CYTOGENETIC ALTERATIONS IN ORAL SQUAMOUS CELL CARCINOMA DETECTED BY KARYOTYPING (G-banding)

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Abstract

Oral carcinogenesis is a multistep process in which multiple genetic events occurs that alter the normal functions of genes. Oral squamous cell carcinoma (OSCC) is one of the most common malignant tumours in India. Many patients still die of the disease in spite of therapeutic procedures being improved. In our study 30 OSCC cases and 10 controls were studied by means of karyotyping using G-band technique, out of which 5 chromosomal abnormal patients were found, in which chromosome 18 deletion being prominent. It is also site specific, 4 out 5 patients with karyotype abnormalities had the tumour on buccal mucosa. It was also noted, most of patients with chromosomal abnormality were tobacco chewers, suggesting chemical carcinogens in tobacco has an impact on the chromosomes.

Key words: Oral squamous cell carcinoma, chromosome 18, karyotype

Introduction

The number of genetic disorders known has grown to a monumental proportion to about 4000. It is estimated that every individual is a carrier of five to eight detrimental genes. Fortunately most of these are recessive and therefore do not cause serious phenotypic defects. The OSCC is the sixth most frequent cancer in the world (1) The genetic changes occurring in OSCC has become a very interesting field in dentistry especially in Oral and Maxillofacial Pathology. Earlier habit, environment, radiation were thought in the etiopathogenesis of carcinoma, but recent advances has revealed genetics also plays a vital role in OSCC.

The Karyotype of OSCC contains different patterns of chromosomal aberrations which includes numerical and structural changes. Recently cytogenetic data obtained from HNSCC(Head and Neck Squamous cell carcinoma) showed gain of chromosomes 3q, 8q, 9q, 20q, 7p, 11q13 & 5p and losses of 3p, 9p, 21q, 9q, 5q, 13q, 18q, 8p (2).

Earlier stage tumors tend to have more simple karyotype. However, within every pathological stage some tumors have more complex karyotype. It has been difficult to assemble a "typical" cascade of genetic evolution that has broad applicability in SCC (3).

Conventional karyotyping analysis, which is highly specialized work and time consuming even for experienced technicians, provides useful information about structural abnormalities in chromosomes, but data concerning chromosomal sites with frequent gains and losses are very limited in oral cancer. In this study we performed cytogenetic analysis of 30 OSCC patients and 10 controls by karyotyping (G-banding).

Materials and Methods

30 samples of peripheral venous blood were taken from the patients reported to Rajah Muthiah Dental College and Hospital, Chidambaram, diagnosed as OSCC. Informed consent was obtained from all patients prior to sampling. This work was performed with authorization of ethical committee (Annamalai University). Medical records of all patients were examined to obtain clinical and histopathological data. None of the patients had undergone radiation, chemotherapy or surgery of the lesion was taken for confirmation of histopathological diagnosis of OSCC. Histopathological classification was based on Broder's classification.

Chromosome preparation and cytogenetic analysis were carried out by standard techniques as previously described by Rooney DE[4]. 0.5 ml of heparinised blood was added to each 5 ml of supplemented RPMI1640 medium. 0.1 ml of phytohaemagglutinin (PHA) [GIBCO] was added to the culture. The vials were incubated for seventy hours at 37ºC to which colchinin was added after centrifugation. 10ml of hypotonic (0.075m KCl) solution was added. Again after incubating for 20mts it is centrifuged at 1500rpm. 10ml of freshly prepared fixative was added in drops to the extracted solution. The tubes are again centrifuged with two or more washings until

a white cell pellet was obtained with clear fixative seen. Two or three drops of cell suspension were dropped onto wet, cold, grease free slide. The slides were left to dry on the hot plate for 7mts. It is then stained with Giemsa's solution which was washed and air dried. The resultant slide was viewed under microscope to evaluate karyotyping. The karyotype description and requirements are based on ISCN (1995). The metaphase cells were digitally imaged with a cytovision ultra system (Applied Imaging, Santa clara-CA). Chromosomal analysis involves first counting the number of chromosomes present in a specified number of cells i.e. metaphase spreads, followed by careful analysis of the banding pattern of each individual chromosome. Usually the total chromosome count is determined in 15-20 metaphase spreads. If the total chromosome count is 46, G group was checked to find the sex of the individual. Detailed analysis of the banding pattern of individual chromosomes were carried out on both members of each pair of homologous in approximately 3-5 metaphase spreads, which showed high quality banding. The chromosomes were carefully analyzed to rule out numerical and structural abnormalities.

STASTICAL ANALYSIS;

All 30 cases were subjected to statistical analysis using logistic regression model and results were obtained.

