ORIGINAL RESEARCH

DNA PLOIDY ANALYSIS OF ORAL SQUAMOUS CELL CARCINOMA - A RETROSPECTIVE FLOW CYTOMETRIC STUDY

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ABSTRACT:
Background- Oral Squamous Cell Carcinoma is the 6th most common malignancy worldwide and 2nd most common malignancy in Indian population. Conventional pathologic tumour classifications have proved to be valuable indicators of prognosis, but do not take into account the individual biologic properties of a distinct tumour. cytometrically determined nuclear DNA content of tumor cells is suggested to be an additional objective parameter for their biologic behavior. AIM- The present study was aimed to analyze the prognostic value of DNA ploidy status in oral squamous cell carcinoma. Materials And Methods-DNA ploidy status of 43 formalin fixed paraffin embedded tissue samples of primary oral squamous cell carcinoma was evaluated using flow cytometry and was correlated with clinical staging and histological grading. Results- Overall incidence of aneuploidy was found to be 44.19%. Association of ploidy status with histopathological grade was statistically significant (p=0.001). Association of ploidy status with clinical stages was not found to be statistically significant (p=0.078) Conclusion-DNA ploidy measurement is a valuable adjunct to clinical and histopathological assessment of oral squamous cell carcinoma.
Key words- DNA ploidy; Flow cytometry; Oral squmous cell carcinoma

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INTRODUCTION

Oral Squamous Cell Carcinoma is the 6th most common malignancy worldwide and 2nd most common malignancy in Indian population. Survival rates for Oral Squamous Cell carcinomas have been nearly unchanged for decades.[1, 2] Conventional pathologic tumour classifications have proved to be valuable indicators of prognosis, but do not take into account the individual biologic properties of a distinct tumour. To get better insight into tumour biology, further information concerning the malignant potential of tumour cells is necessary.[3] cytometrically determined nuclear DNA content of

tumor cells is suggested to be an additional objective parameter for their biologic behavior. Critical early step in the development of at least some of the human cancers is chromosomal rearrangement. The present study was aimed to analyze the prognostic value of DNA ploidy status in oral squamous cell carcinoma.

MATERIALS AND METHODS

Sample selection

Formalin fixed paraffin embedded tumour tissue from 43 patients with primary oral squamous cell carcinoma were randomly chosen. Each tumour was staged according to 1987 TNM classification and graded according to Bryen's histological grading.[4]

Preparation of single cell suspensions

Single cell suspensions were prepared by slight modification of the method given by Hedley DW et al [5]. Cell counts were made using a hemocytometer (Fig 1).The suspension is ready for staining if the cell count is more than 5.0 x 10^5cells.

Staining Procedure-Cell suspensions were centrifuged at 400 x *g* for 5 minutes at room temperature (20° to 25°C). 250 µl of trypsin buffer was added to each tube, gently mixed by tapping the tube by hand and allowed to react for 10 minutes at room temperature (20° to 25°C). 200 µl of Trypsin inhibitor and RNase buffer was added to each tube and gently mixed by tapping the tube by hand. Suspension was incubated for 10 minutes at room temperature (20° to 25°C).200 µL of cold propidium iodide stain solution (2° to 8°C) was added to each tube and incubated for 10 minutes in the dark at 2° to 8°C . The samples were filtered through 50µm nylon mesh into a labeled 12 x 75 mm tube. The samples were now ready to be analyzed on the flow cytometer. The data interpretation and anlysis was done on software generated multi parametric histogram files. In the present study diploid tumours were classified as those having a single G0/G1 peak and the tumours were classified as aneuploid if more than 10% of the total number of cells were located in G2/M peak (Suzuki K, Chen RB, Noruma T and Nakjima T in 1994) [6].(Fig 2 and Fig 3)

Statistical Analysis

The one way ANOVA analysis was performed to compare the mean percentage of cell cycle fractions amongst clinical stages and amongst histological grades. Post-hoc analysis was performed in the form of Scheff's analysis to figure out where the difference lies. Unpaired t-test was applied for comparison of mean ages of patients of diploid tumours to aneuploid tumours. Chi square test was applied to test the statistical significance of association between age, gender of the patient, location of the lesion, clinical staging, histopathological grading and ploidy status.

