

# Histopathological and Cytogenetic analysis in oral potentially malignant disorders and oral squamous cell carcinoma : a hospital based study

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**Abstract-** Tobacco related oral cancer occurs due to accumulation of genetic alterations induced by the genotoxins present in betel quid. The objective of the study was evaluate the cytogenetic damage in the peripheral blood of oral cancer patients and control subjects by analysing the structural aberrations (STAs) .The frequency of STAs was analyzed in 60 histopathologically confirmed oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC) patients. STAs showed a significant P value between the Controls, OPMDs & OSCC cases through ANOVA. Significant P value through t-test was also observed between the pairs of group namely Controls Vs OPMDs cases, Control Vs OSCC cases and OPMDs Vs OSCC cases. STAs showed a significant P value in the patients when compared with the controls and also showed an increased chromosomal alteration in OPMDs and further increased in OSCC thus correlating well with the clinical stages of the disease. CSAs were more than CTAs in the cases with dicentrics being more prevalent. The elevated STAs in the study group could be attributed to the fact they belong to high risk population for oral cancer. A predictor STAs identified in the present study may be used in future for early diagnosis and subsequent disease management of the tobacco related oral cancer patients.

**Index Terms-**structural aberrations, oral potentially malignant disorders, oral squamous cell carcinoma, dicentrics

## I. INTRODUCTION

Oral cancer is the third most common cancer in India. Nearly, 1,30,000 Indians die due to tobacco related oral cancer. Oral cancer is mainly attributed to the use of chewing betel quid and tobacco since, Indians chew tobacco than smoke it, due to which 75,000 to 80,000 new oral cancer cases have been identified in 2012 and these proportions will increase further by 2025.[1] Tobacco related oral potentially malignant disorders (OPMDs) and Oral Squamous Cell Carcinoma (OSCC) arises through an accumulation of genetic alterations, including chromosomal alterations, DNA changes and / or epigenetic alterations due to the toxins present in betel quid and tobacco. Detection, histopathological investigation, genetic tests, and treating tobacco related oral cancer patients especially in their premalignant state are the only hope in reducing the burden of this disease.

Even though the last four decades have witnessed the introduction of a number of relatively rapid genetic tests for detecting tobacco related oral cancer, a sensitive and specific test for early detection seem to be the need of the hour. The present hospital based study to assess the cytogenetic alterations in histopathologically confirmed cases of tobacco related OPMDs and OSCC employed chromosomal aberration test to identify the structural aberration known to initiate tumour progression. Although histopathological and cytogenetic study on OPMDs and OSCC patients has been extensively investigated, to the greatest of our knowledge, this work of combining histopathology and employing chromosomal structural aberration analysis is the first of its kind to be performed in Puducherry.

## II. MATERIALS AND METHODS

60 histopathologically confirmed patients referred from a tertiary care hospital, Mahatma Medical College and Research Institute (MGMCRI) in Pondicherry, with suspected OPMDs and OSCC during the years from September 2011 to May 2014 were included in the study. OPMDs and OSCC patients undergoing radiation treatment and with chromosomal anomalies like Klinefelters, Turners etc were excluded from the study. 2 ml of fresh heparinized venous blood was collected from the patients and equally matched control subjects for Leukocyte culture following standard Hungerford Method [2] to investigated structural chromosome aberrations (STAs) in OPMDs and OSCC cases. Cytogenetic damage was scored from 100 well spread Giemsa stained metaphases. Chromatid type aberration (CTA) and Chromosome type aberration (CSA) of structural aberrations were scored for the total STA. The metaphase cells were digitally imaged with Applied Spectral Imaging, Karyotyping software from ASI, Israel. Written informed consents from these patients and volunteering donors were taken. The study was designed in accordance with the Helsinki II declaration and approved by

the Institutional Human Ethical Committee. Statistical Analysis was done using SPSS 16 Version. The P value less than 0.05 is taken as significant.

