

## A Study of Anticancer Drug Treatment on Satellite Associations in Oral Squamous Cell Carcinoma

Pankaj Gadhia<sup>1\*</sup>, Bhumika Desai<sup>2</sup>, Girish Jani<sup>3</sup> and Rahul Thumbar<sup>1</sup>

<sup>1</sup>*Department of Biosciences, Veer Narmad South Gujarat University, Surat 395007, Gujarat, India*

*E-mail: pankajkgadhia@gmail.com*

<sup>2</sup>*C.K.Pithawalla Institute of Pharmaceutical Science and Research, Surat, 395007, Gujarat, India*

<sup>3</sup>*SSR college of Pharmacy, Silvassa, India*

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**ABSTRACT** Oral squamous cell carcinoma is one of the most prevalent diseases worldwide. Satellite associations are known to play an important role in the pathogenesis of certain diseases including cancer. The present work aimed to study the frequency of satellite association in human peripheral blood lymphocyte culture of freshly diagnosed oral squamous cell carcinoma patients. *In vitro* anticancer drugs (5-Fluorouracil {5-FU} and Cisplatin) were studied for the frequency of satellite association. Results showed significant impact of chemotherapeutic agents on the frequency of satellite associations of different groups of acrocentric chromosomes.

### INTRODUCTION

Squamous cell carcinoma of the head and neck (SCCHN) and its subset, oral squamous cell carcinoma (Oral SCC), arise through an accumulation of genetic alterations, including chromosomal alterations, DNA changes (for example, mutations, amplifications, or deletions), and/or epigenetic alterations, such as changes in methylation that affect genetic regulation (Forastiere et al. 2001; Mork et al. 2001). The nucleolar organizer regions (NORs) of man, located on the short-arm secondary constrictions of chromosomes No. 13-15 and 21-22, are the sites of the 18s and 28s ribosomal RNA (rRNA) genes (Henderson et al. 1972; Evans et al. 1974). The phenomena of satellite association (SA) involving a specific position of the satellite chromosomes with their satellite directed towards each other was first observed in mitotic human chromosomes (Ferguson Smith and Handmaker 1961; Ohno et al. 1961) and was found also in meiotic chromosomes (Ferguson Smith 1964). The formation of SA's has often been attributed

to the involvement of satellite chromosomes in nucleolar formation. The sticky nucleolar material has a tendency to hold the associated chromosomes together through mitosis (Hsu 1965). The fusion of two or more nucleoli mechanically stretches the nucleolar forming chromosome segment with risk of breakage. If breaks occur in more than one of the chromosomes involved, the closeness of the broken ends would predispose to translocations and the SA would thus be active also in the origin of translocation between satellite chromosomes. A high incidence of SA has often been considered as predisposing to an increased tendency of nondisjunction in satellite chromosomes (Anuradha et al. 2002).

The aim of the present investigation was to analyse satellite association frequency in freshly diagnosed oral squamous cell carcinoma patients without chemotherapeutic treatment and further the study was extended to *in vitro* treatment of 5-FU and Cisplatin in the lymphocyte cultures.

### MATERIALS AND METHODS

#### Lymphocyte Culture

Lymphocyte cultures were set up by Hungerford (1965) with slight modifications (Gadhia et al. 2004). Heparinized whole blood (0.5 ml) was added to a mixture containing 5 ml of culture medium RPMI 1640 and 0.1 ml phytohe-

\*Address for correspondence:  
Prof. P. K. Gadhia  
Department of Bioscience,  
Veer Narmad South Gujarat University,  
Udhna Magdalla Road,  
Surat, 395007, Gujarat, India  
Telephone: (0261) 2227141-46  
Fax: (0261) 2227312  
E-mail: pankajkgadhia@gmail.com

maggglutinin (Lectin). Then the culture vials were kept in *HERA cell*<sup>150</sup> CO<sub>2</sub> incubator for 71 hours, at 37 °C with 5 % CO<sub>2</sub>. Then 0.1 ml demecolcine solution was added at last 2 hours of incubation period to arrest cells at metaphase. The cells were collected by centrifugation, resuspended in a prewarmed hypotonic solution (KCL, 0.075 M) for 20-25 minutes and fixed in chilled methanol/ acetic acid (3:1 v/v) solution (Carnoy's fixative). Then drops of cell suspension were allowed to fall from at least 2.5 feet height on pre chilled and chemically cleaned slides. These slides were air dried on a hot plate at 50-60 °C. All slides were blind coded and labelled soon after assuring about well spread chromosome.

