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Analyses of Genomic Changes in Chromosome 17 And P53 Gene In Oral Squamous Cell Carcinoma Patients

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Abstract: *Oral squamous cell carcinoma (OSCC) is widely recognized as the most common type of head and neck cancer and is an important cause of death and morbidity . In Indian population, the situation is more alarming since nearly 10% of the cancers that develop annually belongs to this category. Deactivation and unregulated expression of oncogenes and tumor suppressor genes may be involved in the pathogenesis of oral squamous cell carcinoma. The genomic change results in numerical aberrations in chromosomes 17 & p53 gene. The aim of our study was to identify numerical aberrations of chromosome 17, deletion or amplification of p53 gene. This study was performed retrospectively on 30 cases diagnosed with OSCC through FISH technique. Molecular cytogenetic techniques, using fluorescence in situ hybridization with chromosome-specific DNA probes, facilitate the confirmation of presumed chromosomal aberrations with high sensitivity and specificity. Out of 30 cases 29 represent molecular alteration . About 70 % of cases presented chromosome 17 polysomy and only 20% of cases had chromosome 17 monosomy. 58% of samples revealed p53 gene amplification and 37% of them p53 deletion. High frequency of correlation between molecular changes in chromosome 17 and p53 gene with OSCC indicates towards their critical role in development of this disease.*

Keywords*: Oral squamous cell carcinoma, FISH, Chromosome 17, p53 gene*

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I. Introduction

Oral squamous cell carcinoma (OSCC) is widely recognized as the most common type of head and neck cancer and is an important cause of death and morbidity, with a ~50% survival rate over 5 years despite various treatments[1]. OSCC is more prevalent in developing than developed countries[2]. In Indian population, the situation is more alarming since nearly 10% of the cancers that develop annually belongs to this category.

The International Agency for Research on Cancer (IARC) confirmed that smoking of various forms of tobacco (e.g., bidis, pipes, cigars and cigarettes) is carcinogenic in humans[3]. Ethnicity and socio-economic status are related to OSCC, as there is a marked variation with regard to the incidence and mortality from OSCC between different countries, between different geographic locations and between ethnic/racial groups. This may be attributed to exposure to different environmental factors and to specific high-risk habits[4] . The acquisition of genetic instability is an essential step during carcinogenesis. In most tumors, including OSCC, such a genomic change results in numerical and structural chromosomal alteration. High frequencies of chromosome 17 abnormalities have been reported in some human cancers such as breast carcinoma, colon carcinoma and bladder carcinoma. Different studies revealed that cells with polysomy 17 are significantly increased in squamous cell carcinoma, thus, chromosome 17 abnormalities seems to be correlated with carcinogenesis of OSCC [5].

The development of oral and head and neck squamous cell carcinoma occurs in relation with multiple events including mainly: loss of cycle cell control, evasion from apoptosis, telomerase reactivation. Complex interactions between a set of molecules, cell cycle proteins, tumor suppressor genes, oncogenes and the telomerase occur in the multiple step process of carcinogenesis. The control of the cell cycle relies on tumor

suppressor gene, the p53 gene, located on the short arm of chromosome 17p13. During the development of oral and head and neck squamous cell carcinomas regulation pathway is disabled[6].

Mutation in p53, a tumor suppressor gene, is the most common genetic abnormality in human cancers. p53 plays a crucial role in DNA repair and cell-cycle regulation after carcinogen-induced damage to the DNA of the oral epithelium. When a p53 gene mutation occurs, the protective pathway of the cells fails, and malignant transformation may occur in the epithelium. However, when the p53 has accumulated 2 or more mutations, malignant transformation occurs[7]. The development of molecular cytogenetic techniques, such as fluorescence in situ hybridization (FISH), has had a major impact on studies aiming to detect and characterize the genetic changes involved in constitutional diseases and in hematopoietic and solid tumors[6]. Fluorescence in situ hybridization (FISH or ISH) technique using chromosome specific probes, allows a targeted detection of numerical chromosome aberrations in the interphase nucleus, and is generally referred to as 'interphase cytogenetics'[8]. FISH methodology provides a powerful tool for a rapid and sensitive detection of chromosome abnormalities. In addition, FISH is ideally suited for analysis of single cells, and can greatly contribute to insights into the genetic heterogeneity of biological samples. The aim of present study was to assess the numerical aberrations in chromosome 17 and alterations in p53 gene in Oral Squamous Cell Carcinoma Patients in Indian Population, to determine the role of these genetic changes in development of the disease.

