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FLOW CYTOMETRIC ANALYSIS IN TONGUE SQUAMOUS CELL CARCINOMA: RELATION TO DISEASE FREE SURVIVAL

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ABSTRACT

DNA ploidy was investigated in 19 formalin fixed-paraffin embedded blocks of tongue squamous cell carcinoma. Single cell suspensions for flow cytometric analysis were prepared according to Hedley, (1989). The DNA ploidy and histogram were calculated and compared with the incidence of tumor recurrence. DNA aneuploidy was observed in 8 (42%) tumors. The mean DNA index was 1.46 with a range of 1.74 to 1.89. Recurrence of the primary lesions was seen in ten cases, distant metastasis found in three cases while only one case showed both local recurrence and distant metastasis. The results indicate positive relationship between aneuploidy and recurrence of tongue squamous cell carcinoma.

INTRODUCTION

Squamous cell carcinoma is the most common form of malignancy of the oropharyngeal region, representing 90% of all tumors in this area. The tongue was represent the most common site (36.10%) for squamous cell carcinoma [12]. it has been well documented that the 5-year survival rate for these tumors vary according to their anatomic location, clinical stage and histologic grade [10,11].

DNA ploidy is a sensitive and reliable marker of the premalignant[14] and the malignant states [1,4,5,7,9]. Several studies have shown the prognostic significance of DNA ploidy in various malignancies [4,5,7,9]. Some reports have demonstrated a positive relationship between DNA aneuploidy and incidence of recurrence in tongue squamous cell carcinoma (SCC) [2, 17]. However this relation is not shown in other study [3]

The purpose of this study was to ascertain whether the DNA ploidy is a prognostic indicator in tongue SCC.

MATERIAL AND METHODS

Nineteen formalin-fixed and paraffin-embedded surgical specimens, previously histologically diagnosed as tongue SCC were selected from archives of Surgical Pathology unit, Pathology Department, National Cancer Institute (NCI), Cairo University from January 2002 to December 2007. Charts were reviewed for evidence of local recurrence, regional metastasis and status at last follow up.

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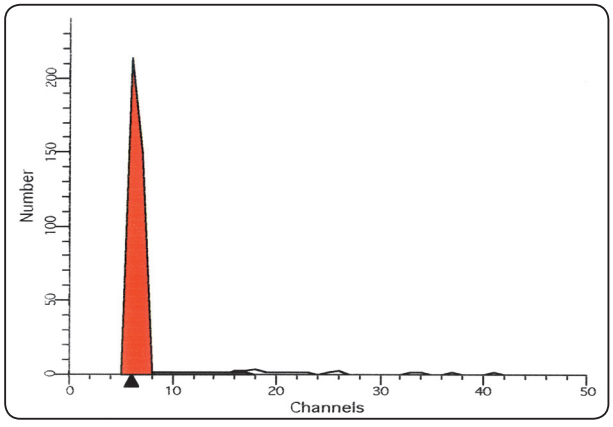
Tumor specimens were examined to verify the existence of at least 50% of tumor tissue. For flow cytometric analysis, single cell suspensions were prepared by the method described by Hedley[6]. The sections were deparafinized in xylene for 10 minutes, rehydrated in ethanol at decreasing concentrations (100%, 95%, 70%, and 50%), washed with phosphate buffered saline, and then incubated in 2 ml of 0.5% pepsin solution ((Sigma p6887) adjusted to pH 1.5 for 90 minutes at 37C° in water bath, with intermittent tapping every 5 minute for mixing. Next, cells were filtered twice through a 50 μm pore size nylon mesh to remove debris and cell clumps that may give abnormal extra peaks or background noise during analysis. After centrifugation (10 minutes at 2500 rpm), nuclear pellets were treated with 2 ml of a ribonuclease solution (Sigma). The cell suspension was incubated at 37c° for 1 hour. At the completion of incubation, the cell suspension was centrifuged and resuspended in a propodium iodide solution. The stained specimen was kept in the dark for 6-8 hours at 4 c° before flow cytometry.

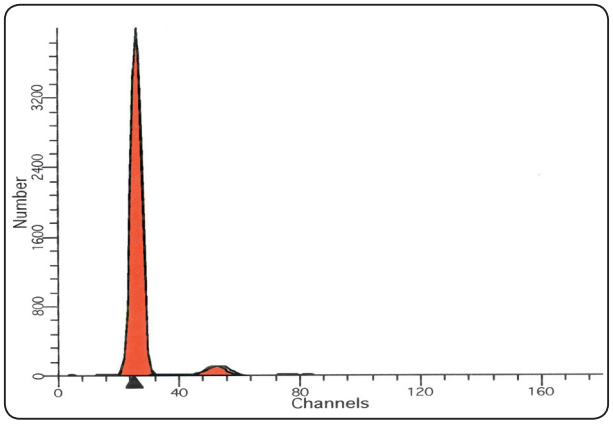
Analysis was performed using the Mod fit software in the FAC scan flow cytometer, Becton & Dickinson (B-D) sunny vale, CA. criteria for DNA analysis were performed following the guidelines described by Skankey et al [15]. The DNA histograms were classified as diploid when only one symmetrical G0/G1 peak was present. If one asymmetrical or two G0/G1 peaks were present, the DNA histograms were considered aneuploid. The DNA index was calculated from the ratio of the modal channel numbers of aneuploid peaks to the modal channel number of the control peak. Samples with a DNA index between 0.9 and 1.1 were considered diploid.