### Nucleolar Organizing Regions Staining by AgNO<sub>3</sub>

Nucleolar Organizing Regions (NOR) staining was performed according to the silver nitrate (AgNO<sub>3</sub>) method of Verma and Babu (1995). AgNO<sub>3</sub> Solution was prepared by mixing 4g AgNO<sub>3</sub> in 8 ml distilled water and stored light protected at 4°C. Few drops of silver nitrate solution were applied on slide along with 2 % gelatine solution mixed with formic acid. Heat was applied till brownish colour appeared. Prepared slides were blind coded and scored for observations of NORs.

### Criteria for Consideration of Satellite Associations

- All chromosomes associated were confirmed to be acrocentric.
- All associated chromosomes were inter-oriented.
- Distance between their centromeres was not more than the thickness of chromatids of the same.

### Experimental Protocol

Total of 32 oral squamous cell carcinoma patients (blood was collected from Lions Cancer Detection Centre, Surat) were studied along with 32 age and sex matched controls. Written consent of patients was taken. All control and patients were divided in 6 groups.

**Group A:** Total 12 PBL cultures of healthy individuals (control) without chemotherapy.

**Group B:** Total 12 PBL cultures of freshly diagnosed oral SCC patients. (Without chemotherapy)

**Group C:** Total 10 PBL cultures of healthy individuals exposed to 30 ng/30µl 5-FU added at 24 hours in lymphocyte culture.

**Group D:** Total 10 PBL cultures of oral SCC patients exposed to 30 ng/30µl 5-FU added at 24 hours in lymphocyte culture.

**Group E:** Total 10 PBL cultures of healthy individuals exposed to 15 ng/15µl cisplatin added at 24 hours in lymphocyte culture.

**Group F:** Total 10 PBL cultures of oral SCC patients exposed to 15 ng/15µl cisplatin added at 24 hours in lymphocyte culture.

Slides were prepared from all 64 cultures and scored. Results were analysed using student t-test with aid of SPSS software.