II. Materials & Method

Present research was carried out in Department of Anatomy, S.P. Medical College and Associated Group of Hospitals, Bikaner, Rajasthan. Prior approval of hospital's ethical committee was taken. Total 30 OSCC patients were incorporated in the study with their prior approval in the form of Informed Consent Form. Tumor samples were processed by usual techniques for inclusion in paraffin. For each subject, 5 µm sections from the paraffin blocks were stained with hematoxiline-eosine for the establishment of the histopathological type and differentiation stage.

Interphase FISH technique was performed on tissue sections after optimization of the protocol using commercially available probe from Vysis, locus specific identifier LSI TP53/CEP 17 FISH Probe Kit which is intended to detect the copy number of the LSI TP53 probe Spectrum Orange target located at chromosome 17p13.1 and of the CEP 17 (17p11.1-q11.1 Alpha Satellite) probe Spectrum Green Dual Colour target located at the centromere of chromosome 17 according manufacturer's protocol Abbott/Vysis.

Specimen slide of paraffin section is preheated at 56°C & immersed in xylene for 15 min at 40°C. Then the slides were dehydrated by immersion in 100% EtOH . The slides were then air-dried. For pre treatment slide was immersed in 1M NaSCN at 80°C for 30-40 minutes followed by immersion in wash buffer 2 X SSC. Pre hybridization is done with protease buffer for 7 minute at 37°C followed by washing & dehydration in EtOH. The slides were then air-dried and on each plate 10 µl of the probe was added in the selected hybridization area. The smears were covered with a 22x22 mm coverslip, sealed and incubated at 78°C for 10 minutes & then at 37°C for 18 hours. Then, two washes were performed post hybridization, using washing solutions: 0.4 × SSC/0.3% NP40 at 73°C for 2 minutes, and 2 × SSC/0.1% NP40 for 1 minute. The slides were then dehydrated & air-dried and 4',6-Diamidino-2-phenylindole (DAPI II) was added for counterstaining.

The slides were analyzed using a fluorescence microscope equipped with filter sets for DAPI at a magnification of ×100. Images were captured using digital camera. For each subject hybridized signals were counted in 200 interphase nuclei. Using in situ hybridization we analyzed the numerical aberrations of chromosome 17 and p53 gene deletions/amplification in 30 paraffin embedded OSCC samples.

III. Results

In situ hybridization for revealing the numerical aberrations of chromosome 17 and p53 gene deletion/amplification in 30 paraffin embedded OSCC samples was performed **.** 200 nuclei were scored under x100 magnification, using an oil immersion objective and the fluorescent microscope for each defined histological area from the tumor , each nucleus being assessed for the chromosome copy number. The numerical aberrations of chromosome 17 varied from individual to individual. Specifically, only distinct isolated nuclei were counted, and paired signals were scored as single events. We interpreted as monosomy 17 if the mean number of signals in analyzed cells for each subject was lower than two. Chromosome polysomy was defined as the fraction of the cells demonstrating three or more signals in each nucleus [Fig 1].

In our study, a significant number of aneuploidies were detected in most of the neoplastic cells, Out of 30 cases, 29 cases (97%) showed molecular alterations ,of the 29 cases 25 represent aberration of chromosome 17 along with p53 mutation, 3 cases showed p53 mutation only & a single case represent aberration of chromosome 17 only. Aberrations in chromosome 17 was found in 26 cases (86.66%) it is frequently represented by polysomy, rather than monosomy of chromosome 17. Out of 30 cases 20 cases (66.66%) represented polysomy of chromosome 17 and 6 cases (20%) showed monosomy of chromosome17. Of the 20

subjects with polysomy, 17 represent polysomy along with amplification of p53 gene, 2 cases represent polysomy along with deletion of p53 gene and 1 case represent only polysomy of chromosome 17. Out of 30 cases 28 (93.33 %) represent alteration in p53 gene, 17 (56.66%) cases represent amplification of p53 gene whereas 11 (36.66%) cases represent deletion of p53 gene. No aberrations were found in 1 (3.33%) case.