Result

The study group comprised of 10 controls and 30 OSCC patients of which 16 were females and 14 male patients, 5 (17%) patients of the study group exhibited chromosomal abnormalities and they were more than 45 years of age. Out of which 3 (60%) were females and 2 (40%) male. Out of above 5 cases 4 (80%) were tobacco chewers while 1(20%) was a smoker. Among this group who had chromosomal abnormality 4 (80%) patients had OSCC on buccal mucosa and 1(20%) in lateral border of tongue.

Among 5 chromosomal abnormal patients numerical aberration was noted in 3(60%) cases and structural abnormality was noted in 2(40%) cases. The Chromosome 18 loss (monosomy) was noted in 2(40%) cases. Chromosome X addition (trisomy) was documented in 2 cases. Case no 1 showed monosomy of chromosome 18 alone while case 2 showed monosomy of chromosome 18,16 and trisomy of chromosome X. Trisomy of chromosome X alone was noted in case no 21. Translocation was noted in two patients (both were males) (case 9 and case 26). Case 9 showed deletion of 1q23-25 arm and translocation of chromosome 1 and 22. The region of translocation being q31 region of chromosome 1 and qter region of chromosome 22. Case 26 showed translocation between chromosome 9 and 18 with region of translocation between pter and q21 respectively. The entire control group showed normal karyotype.

STASTICAL DATA;

Logistic Regression Test

The goal of logistic regression is to find the best fitting model is describe the relationship between the dichotomous characteristic of interest (response or outcome variable) and a set of independent (Predictor or Explanatory) variable. Logistic regression generates the coefficients to predict the probability of presence of the character of interest.

Logic (P) = b0 – b1 age + b2 Sex + b3 Habit where b0, b1, b2 are the coefficient for estimating the probability of positivity. Logistic regression with 3 predictor independent variables namely Age, Sex and habit was applied to predict the chances of chromosomal abnormality

From the results it was found logistic regression model can predict the symptom of positivity i.e. chromosomal abnormality at 95% confident level. (P = 0.05). Since the model is statistically significant the coefficient of each predictor variable used to predict the chances of chromosomal abnormality in OSSC. Based on the result it was found female patient the age above 45 years with tobacco abuse have 42.8% of getting chromosomal abnormality.

Discussion

Cytogenetic analysis of OSCC has revealed high complexity of karyotype which is highest among the solid tumors[5]. In our study out of 30 cases of OSCC, 5(17%) cases showed chromosomal abnormality by G-banding whereas control group (10 cases) did not show any chromosomal abnormality. This finding is almost consistent with the study done by Ravindran[6] et al. In their study on tissue samples of 75 OSCC patients they noted chromosomal abnormalities in 12(16%) patients. Their study was conducted in South Indian population as was the present study. In most of the studies tissue samples were used for harvesting cultures. We used peripheral blood for our study which gave promising results? This was based on the proposition made by Johanson[7] et al., It states that heritable acquired characteristics of neoplastic cells brought about by changes in the genetic material, does not imply that their neighboring non neoplastic cells are without importance. Tumour cells face not only each other but also surrounding stromal tissue and the systemic antitumor response including the 'immune surveillance". This proposition supports

even peripheral blood which is a non-neoplastic tissue can be used for cytogenetics. Schantz et al[8] used bleomycin a mutagenic agent on peripheral lymphocytes of OSCC patients and found chromosomal aberrations.

In the present study loss of chromosome 18 (2 cases) was frequently found which is consistent with the study done by Jin F & Mertens F, one case showed translocation between q arm of chromosome 18 and p arm of chromosome 9. Chromosome 18 is numerically and structurally altered in the present study. Translocation was also noted in chromosome 1 and 22. 18q deletion was noted by Sreekantaiah C[10] et al in OSCC and suggested that there may be putative tumour suppressor gene, loss of which may play role in pathogenesis of tumor. Adding to this Vandyke[11] also found that loss of 18q is very poor prognostic indicator in SCC at many sites including head and neck. He suggested primary target gene is unknown but probably smad2, smad4 and DCC. 18q deletion was also noted by Gollin[12], Bockmuhl U[13], Mitelman F[14]. 18q deletion was frequently noted in colorectal cancer by Jen J[15] et al. They concluded 18q deletion is associated with tumor progression which is prognostic marker for that disease. The chromosome 18 monosomy is seen only in 2 cases. Further studies on south Indian population with respect to chromosome18 will reveal facts about OSCC and chromosome 18.

In the present study 2 cases showed trisomy of chromosome X. This is consistent with study done by Chen YJ[16] et al., They found gains of chromosome X in OSCC by comparative genomic hybridization. Their study was done in Asian population where areca nut quid chewing is common.