RESULTS

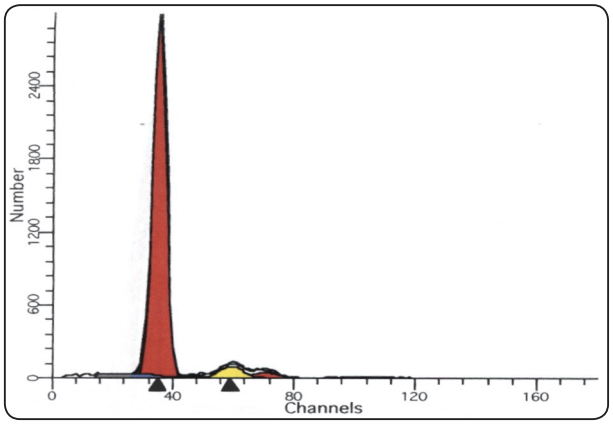
At the end of the follow up period, 10 patients had local recurrence, 3 had distant metastasis and one patient had both local recurrence and distant metastasis. Only 5 cases had no local recurrence. Flow cytometric analysis of the 19 cases with tongue SCC revealed that abnormal DNA content or aneuploidy was identified in 8 tumors (42%). The mean DNA index was 1.46 with a range of 1.74 to 1.89. The mean coefficient of variation was 5 with a range of 2.6 to 6.3. The remainder of patients had diploid tumors (58%) with a mean coefficient of variation of 4 and a range of 3.7 to 5.8. Among patients with aneuploid tumors, 4 cases had recurrent disease, 2 cases had distant metastasis and 1 case had both local recurrence and distant metastasis. Only one case with aneuploid tumor showed no local recurrence or distant metastasis. Whereas, patients with diploid tumors showed local recurrence in 5 cases, distant metastasis in 1 case and 5 cases had no local recurrence or distant metastasis.



DNA frequency histogram of a diploid standard (normal human lymphocytes),



DNA frequency histogram showing diploid cell population



DNA frequency histogram showing aneuploid cell populations

DISCUSSION

Flow cytometric DNA analysis of solid tumors has gained favor in the past decade as a prognostic indicator. Interest in defining the use of this technique arises because of the varied biologic behavior of squamous cell carcinomas of the head and neck, specifically the tongue. Even in those with localized diseases, the clinician often cannot predict which patients will develop local recurrence, cervical disease or a second primary tumor. Since much clinical information has already been accumulated and squamous cell carcinoma of the tongue will be detected in only a few patients in any one year, most studies have been retrospective [8, 13].

In this study of 19 cases of tongue SCCs, the aneuploidy rate was 42%. This is similar to that 42% incidence seen in 36 tongue SCCs reviewed by **Saito et al** [16] but this is less than the 66% incidence seen in 15 paraffin-embedded tumor specimens of tongue SCCs examined by **Sickle Santanello et al** [13]. However, **Farrar et al** [3] found an aneuploidy rate of only 30% in their review of 60 cases with tongue SCCs

The present study has shown that among tongue SCCs, the frequency of local recurrence was higher in the aneuploid cases than in the diploid cases. The frequency of the distant metastasis was also higher in the aneuploid cases than in the diploid cases. These data indicate that ploidy status is a useful diagnostic variable which predicts the metastatic propensity of tongue SCCs.

This was in agreement with the study of squamous cell carcinoma of the tongue by **Sickle Santanello et al** [13]. Fifteen paraffin-embedded tumor specimens were examined, with 10 being aneuploid and 5 diploid. Patients with aneuploid tumors had a 5-year survival rate of 33% whereas patients with diploid tumors had a 5-year survival rate of 80%.

In contrast to this result**, Saito et al** [16] found no obvious difference in the incidence of the recurrence was noted between the diploid and the aneuploid cases. Also, **Farrar et al** [3] reported no significant differences between diploid and aneuploid patients when disease free survival, local recurrence, regional metastasis and incidence of second aerodigestive tract primary tumors were analyzed.

Based on preliminary data from this study, however, we are of the opinion that the ploidy status may be used as a prognostic indicator in squamous cell carcinoma of the tongue.

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