OBSERVATION AND RESULTS

Overall incidence of aneuploidy was found to be 44.19%. The patients with aneuploid tumours were found to be older (mean age=58.74 years) than patients with diploid tumours (mean age=50.74years).The difference was found to be statistically significant (p=0.020). Association of ploidy status with histopathological grade was statistically significant (p=0.001) (Table 1). All the stage I carcinomas were found to be diploid. 64.29% (9 out of 14) of stage II carcinomas were found to be aneuploid. 29.17% (7 out of 24) of stage III carcinomas were found to be aneuploid. 75% (3 out of 4) of stage IV carcinomas were found to be aneuploid. Association of ploidy status with clinical stages was not found to be statistically significant (p=0.078) (Table 2). A decrease in the G0/G1 fraction was noticed with decrease in the degree of differentiation (p=0.012) (Table 3).

S phase fraction was significantly high in poorly differentiated tumours. There was a progressive increase in the G2+M fraction with decrease in the degree of differentiation(p=0.004) (Table No.4).The results of Post-hoc analysis showed statistically significant difference between mean G0/G1 and G2/M phase fractions of well differentiated tumours and poorly differentiated tumours.

DISCUSSION

Hedley DW et al (1983)[5-8] stated that use of archival, formalin fixed, paraffin embedded tumour material can be used successfully for flow cytometric DNA content analysis of solid tumours. Our results confirmed these observations. In the present study it was found that patients with aneuploid tumours were older than patients with diploid tumours. Pietrusky GR & Hornstein OP (1982)[9] , Kokal WA et al (1988) (quoted by Mahmood JU)[10] reported similar findings but these findings were in contrast with findings of Franzen G et al (1987) (quoted by Stell PM)[8].They stated that there was no relationship between age and ploidy but gave no details to support this view. Our study could not establish a statistically significant

Table 1: Comparison of cell cycle fractions and Histological grading

	G0/G1 Phase	S Phase	G2+M Phase
Well Diff	68.60 %	24.42 %	7.72 %
Mod Diff	55.91 %	19.68 %	22.62 %
Poorly Diff	31.44 %	32.83 %	45.60 %

Table: 2 Comparison of Cell Cycle Fractions and Clinical staging

	G0/G1 Phase	S Phase	G2+M Phase
Stage I	100%	2.09%	0%
Stage II	46.94%	25.64%	32.66%
Stage III	58.42%	26.20%	18.23%
Stage IV	53.93%	32.50%	13.57%

Table 3: Comparison of mean G0/G1 phase fractions among histopathological grades of oral squamous cell carcinoma.

Histopathological grade	Mean (%)	Std. Deviation	F value	p Value	Result
Well differentiated SCC (n=17)	68.60	29.44	4.979	0.012	Significant
Moderately differentiated SCC (n=16)	55.90	33.91			
Poorly differentiated SCC (n=10)	31.43	20.68			

Table 4: Comparison of mean G2/M phase fractions among histopathological grades of oral squamous cell carcinoma

Histopathological grade	Mean (%)	Std. Deviation	F value	p Value	Result
Well differentiated SCC (n=17)	7.71	18.45	6.307	0.004	**Significant**
Moderately differentiated SCC (n=16)	22.62	27.45			
Poorly differentiated SCC (n=10)	45.60	36.40			

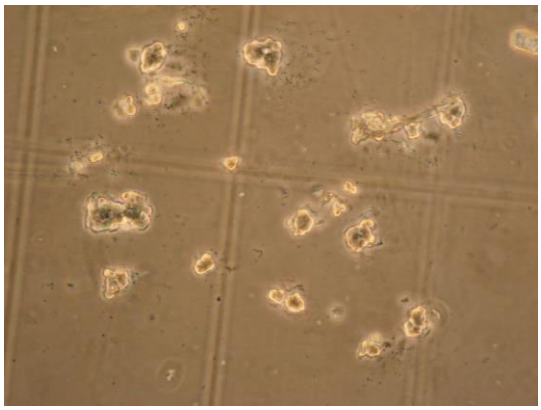

Figure 1: Unstained Squamous cells on Hemocytometer.