In the present study we found deletion of 1q23–25 in one case and translocation in 2 cases. Jin and Mertens [9] found 1q deletion in OSCC which is consistent with our study. They found rearrangement of chromosome1 have been most prevalent in OSCC. On contrary Rao PH[17] et al.. found recurrent deletion at 1p13 in 4 cases out of 11 cases.

In the present study 4 out 5 patients with karyotype abnormalities had the tumor on buccal mucosa. It was also noted that most of patients with chromosomal abnormality were tobacco chewers, suggesting chemical carcinogens in tobacco has an impact on the chromosomes. This view is supported by study done by Saranath D[18] Jussawalla DJ and Desphande[19] documented that there is 7.7 times high risk for OSCC in chewers compared to nonchewers. Scully[5] in his review article suggested herpes simplex virus can act synergistically in vitro with tobacco specific chemical carcinogens in inducing malignant transformation. These findings support the view that chewing habit and poor oral hygiene have an additive effect on oral carcinoma. Ravindran[6] et al. found an association of the DNA oncogenic virus herpes simplex type 1(HSV-1) with oral cancer. Yunis JJ[20] et al., demonstrated cultured human lymphocytes exposed to tobacco carcinogens which are subsequently treated with mutagens induced, deletions and inter chromosomal recombination at fragile sites. He also suggested that tobacco carcinogens increase the potential damage at fragile sites.

In the present study all the patients with chromosomal abnormalities were above 45 years of age. This suggests that genetic alteration in OSCC is a multistep process, in which the older patients have more chance of exposure to mutagens, there by acquiring cancer. Hosseini[21] et al in their study documented all the chromosomal abnormal patients were above 40 years of age which is consistent with our study.

Johanson S[7] et al suggested that two quite distinct neoplasia associated karyotype were found. One is characterized by simple and disease specific abnormality as seen in leukemia and lymphoma, while the other is characterized by multiple and non-specific aberrations as seen in epithelial neoplasia. This view is supported by as Gray JW and Collins C[22]. They suggested banding analysis has been especially useful in identifying causative chromosomal rearrangement in leukemia and lymphomas. However, this approach has been less successful in solid tumor because of the difficulty of obtaining high quality, metaphase representative chromosome preparations and because the high level of chromosomal rearrangement complicates karyotype interpretation. Saunders WS et al, has found no two karyotype were identical which vary in chromosome copy number and structure. This is consistent with our study where different karyotype was seen in all 5 cases.

Conclusion

In the present study because of economical and technical difficulties only fewer samples (30 patients and 10 controls) were taken for the study. In future larger samples of patients can be taken to standardize the chromosome of interest in the same population. Though we got promising results with peripheral blood samples, tissue samples will give better results than blood as suggested by Hosseini FA[21] et al.

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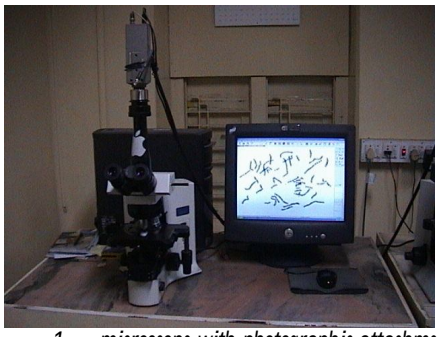
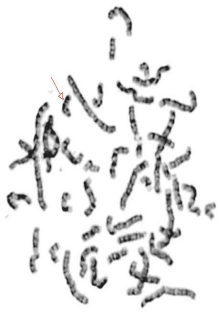
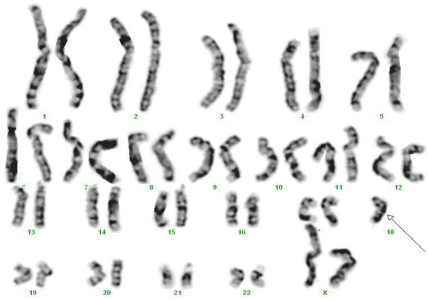


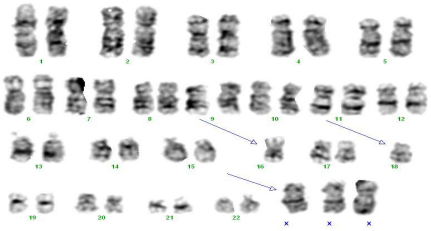
1. microscope with photographic attachments and cytovision software for karyotyping



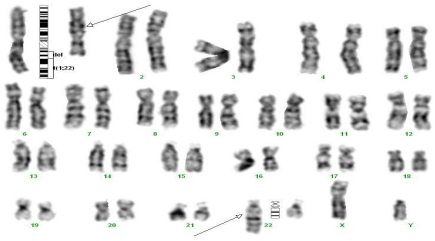
2. metaphase spread showing monosomy of chromosome 18