IV. Discussion

An extensive amount of chromosomal abnormalities have been previously described also in head and neck squamous cell carcinoma (HNSCC). There are various methods, including classical and molecular cytogenetics (CGH and interphase FISH) [9] and loss of heterozygosity (LOH) that can be used to detect them[10]. FISH method can be used for evaluating the degree of genomic instability and aneuploidy, in OSCC. Genetic instability is putatively involved in the multistep process of carcinogenesis of most cancers. Current evidence suggests that, genomic instability occurs at two levels: the nucleotide level and the chromosome level[11]. Gains or losses of whole or large parts of human chromosomes in tumor cells are found in most cancers[12]. This has been proposed as a major driving force for determining the rate of accumulation of specific genetic hits in several human cancers[13]. Chromosome 17 abnormality has been shown to have a strong correlation with neoplastic development and progression[14]. FISH method can be used for evaluating the degree of genomic instability and aneuploidy, possible prognostic markers, in OSCC. In the present study, a significant population of aneuploid cells was detected in most of the tumor cells, and this was frequently represented by chromosome gain rather than loss. In our study, a significant number of aneuploidies were detected in most of the neoplastic cells, about 97% showed molecular alterations and this was frequently represented by polysomy, rather than monosomy of chromosome 17. In our study, 20 cases (66.66%) represented polysomy and about 6 cases (20%) represented monosomy and this implies that chromosome gains was more frequently encountered than chromosome loss in the series of oral SCCs studied here. This result was within the range of other published findings by Meszaros et al. in 2010, which presented 80% with chromosome 17 polysomy and 20% with chromosome 17 monosomy in OSCC patients.[5] Our study is also in accordance with other studies as reported by Khor et.al (2009)[15]& Zedan et.al (2015)[16] . Chromosome polysomy might be considered as quantitative marker of ongoing or accumulated genomic instability in tumors . Although in our study there were only 6 subjects of OSCC showing monosomy of chromosome 17, this seems to suggest that the loss of chromosome 17 may have occurred as an early event before its transformation to OSCC.

The p53 gene is a tumor suppresor gene which induces a G1 arrest and is involved in DNA repair and apoptosis. Abnormalities in the p53 gene cause an inefficient checkpoint system for the repair and destruction of mutant cells. These result in an increased genomic instability. In addition, it were noticed that the characteristic feature of the p53 gene mutational map is the high frequency of missense point mutations. Unlike many other tumor suppressor genes, more than 80% of p53 gene mutations result in single amino-acid substitutions which lead to the synthesis of a stable full-length protein rather than deletions. These missense mutations lead to the synthesis of a protein that lacks specific DNA binding site and accumulates in the nucleus of tumor cells[17].

In present study, 28 cases (93.33%) presented p53 gene alterations. 17 cases (56.66%) had gene amplification and 11 cases (36.66%) had gene deletion. In accordance with the findings of our study, Zedan et al. reported that 70% cases present p53 amplification while 20% cases present p53 gene deletion[16] and Meszaros et al. in 2010 presented p53 amplification in 46.6% cases & p53 deletion in 33.3% cases[5]. This finding is also in correlation with the previous study which concluded p53 aberrations in OSCC ranges between 19-94% of the cases[18,19] ·Furthermore, it was found that p53 gene to be overexpressed in 63% of oral carcinomas[20]. Another study held in 1994 suggests that, in oral squamous cell carcinoma, p53 abnormalities may result in increased genomic instability and contribute to carcinogenesis. It is estimated that in head and neck cancer, p53 mutations are present in 33% to 59% of tumors[20]. Several researches worldwide have proved that p53 tumor suppressor gene is altered very early in the process of carcinogenesis.[21]