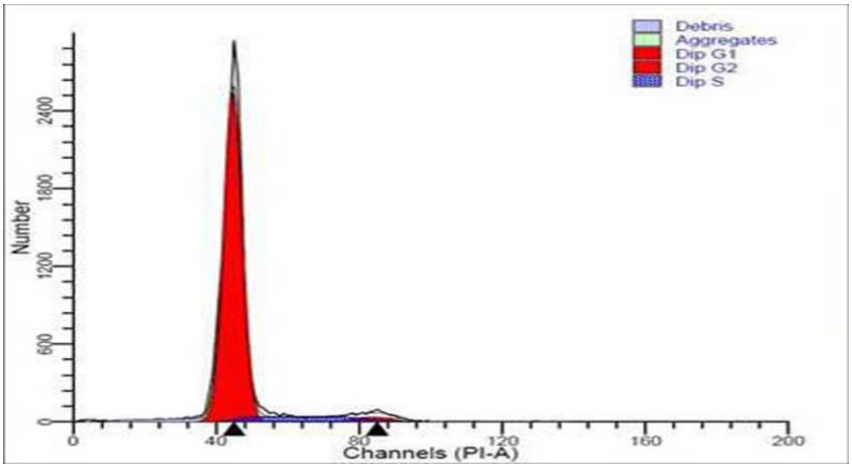

Figure 2: Histogram of a Diploid Tumour

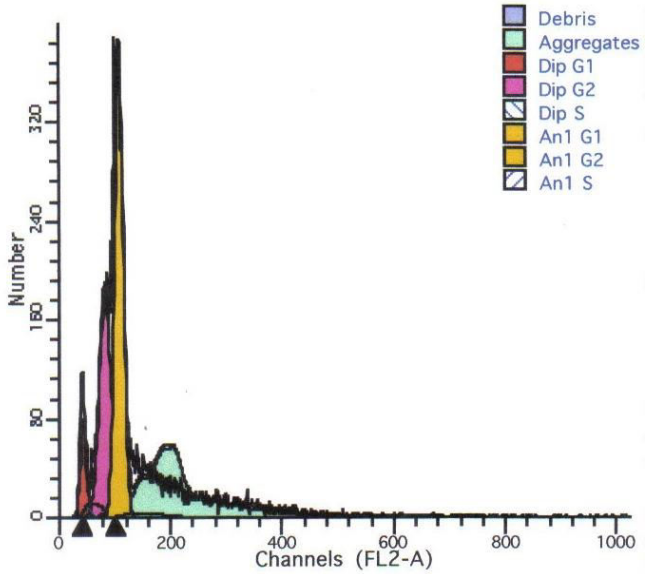

Figure 3: Histogram of an Aneuploid Tumour

correlation between clinical staging and ploidy status. Wang XL (1990)[11], Fukushima T (1997)[12] studied 145 patients of oral squamous cell carcinoma and found no correlation between clinical stage and ploidy status. King W.W et al (1995)[13] found 70% aneuploid tumours in stages I and II and 62.5% aneuploid tumours in stage III and IV. Thus the incidence of aneuploidy was not found to increase with clinical stage. Baretton G et al (1995)[14] in their study on paraffin embedded specimens of oral squamous cell carcinomas, failed to establish any relationship between tumour stage and ploidy. Our results are in contrast with Chen RB (1989) (quoted by Stell PM)[8] and Suzuki K (1994)[15], who reported a strong correlation between ploidy and clinical staging. There was no significant difference in the mean G0/G1 phase fractions of stage II, stage III and stage IV tumours (0.05<P).The mean G2/M fractions showed a progressive decrease as the clinical stage increased (stage II-32.66%, stage III - 18.23%, Stage IV - 13.57%) but this difference was not statistically significant. We could not find any previous study for comparing our results. Despite the progressive increase there was no significant difference in the mean S phase fractions of stage II, stage III and stage IV tumours (0.05<P).These results are in contrast with Johnson TS et al and Chen RB et al. Our findings are similar to those of Mohr C et al who found no correlation between S phase fraction and clinical staging and Suzuki K et al (1994).[15]

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