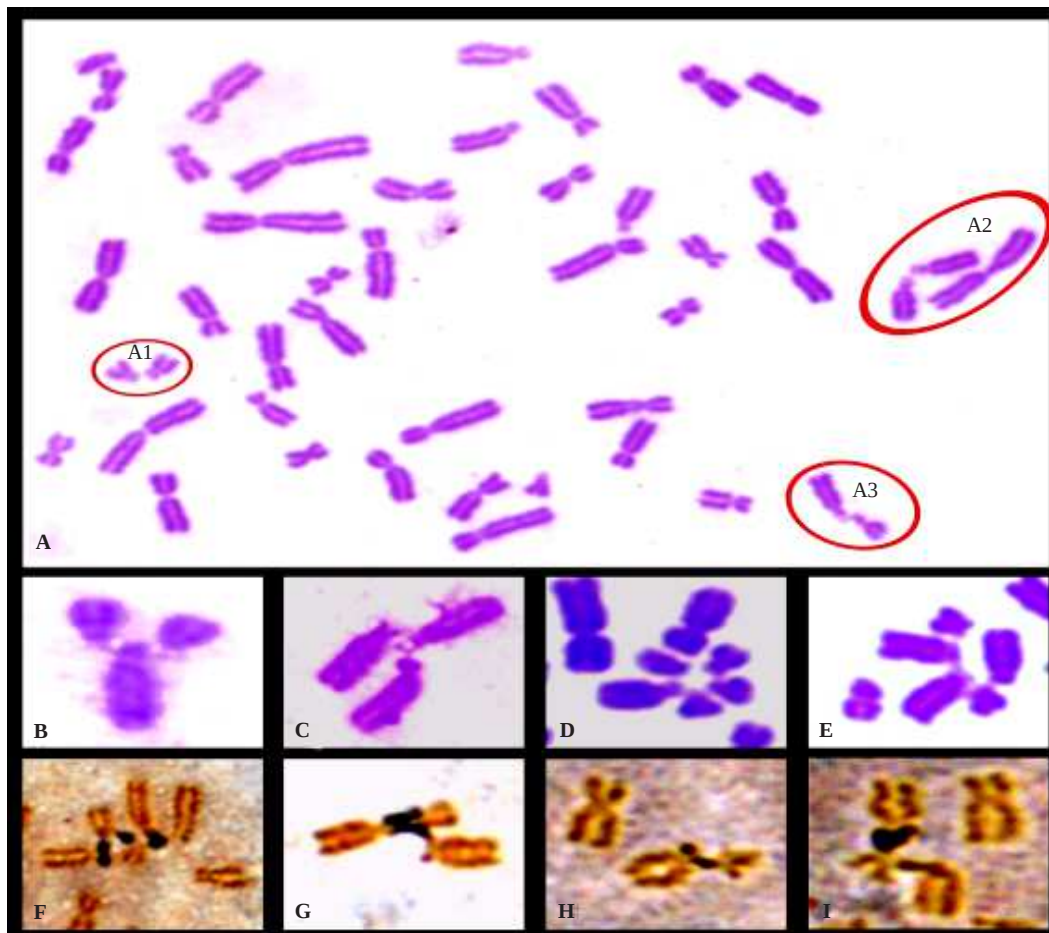
### RESULTS

Table 1 shows distribution of satellite associations in various study groups. It shows total number of metaphase analysed and percentage satellite association amongst above mentioned study groups. Figure 1 shows various types of satellite associations found in oral SCC patients.

It has been observed that high percentage of satellite association (29.65 %) was found in group D patients exposed to 5-FU *in vitro* in comparison to control (15.02 %). In case of patients exposed to Cisplatin percentage satellite

**Table 1: Distribution of satellite associations of D and G group chromosomes in various study groups**

| Groups                                       | Total no. of metaphases analysed | Total no. of S.A found |       | Association between 2 chromosomes |       | Association between 3 chromosomes |      | Association of more than 3 chromosomes |      |
|----------------------------------------------|----------------------------------|------------------------|-------|-----------------------------------|-------|-----------------------------------|------|----------------------------------------|------|
|                                              |                                  | No.                    | %     | No.                               | %     | No.                               | %    | No.                                    | %    |
| Group A Control (without Cx)                 | 1187                             | 89                     | 7.49  | 74                                | 6.23  | 15                                | 1.26 | -                                      | -    |
| Group B Cancer Patient (without Cx)          | 1183                             | 209                    | 17.66 | 189                               | 15.97 | 17                                | 1.43 | 03                                     | 0.25 |
| Group C Control (effect of 5-FU)             | 965                              | 145                    | 15.02 | 129                               | 13.36 | 16                                | 1.65 | -                                      | -    |
| Group D Cancer Patient (effect of 5-FU)      | 951                              | 282                    | 29.65 | 241                               | 25.34 | 39                                | 4.1  | 02                                     | 0.21 |
| Group E Control (effect of cisplatin)        | 979                              | 189                    | 19.30 | 152                               | 15.52 | 32                                | 3.26 | 5                                      | 0.51 |
| Group F Cancer Patient (effect of cisplatin) | 948                              | 258                    | 27.21 | 204                               | 21.51 | 47                                | 4.95 | 7                                      | 0.73 |



**Fig. 1. Some examples of satellite associations**

A- Satellite association between GG (A1), DD (A2) and DG (A3), B- DGG association, C- DDD association, D- 4GD association, E- 3D2G association, F- 3D2G association (NOR staining), G- DDG association (NOR staining), H- DG association (NOR staining), I- DDG association (NOR staining).

association was found to be 27.21 in comparison to that of control, that is, 19.30%.

Without any chemotherapeutic exposure, the percentage values of satellite associations were found to be 7.49 and 17.66 for control and cancer group respectively. Further the pattern of association between any 2 of D and/or G group chromosomes was observed as

5-FU > Cisplatin > without chemotherapy, for both control and cancer patients.

Whereas in case of association between 3 acrocentric chromosomes the pattern differed as Cisplatin > 5-FU > without chemotherapy. A pattern of SA distribution was not in higher frequency as expected in case of a rare type of association of more than 3 chromosomes.

Table 2A shows mean distribution of satellite associations of acrocentric chromosomes in oral SCC patients and controls without exposing their lymphocytes to chemotherapy.

A significant difference was observed in DD, DG, GG and DDD associations in cancer patients in comparison to that of control (P value < 0.05).

Table 2B shows mean distribution of satellite associations of acrocentric chromosomes in lymphocytes of oral SCC patients and controls exposed to 5-FU. A significant difference was observed in DD, DDG and DDD associations in oral SCC patients in comparison to that of control (P value < 0.05). Table 2C shows mean distribution of satellite associations of acrocentric

**Table 2A: Mean distribution of satellite associations in control and patients without chemotherapy**

| Groups         | Type of satellite association |        |       |       |       |        |       |         |       |
|----------------|-------------------------------|--------|-------|-------|-------|--------|-------|---------|-------|
|                | DD                            | DG     | GG    | DDG   | DGG   | DDD    | GGG   | 3DG/3GD | DDGG  |
| Control        | 1.6                           | 3.45   | 1.17  | 0.25  | 1.01  | 00     | 00    | 00      | 00    |
| Cancer patient | 4.73                          | 8.03   | 3.21  | 0.42  | 0.5   | 0.33   | 0.16  | 0.16    | 0.084 |
| P value        | 0.017*                        | 0.027* | 0.03* | 0.204 | 0.144 | 0.014* | 0.278 | 0.076   | 0.164 |