From the previous finding we can hypothesize that accumulation of the P53 protein in an inactive form might has a role in the development of OSCC. There is now strong evidence that mutation not only abrogates p53 tumor suppressive functions, but in some instances can also endow the mutant proteins with novel activities. Such neomorphic p53 proteins are capable of dramatically altering tumor cell behaviour, primarily through their interactions with other cellular proteins and regulation of cancer cell transcriptional programs. [22]

V. Conclusion

In the present study we found molecular changes in chromosome 17 and p53 gene in 97% of OSCC patients in which maximum cases expressed polysomy of chromosome 17 (66.6%) and amplification of p53

gene (56.66%). High frequency of correlation between molecular changes in chromosome 17 and p53 gene with OSCC indicate towards their critical role in development of this disease. These genomic changes are indicated to have massive influence on various cellular processes including differentiation and carcinogenesis , as mutant p53 can induce an increased epigenetic instability of tumor cells thus facilitating and accelerating the evolution of the tumor. On the basis of above findings, it is suggested to analyze these molecular changes in premalignant lesions and resection margins which can help to improve the early diagnosis, prognosis, to predict the probability of recurrence and also improve the survival rate of OSCC patients.

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Table 1 : Distribution of cases according to type of numerical aberrations

Type of Numerical aberrations	**Number**	**Percentage %**
Chromosome 17 Polysomy and p53 Gene Amplification	17	56.66%
Chromosome 17 Monosomy and p53 Gene Deletion	6	20%
Chromosome 17 Polysomy only	1	3.33%
P53 Gene Deletion only	3	10%
Chromosome 17 Polysomy and p53 Gene Deletion	2	6.66%
No Aberrations	1	3.33%
Total (n=30)	30	100%

Table 2 : Comparison of distribution of numerical aberrations in present study & previous studies

Type of Numerical aberrations	**Present study**	**Khor et.al (2009)**	**Meszaros et.al (2010)**	**Zedan et.al (2015)**
Chromosome 17 Monosomy	20%	10%	20%	20%
Chromosome 17 Polysomy	66.66%	85%	80%	70%
p53 Gene amplification	56.66%	-	46.6%	70%
p53 Gene Deletion	36.66%	-	33.3%	20%

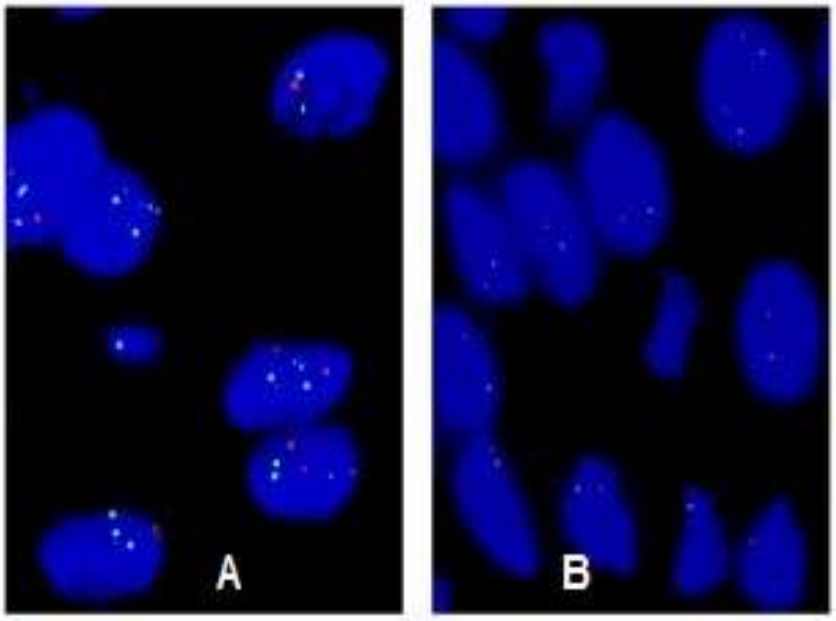

Fig.1 – A: Showing three green signals (chromosome 17 polysomy) and three red signals (p53 gene amplification).
B : Showing one green signal (chromosome 17 monosomy) and one red signal (p53 gene deletion).

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