### III. RESULTS

60 histopathologically confirmed oral cancer cases consisted of 15 OPMDs and 45 OSCC at an incidence of 1OPMDs:2 OSCC ratio. OPMDs consisted of 8 patients with leukoplakia (3 mild dysplasia and 5 severe dysplasia) and 7 patients with sub mucosal fibrosis (2 early stage and 5 advanced stage). As per Broders' grading system, out of 45 OSCC cases, 12 belonged to well differentiated squamous cell carcinoma (WDSCC), 24 to moderately differentiated squamous cell carcinoma (MDSCC) and 9 to poorly differentiated squamous cell carcinoma (PDSCC). Prevalence of moderately differentiated OSCC was noted in the study population (53.33%). The study group comprised of 41 males and 19 females with a mean age  $58.7 \pm 12.7$  years. 41% belonged to  $\geq 60$  while 59% belonged  $< 60$  age group. The mean age of 13 males and 2 females of OPMDs was  $50.5 \pm 13.8$  years with 10 patients  $< 60$  years and 5 patients  $\geq 60$  years of age. The mean age in 28 males and 17 females with OSCC was  $61 \pm 11$  years with 21 patients  $< 60$  years and 24 patients  $\geq 60$  years of age. The patients were characterized into three groups based on their types of habits (risk factors). 40 patients chewed only betel quid (67%), 11 patients chewed betel quid and smoked (18%), while 9 patients smoked and consumed alcohol (15%). The 'betel quid' ingredients in the study group consisted of betel leaf, areca nut, slaked lime, and sun-dried tobacco. All cases in the study group belonged to tobacco related oral cancer. The duration of their habits was  $30.2 \pm 11.1$  years (OPMDs -  $22.33 \pm 12.34$  years and OSCC -  $32.82 \pm 9.4$  years). The site of the oral cancer was also noted. 43 patients suffered oral cancer from buccal mucosa region, 13 from tongue, 2 from lip and 2 from palatal region.

Structural aberrations (STAs) showed a significant P value between the Controls, OPMDs & OSCC cases through ANOVA. Significant P value through t-test was also observed between the pairs of group namely Controls Vs OPMDs cases, Control Vs OSCC cases and OPMDs Vs OSCC cases. (Table 1) STAs showed a significant P value in the patients when compared with the controls and also showed an increased chromosomal alteration in OPMDs and further increased in OSCC thus correlating well with the clinical stages of the disease. CSAs (Table 2) were more than CTAs (Table 3) in the cases with dicentrics (Figure1) being more prevalent.

**Table 1: Frequency of structural aberrations (STAs) in oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC)**

Group	N	CTAs*	CSAs*	STAs*
Control	60	$0.12 \pm 0.32$	$0.23 \pm 0.56$	$0.35 \pm 0.66$
OPMDs	60	$2.6 \pm 5.42a$	$9.8 \pm 7.11a$	$12.4 \pm 7.36a$
OSCC	60	$1.27 \pm 1.97b$	$19.93 \pm 15.43bc$	$21.2 \pm 15.42bc$

'a' denotes Significant 'p' value between the Controls & OPMDs cases

'b' denotes Significant 'p' value between the Controls & OSCC cases

'c' denotes Significant 'p' value between the OPMDs & OSCC cases

In Chromatid type aberrations there is no significant 'p' value between the OPMDs & OSCC

