prognosis of advanced cases has not meliorated much over the past three decades.1 Therefore, early diagnosis and use of reliable prognostic biomarkers forms the mainstay of the disease process. Currently, in addition to the clinicopathological parameters, molecular markers are being intensively used to predict the outcome of the disease progression.2 However, the fact that more than 50% of the cases of head and neck squamous cell carcinoma (HNSCC) usually have advanced disease at the time of diagnosis, suggests a lack of clinically useful markers.3

Lately experimental results indicate that cell-adhesion molecules modulate signal transduction directly. The alteration in the function or expression of cell-adhesion molecules can lead to tumour progression both by commuting the adhesion status of the cell and by having effect on cell signalling. The molecules of cell adhesion of various classes including cadherins, immunoglobulin-like cell-adhesion molecules (Ig-CAMs), the hyaluronan receptor CD44 and integrins, can regulate several signalling pathways. Changes within interactions of cell-cell and cell-matrix are directed by cell molecules which can directly affect the function of adhesion molecules. In various types of cancer, loss of epithelial cadherin function is followed by the expression gain of mesenchymal cadherins in a process called as Cadherin switch.4 N-cadherin is a cell adhesion molecule which is calcium dependent that regulates cell migration and tumor invasion with significant expression observed in prostate and breast cancer.5 As the tumor develops, N-cadherin helps in the formation of new blood vessels and conversion of epithelial phenotype into mesenchymal cadherins in a process called as Cadherin switch.4

The aim of the present retrospective study was to evaluate N-cadherin expression in oral epithelial dysplasias and OSCC and to analyse the relation of expression between different grades.
METHODOLOGY

A total of 100 cases were analyzed, selected from the Department of Oral and Maxillofacial Pathology. The cases were diagnosed on the basis of histopathologic features, reporting the grade of dysplasia and OSCC. The cases were divided into three groups. Group I comprised of the control Group which included ten cases of the oral epithelium which were diagnosed as normal epithelium that did not show any dysplastic changes in the epithelial cells. These were those tissues which were left out during surgery of third molar extraction. Group II comprised of 60 diagnosed cases (60%) of oral epithelial dysplasia which were further divided into three subgroups (II a, b and c) comprising 20 cases (33%) each of mild, moderate and severe dysplasia, respectively. Group III comprised of total 30 diagnosed cases (30%) of OSCC. This group was further divided into two subgroups (III a and b) consisting 15 cases (50%) each of well and moderately differentiated OSCC. Poorly differentiated cases were not taken due to insufficient sample size in the department. Hematoxylin and Eosin staining was carried out in another set for confirmation of diagnosis of the lesions.

The tissues fixed in formalin and embedded in paraffin were stained with N-cadherin antibody. 4μm thick sections were procured and put on slides coated with poly-L-lysine kept at 60°C in oven for 15 minutes to increase the adhesion and deparaffinized by transferring xylene with three changes. Next the section was hydrated through ethyl alcohol by descending grading. Antigen was retrieved in EZ retriever system (Biogenex) using citrate buffer (pH - 6.0) in 2 cycles (cycle 1: 98°C - 5 minutes and cycle 2: 95°C - 6 minutes). It was then cooled to room temperature followed by washing in PBS. To block the endogenous peroxidase activity, 0.3% peroxide stock solution was used followed by a PBS wash for a duration of 10-15 minutes followed by addition of power block for 10-15 minutes. For one hour, the N-cadherin antibody was added on to the slides. The sections were then washed in PBS and secondary antibody (HRP polymer) was added. Again PBS wash was done followed by staining with DAB chromogen for duration of 20 minutes. After that the slides were dehydrated in alcohol in graded manner followed by clearing in xylene for 5 minutes. Mounting was done with DPX.8