(\* - Significant at P &lt; 0.05)

**Table 2B: Mean distribution of satellite associations in control and patients exposed to 5-FU**

| Groups         | Type of satellite association |      |       |        |       |        |     |         |      |
|----------------|-------------------------------|------|-------|--------|-------|--------|-----|---------|------|
|                | DD                            | DG   | GG    | DDG    | DGG   | DDD    | GGG | 3DG/3GD | DDGG |
| Control        | 3.8                           | 6.4  | 2.7   | 0.6    | 0.7   | 0.1    | 0.2 | 0.0     | 0.0  |
| Cancer patient | 9.3                           | 11.2 | 3.6   | 1.7    | 0.8   | 1.2    | 0.2 | 0.2     | 0.0  |
| P value        | 0.024*                        | 0.08 | 0.279 | 0.046* | 0.392 | 0.013* | 0.5 | 0.165   | -    |

(\* - Significant at P &lt; 0.05)

**Table 2C: Mean distribution of satellite associations in control and patients exposed to Cisplatin**

| Groups         | Type of satellite association |       |        |       |       |       |        |         |       |
|----------------|-------------------------------|-------|--------|-------|-------|-------|--------|---------|-------|
|                | DD                            | DG    | GG     | DDG   | DGG   | DDD   | GGG    | 3DG/3GD | DDGG  |
| Control        | 6.5                           | 7.4   | 1.3    | 1.6   | 0.7   | 0.8   | 0.1    | 0.4     | 0.1   |
| Cancer patient | 5.4                           | 9.9   | 5.1    | 1.7   | 1.0   | 0.7   | 1.3    | 0.3     | 0.4   |
| P value        | 0.317                         | 0.233 | 0.054* | 0.452 | 0.336 | 0.416 | 0.008* | 0.357   | 0.181 |

(\* - Significant at P &lt; 0.05)

tric chromosomes in lymphocytes of oral SCC patients and controls exposed to Cisplatin. A significant difference was observed in associations between only G group chromosomes in Cancer patients compared to control (P value < 0.05).

## DISCUSSION

There have been few reports on frequency of satellite associations in healthy individuals (Rosenkrans and Hozler 1972; Ray and Pearson 1979; Kumagai 1982; Melaragno et al. 1990); however, the frequency of satellite associations was studied in various age groups (Mattevi and Salzano 1975; Liem et al. 1977; Vormittag 1980; Lezhava 1984). There is a paucity of information on the study of satellite association with special reference to cancer (Guleria et al. 2005). In present study we have selected Oral SCC since the prevalence of this cancer is higher in region of south Gujarat (Gadhia et al. 1995). We have studied 12 freshly diagnosed Oral SCC patients along with 12 age and sex matched controls. The PBL cultures were set up in case of freshly diagnosed cancer patients and the same was treated with anticancer drugs namely 5- Fluor

ouracil and cisplatin, where the frequency of satellite association of 2 acrocentric chromosomes and 3 acrocentric chromosomes were higher compared to non-treated cancer control patients (Table 1). Results showed that there is a significant difference in the frequency of SA involved in D group acrocentric chromosomes (Table 2A).

Further the study was extended in total of 10 PBL cultures of Oral SCC patients along with 10 age and sex matched controls. 5-FU was added in both set of cultures at the final concentration of 30 ng/30 µl after 24 hours of initiation of culture. It is known that 5-FU is an antimetabolite. In case of 5-FU treatment we have noted higher frequency of SA between DD, DDG and DDD type of acrocentric chromosomes as compared to control (Table 2B), which was significant at the level P < 0.05.

On the other hand exposure to cisplatin at the concentration of 15 ng/15 µl after initiation of 24 hours of culture showed significant involvement of G group chromosomes. In addition the frequency of GGG association in oral cancer patients was significantly higher compared to that of control. We have also noted the frequency of GG type of association was high when exposed to cisplatin.

It is interesting to note here that the association between the D and G group chromosomes vary after exposure to different anticancer drugs. It is already established that higher frequency of SA has been observed in case of certain pathological conditions, for example, parents of Down syndrome patient. Similarly high frequency of SA in present study could throw some light on the effect of anticancer drugs on the selective D and G group chromosomes with special reference to SA. It could suggest a selective inhibition of rRNA synthesis especially on acrocentric part of chromosome which could lead to a high frequency of SA. More such studies are required before we arrive to a meaningful conclusion.

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