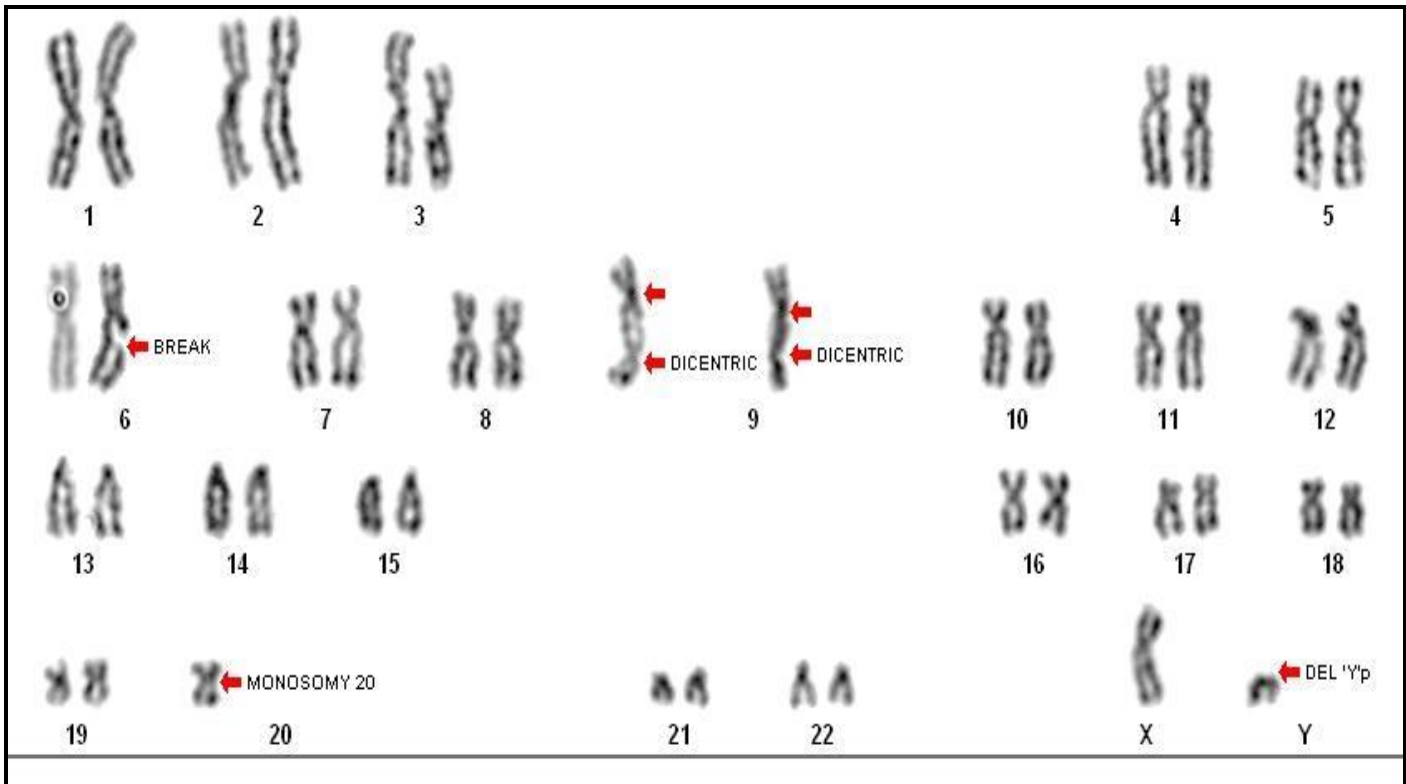
\* ANOVA shows significant 'p' value for all types of structural aberrations for 'a', 'b' & 'c'.

**Table 2: Chromosomal type aberration (CSAs) frequency in oral cancer cases and control subjects**

Group	N	Breaks	Dicentrics	Rings	Fragments	Total CSAs%
Cases	60	$1.9 \pm 2.5$	$8.5 \pm 6.6$	$2.82 \pm 10.2$	$4.1 \pm 6.1$	<b><math>17.4 \pm 14.5</math></b>
Control	60	$0.08 \pm 0.28$	$0.07 \pm 0.25$	$0.02 \pm 0.13$	$0.07 \pm 0.3$	<b><math>0.24 \pm 0.56</math></b>

**Table 3: Chromatid type aberration frequency in oral cancer cases & control**

Group	N	Breaks	Interchanges	Total CTAs%
Cases	60	$1.5 \pm 0.3$	$0.12 \pm 0.38$	<b><math>1.6 \pm 3.2</math></b>
Control	60	$0.1 \pm 0.3$	$0.02 \pm 0.13$	<b><math>0.12 \pm 0.32</math></b>



**Figure 1: A composite karyotype showing various structural chromosomal aberrations**

#### v DISCUSSION

Worldwide, one of the highest incidence rates of mouth cancer among men is found in Pondicherry (8.9 per 100,000)[3]. This hospital based study in tobacco related oral cancer was thus under taken at a MGMCRI in Pondicherry to identify a feasible method and to identify a cytogenetic predictor which could be used as a mass screening test in this high risk oral cancer population.

Cytogenetic study in the peripheral blood of oral cancer patients was employed not only for identifying the different genomic instabilities but to also introduce timely interventional strategies to combat and control the epidemic. Many cytogenetic studies have been carried out in oral cancers using tissue biopsy than leukocyte cultures. We used peripheral blood for our study which gave promising results. This was based on the proposition made by Johanson [4] 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 and was thus taken for the cytogenetic analysis. A conclusion section is not required. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions. In the present study there is no association between the age & gender and the occurrence of chromosomal damage. In PMDs, leukoplakia showed an increased chromosomal damage than OSMF indicating a greater malignant transformation than OSMF. It is in concordance with several studies in India as the frequency of malignant alterations in oral leukoplakia has ranged from 0.13 to 2.2 % while in OSMF it ranged from 0.2 to 1.2%. Prevalence of moderately differentiated OSCC was noted in our study population (53.33%) while Agarwal and Rajderkar [5] and Ghour et al[6] in their study group have noted a high incidence of well differentiated OSCC.