The slide was evaluated using scanner view, low power and high power of microscope to demonstrate N-cadherin expression positivity. Brownish staining in the membrane of cell and cytoplasm was considered as positive staining. On the basis of the intensity of staining and the percentage of cells exhibiting membranous staining, the expression was classified. The sections were observed at low magnification to identify positively stained areas (hot spots). Hot spot area chosen was the area which covered the maximum staining area. Ten}

hotspots (positive fields) per slide for quantitative score and the whole tissue section were considered for quantitative scores. In the control and dysplasia groups, the positivity of N-cadherin expression was analyzed in epithelium in basal and spinous layer. In the carcinoma group, the tumor cells were divided into two areas of tumor islands present in the central area of the incisional biopsy tissue section and the peripheral area of the incisional biopsy tissue section. According to the staining intensity and the percentage of positive cells, the scoring was done. Immunostaining was scored using a scoring system described by Shen et al.9 Both the scores were multiplied and total score was calculated. On the basis of the total score, the final expression was graded as negligible (0-2; +), mild (3-5; ++), moderate (6-8; ++++) and intense (9-12; ++++). For statistical significance, SPSS 13.0 was used to calculate the data by non-parametric tests (Mann-Whitney Test & Kruskal-Wallis Test). Level of significance was kept at p value <0.05.

RESULTS

In the ten cases (10%) of control group, negligible staining was found in 4 cases (40%), mild membranous staining in 4 cases (40%) and moderate membranous staining in the rest 2 cases (20%, Figure 1a). In the 20 cases (33%) of mild dysplasia, 14 cases (70%) showed negligible staining, four cases (20%) showed mild membranous staining and two cases (10%) showed moderate membranous staining (Figure 1b).

In the 20 cases (33%) of moderate dysplasia, 3 cases (15%) showed negligible staining, 11 cases (55%) showed mild membranous staining and six cases (30%) showed moderate membranous staining (Figure 1c). In the 20 cases (33%) of severe dysplasia, two cases (10%) showed negligible staining, 17 cases (85%) showed mild membranous staining and one case (5%) showed moderate membranous staining (Figure 1d).

In total, out of the 60 cases (60%) of dysplasia, one case (31.6%) showed negligible staining, 32 cases (53.3%) showed mild membranous staining, and 9 case (15%) showed moderate membranous staining.

In the 15 cases (50%) of well-differentiated OSCC, 1 case (6.6%) showed negligible staining, 5 cases (33.3%) showed mild membranous staining, 7 cases (46.8%) showed moderate membranous staining and 2 cases (13.3%) showed intense membranous staining (Figure 2a).

In the 15 cases (50%) of moderately-differentiated OSCC, two cases (13.3%) showed negligible staining, 6 cases (40%) showed mild membranous staining, 4 cases (26.6%) showed moderate membranous staining and 3 cases (20%) showed intense membranous staining (Figure 2b).

In total 30 cases (50%) of OSCC, 3 cases (10%) showed negligible staining, 11 cases (36.6%) showed mild membranous staining, 11 cases (36.6%) showed moderate membranous staining and 5 cases (16.6%) showed intense membranous staining.
Table I shows the comparison between all the major groups (control group, dysplasia and OSCC) and their subgroups (mild dysplasia, moderate dysplasia, severe dysplasia, well-differentiated OSCC and moderately differentiated OSCC). The median value [3(3.687)] of control group was greater as compared to mild dysplasia [median=2.375(1.375)] while the median value of moderate dysplasia [median=3(3)] was more as compared to mild dysplasia [median=2.375(1.375)] and equivalent to severe dysplasia [median=3(3)] both. the median value of moderately differentiated carcinoma [median=5(4.5)] was less as compared to well-differentiated carcinoma [median=6.25(3)]. Comparative analysis between mild vs. moderate dysplasia, mild vs. severe dysplasia, severe dysplasia vs. OSCC, severe dysplasia vs. well-differentiated OSCC, and severe dysplasia vs. moderately differentiated OSCC was found to be statistically significant. Rest of the comparisons were found to be statistically insignificant (Table II).