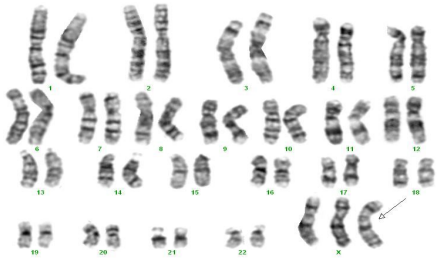
3. karyotype showing monosomy of chromosome 18



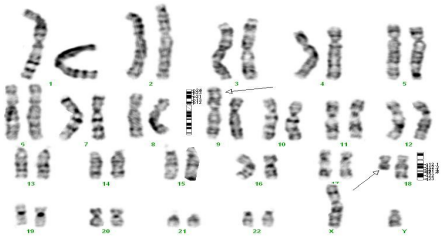
4. karyotype showing monosomy of chromosome 16 and 18 and trisomy of chromosome x



5. karyotype showing deletion of 1q23-25 and translocation between chromosome 1 and 22



6. karyotype showing trisomy of chromosome x



7. karyotype showing translocation between chromosome 9 and 18

Features of OSCC cases- Age, Sex, Habit, Site Histopathologic Diagnosis, composite karyotype Results

Sl. No	Age/ Sex	Habits	Site	Histo Pathological Diagnosis	Composite Karyotype	
1	55/F	Tobacco and Betel Nut Chewing	Right Buccal Mucosa	PD SCC	45,XX – 18	
2	50/F	Tobacco and Betel Nut Chewing	Left Buccal Mucosa	MDSSC	45,XXX,-16,-18	
3	60/ M	No Habits	Right Maxilla	WDSCC	46, XY	
4	60/F	No Habits	Lower Lip	WDSCC	46, XX	
5	52/ M	Smoking Cigarette	Right Buccal Mucosa	WDSCC	46, XY	
6	40/F	No Habits	Left Buccal Mucosa	WDSCC	46, XX	
7	45/F	No Habits	Left Buccal Mucosa	WDSCC	46, XX	
8	60/ M	Tobacco and Betel Nut Chewing	Lower Lip	WDSCC	46, XY	
9	55/ M	Smoking Bidi and Tobacco Chewing	Lateral Border of Tongue	WDSCC	46,XY del (1) (q23-q25) t(1:22) (q31:qter)	
10	65/ M	Tobacco and Betel Nut Chewing	Right Buccal Mucosa	WDSCC	46, XY	
11	56/ M	Bidi Smoking	Right Maxilla	WDSCC	46, XY	
12	45/ M	Tobacco and Betel Nut Chewing	Right Buccal Mucosa	WDSCC	46, XY	
13	45/ M	No Habits	Right Buccal Mucosa	WDSCC	46, XY	
14	58/ M	Cigarette Smoking	Left Buccal Mucosa	WDSCC	46, XY	
15	55/F	Tobacco and Betel Nut Chewing	Right Buccal Mucosa	WDSCC	46, XX	
16	60/ M	Bidi Smoking	Right Buccal Mucosa	WDSCC	46, XY	
17	65/ M	Tobacco and Betel Nut Chewing	Right Buccal Mucosa	WDSCC	46, XY	
18	70/F	Tobacco and Betel Nut Chewing	Left Buccal Mucosa	WDSCC	46, XX	
19	45/F	No Habits	Left Buccal Mucosa	WDSCC	46, XX	
20	58/F	Tobacco and Betel Nut Chewing	Right Buccal Mucosa	WDSCC	46, XX	

21	48/F	Tobacco and Betel Nut Chewing	Left Buccal Mucosa	WDSCC	47,XXX
22	95/F	No Habits	Left Buccal Mucosa	WDSCC	46, XX
23	50/F	No Habits	Left border of Tongue	WDSCC	46, XX
24	38/ M	cigarette Smoking	Right Buccal Mucosa	WDSCC	46, XY
25	35/F	Tobacco and Betel Nut Chewing	Left Buccal Mucosa	WDSCC	46, XX
26	76/ M	Bidi Smoking	Left Buccal Mucosa	WDSCC	46,XY ,t(9:18)(pter:q21)
27	45/F	No Habits	Soft Palate	WDSCC	46,XX
28	40/F	No Habits	Lower Left alveolar region	WDSCC	46, XX
29	61/ M	Tobacco and Betel Nut Chewing	Right Buccal Mucosa	WDSCC	46, XY
30	55/F	Tobacco and Betel Nut Chewing	Left Buccal Mucosa	WDSCC	46, XX

VDSCC Well Differentiated Squamous Cell Carcinoma

MDSCC Moderately Differentiated Squamous Cell Carcinoma

PDSCC Poorly Differentiated Squamous Cell Carcinoma.