In this study we found that oral cancer in males was more common than females (2 fold) and this finding is comparable to others.[7,8]100% of the patients were of tobacco related oral cancer [ 8] but many studies have reported 25% to 80 % of cases belonging to tobacco related oral cancer [9,10,11] Since all the patients in the study group chewed and/or smoked tobacco high frequency of structural was seen which could be attributed to the synergism of the toxic effects present in betel quid taken with dried tobacco. In the present study 55 of the oral cancer patients (91.6 %) were over 40 years old, which is consistent with previous reports. [7,8] The consumption of tobacco and alcohol appear as the most important non-genetic risk factor associated with the development of head and neck squamous cell carcinomas (HNSCC).[12] Even though the present study showed that the site of the oral cancer showed no association with the chromosomal damage, we have observed 72 % of oral cancer cases having cancer in the buccal mucosa. This

could be attributed to the chronic use and the individual habit of the placement of betel quid in a particular site of buccal mucosa. Our study also showed that there is no correlation between the years of habits and their CA in regard to their different types of habits.

The frequency of STAs was not related to duration of the habits and is in agreement with Bhuvansesh et al.[13] who reported that the accumulated chromosomal aberrations in head and neck squamous cell carcinoma are not significantly influenced by the severity of tobacco/alcohol exposure. The site of cancer had no influence in the frequency of STAs. We found 72% of the cases with buccal mucosa as site for oral cancer but Patrizio et al.[14] 2011 identified tongue to be more associated with chromosomal aberration in OPMDs than other sites. Van Dyke et al.[15] reported other cancer abnormalities appeared to be site specific but suggested a pattern of genetic evolution in squamous cell carcinoma that is independent of anatomic site.

Comparing the frequency of CA between the cases and the controls, it showed a significant higher p value in the patients than those of controls. Our findings were in accordance with several recent case-control studies; they revealed that spontaneous genetic damage in chromosomal aberrations of oral cancer patients was significantly higher than that of controls and thus genetic instability appeared to exist in oral cancer patients.[4-14] Sunil et al.[8] and Ravindaran et al.[16] reported 15-16% of CA in their OSCC case, almost consistent with our STAs at 14.32%.

The present study showed varied structural disorders (chromatid interchanges, breaks, dicentrics, rings and fragment) which initiate tumor progression leading to cancer. Not many studies have had reported structural aberrations in oral cancer in leukocyte cultures. Some authors have reported that no two karyotypes were identical which vary in chromosome copy number and structure.[8,9] This was in concordance with our study where different structural chromosomal disorders were seen in all 60 cases. In the present study CSAs were more predominantly seen than CTAs and is similar to some studies[17] but others [18] have shown that there was no clear indication for cancer prediction between CSAs and CTAs suggesting that both DNA double-strand breaks and other initial DNA lesions responsible for CSAs and CTAs are associated with cancer risk. A high prevalence of dicentrics and ring chromosomes was noted. The cause and process of this phenomenon are unknown, but the malignant cell may contain an amplified area that has many transcriptions from proto-oncogenes. Indeed, in the carcinogenesis process, any cytogenetic disorders activate proto-oncogenes, or inactivate suppress tumor suppressor genes.

## VI. CONCLUSION

Our study shows that the STAs increase with the increase in its clinical manifestation i.e. from OPMDs to OSCC and between the grades of OSCC. High frequency of STA especially dicentrics present in the control population clearly indicates that the population belongs to a high risk for cancer. Structural aberrations in the chromosomes of an individual are thus clear predictors to screen any population at risk of cancer. Elevated STA levels in the oral cancer patients in the present study indicate that these structurally aberrant chromosomes may represent sites of putative tumor suppressor genes, a lesion or loss in them play a major role in the pathogenesis of oral cancer. The findings identified in our study which may be used in future for early diagnosis and subsequent disease management of the tobacco related oral cancer patients, which is the only hope in lessening the mortality and morbidity associated with the disease and has thus paved way for such chromosomal studies in various other types of cancer too.

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