DISCUSSION

In the control group, minimal to moderately positive membranous expression of N-cadherin was observed. This could be the normal N-cadherin expression observed in the normal epithelial cells as small amount of N-cadherin is known to be expressed by the normal cells. However, it has been reported that the precursor or adult normal cells progressing to cancer cells do not express N-cadherin and the expression is 'de novo' in the cancer cell.

In the present cases, positive N-cadherin membranous expression was found in 6 cases out of ten in normal epithelium of oral mucosa. This was in accordance with an earlier study that found positive signals of N-cadherin in 18 cases out of 62 in normal esophageal mucous membrane.

Sixty-eight cases of dysplasia showed positive N-cadherin membranous staining and 32 cases of dysplasia showed negative staining (Figures 1b, c and d). According to a study, 33 cases out of 53 cases were N-cadherin positive in para-cancer tissues of the tongue. Compared to that of normal epithelium of tongue tissues, an evident increase was found in expression of N-cadherin in para-cancer tissues in their study; whereas, in the present study, no significant difference was found between N-cadherin expression between epithelium of normal oral mucosa (control) and dysplastic tissues.

Out of 30 cases of oral squamous cell carcinoma, 27 cases showed positive N-cadherin expression, out of which 5 cases showed intense N-cadherin positivity which included two cases of well-differentiated squamous cell carcinoma (WDSCC) and 3 cases of moderately-differentiated squamous cell carcinoma (MDSCC).
of 53 of squamous cell carcinoma of tongue tissues showed positive cytoplasmic N-cadherin expression. In a study of esophageal squamous cell carcinoma, 47 cases out of 62 were found to be N-cadherin positive in the cytoplasm. In urothelial tumors, 60% cases showed positive membranous expression for N-cadherin in invasive tumors. Membranous N-cadherin expression was observed in 51.9% of carcinomas. Heterogenous expression of N-cadherin in primary pancreatic cancers was observed when compared to normal epithelial tissues with 43% of the primary tumors showing a positive N-cadherin expression. Positive membranous N-cadherin expression was found in 34% of cases of tissues of prostate cancer. On the contrary, 12.3% of tumors of ductal carcinomas of breast showed a positive N-cadherin expression and its expression was limited to a small number of cells, which presented a faint membranous staining always associated with a strong expression in the cytoplasm.

The difference in the N-cadherin expression of severe dysplasia and WDSCC; and severe dysplasia and MDSCC was found to be significant statistically. This was in accordance with the study of Paredes et al., who found no correlation between N-cadherin expression and histological grade. Li et al. did not found any correlation of N-cadherin expression with the histological grade. On the contrary, Gravdal et al. stated that positive N-cadherin expression was associated with poor WHO histologic differentiation, seminal vesicle invasion, and pelvic lymph node infiltration in prostate carcinoma. Nakajima et al. observed that the N-cadherin expression in primary tumors correlated significantly with histological grade. ElMoneim and Zaghloul found N-cadherin expression to be significantly more in poorly differentiated carcinomas as compared to moderately or well-differentiated invasive ductal carcinomas.

Thus, it was observed that N-cadherin expression was greater in oral squamous cell carcinoma as compared to oral epithelial dysplasia and normal epithelium of oral mucosa. As the loss of adhesion due to downregulation of E-cadherin causes gain of N-cadherin expression, and since the cells become motile and invasive, it may be suggested that the increased expression of N-cadherin in oral squamous cell carcinoma is associated with cell migration and invasion and correlates with poor prognosis.

CONCLUSION
This study suggests that N-cadherin is upregulated in oral squamous cell carcinoma. Its expression does not correlate with the histological grade of oral squamous cell carcinoma. N-cadherin expression strongly suggests the importance of epithelial-mesenchymal transition featuring its increased expression in the progression of tumor.

REFERENCES