



Chromosome 17 Aberration of Oral Squamous Cell Carcinoma in Malaysia

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Abstract

In most tumours including Oral squamous cell carcinoma (SCC), acquisition of genetic instability is an essential step during carcinogenesis that involves generalized increased rate of errors during DNA replication, and defective repair of DNA. The genomic change results in numerical and structural chromosomal alterations, particularly in chromosomes 3, 9, 11 and 17. Chromosome 17 abnormality was chosen in my study because it shown strong correlation with neoplastic development and progression. In my study, twenty Oral SCC archival blocks were selected retrospectively from two referred Malaysian oral cancer centres. Histopathological findings were prior diagnosed by experienced Oral Pathologist, then Fluorescent in-situ Hybridization (FISH) technique was conducted subsequently by using specific probe of chromosome 17 in these oral SCC cases. In this study, the numerical aberration of chromosome 17 by FISH method in oral SCC in apparently normal tissues was evaluated. In this study, normal control cases showed $67.8 \pm 11\%$ cells of disomic signals in their nuclei, whereas the numerical aberration of chromosome 17 is frequent demonstrated in oral SCC (17/20, 85%) cases. In hence, FISH can be used as a possible prognostic marker for the degree of genomic instability and aneuploidy in excised specimens of oral SCC containing invasive cancer in Malaysia.

Keywords: Aneuploidy, Carcinogenesis, Chromosome, Fluorescent in-situ Hybridization, Oral squamous cell carcinoma

1. Introduction

Oral SCC is the sixth most common malignancy in developed countries accounting for 3% of all malignancies, (Joanna and Zakrzewska, 1999) but third in the developing countries. (Parkin et al., 1988) In Malaysia, oral cancer is the sixteenth most common cancer for female and twenty-one for male of overall cancer incidence per 1,000,000 populations in the year of 2002, according to the governance manual of National Cancer Registry. (Lim, 2002) Despite that, the most common form of oral cancer is oral SCC in Malaysia. (Ng and Siar, 1997). The acquisition of genetic instability is an essential step during carcinogenesis. In most tumours, including oral SCCs, such a genomic change results in numerical and structural chromosomal alteration. A high frequency of chromosome 17 abnormality has been reported in some human such as breast carcinoma, (Marta et al., 2005) colon carcinoma, (Petra et al., 2002) and bladder

carcinoma. (Fadl-Elmula, 2005) Chromosome 17 abnormality has been shown to have a strong correlation with neoplastic development and progression. (Fadl-Elmula, 2005; Naoko et al., 2003) FISH can be used as possible prognostic markers for the degree of genomic instability and aneuploidy in excised specimens of oral SCC containing invasive cancer. In this study, the numerical aberrations of chromosome 17 by FISH method in oral SCC were evaluated.

2. Materials and methods

2.1 Sample collection

The samples of 20 oral SCC cases were retrieved from the archives of two referred oral cancer centres in Malaysia, namely the Department of Oral Pathology, Oral Medicine & Periodontology, Faculty of Dentistry, University of Malaya, and the Unit of Stomatology, Institute for Medical Research, Kuala Lumpur, Malaysia. All cases were representative specimens of the free resection margins of oral mucosa of oral SCC patients. 5 normal controls from mucosal specimens of non oral cancer patients were also obtained for this study as a comparative study.

2.2 Histological grading

For all cases, 4 um thick paraffinized sections were stained with Haematoxyline & Eosin stain for detailed histological examination. Histopathological diagnosis of SCC was verified by an experienced Oral Pathologist according to the classification of the World Health Organization (WHO). (Pinborg et al., 1997)

2.3 FISH analysis

A directly labeled probe for the α satellite probe specific for centromeric region of chromosome 17 (Fluorescein labelled CEP-17; VYSIS, USA) were applied to 4 um thick paraffinized sections of all cases, as well as reagents necessary for hybridization were purchased. Color hybridization of Fluorescein Isothiocyanate (FITC) was performed using a supplement probe kit (VYSIS) by following the manufacturer's instructions.

2.4 Analysis of chromosome copy number

Areas for analysis were selected by the pathologist by comparing hybridized slides to a corresponding H&E stained section. The hybridized signals appear as small spots since the region of a chromosome occupies only a small region of the interphase nucleus. At least 200 nuclei were scored using a 100X objective in each defined histological area, and each nucleus was assessed for the chromosome copy number. Chromosome polysomy was defined as the fraction of the cells demonstrating three or more signals in each nucleus.

2.5 Evaluation of FISH

Evaluation of the preparations was performed by counting 200 nuclei per slide, according to criteria described before (Soder et al., 1995). Normal control from mucosal specimen of non tumor patients were obtained, yielding disomic hybridization signals. For the tumor specimens under study, a significant deviation from the disomy observed in the control hybridization required a percentage of cells with the respective number of FISH signals which was greater than the mean+2SD (standard deviation) of the controls.

For chromosome 17 aneusomy (Table 1), the resulting cut-off value for monosomy was set at 55% ($29+2 \times 12.8$) therefore, when the cell numbers with one signal in nucleus was above 55% it was evaluated as monosomy 17. On the other hand, polysomy 17 were considered when cell numbers with 3 or 4 signals in nucleus above value of 7% ($3.2+2 \times 1.8$). Chromosomes were designated as polysomic (and tumour polyploidy) if more than three chromosome signals/cell were counted.

<<Table 1>>

2.6 Image analysis

The number of hybridization signals was determined by observing 200 nuclei per slide under the objective power of 100X, by using a Nikon fluorescent microscope. Images for documentation were then captured by using a photometrics cooled CCD camera and processed by Image-Pro Express version 4.01.

2.7 Interpretation

Normal: Disomy, when nuclei with 3 or more signals were not observed for 1 or both chromosomes.

Abnormal: Aneusomy, when multiple nuclei with 3 or more signals were observed for 1 or both chromosomes.

2.8 Statistical analysis

Descriptive statistics was performed by Statistical Package of Social Sciences (SPSS) software program for window student version 12.0 to analyze the data obtained. The significance level for chromosome 17 aneusomy was considered at p value < 0.05.

3. Results and discussion

3.1 Histopathological

In this study, normal control samples shown normal mucosa under microscopic histological examination according to the classification of the WHO. Test sample consisted of 13(65%) cases of well-differentiated (WD) oral SCC and 7(35%) cases of moderately-differentiated (MD) oral SCC (Table 2, Figure 1).

<<Table 2>>

<< Figure 1>>

3.2 Fluorescent in-Situ (FISH)

In this findings, the signals showed in the pericentromere of chromosome 17, were detected in interphase cells. In the 5 normal control cases, 67.8±11.0% cells had two hybridized signals in their nuclei indicative of disomy, whereas cells with more than two signals were rare (3.2±1.8%). For the test sample, the mean FISH signals were 43.7±13.0% for monosomy 17, 40.9±11.1% for disomy 17 and 15.3±9.03% for polysomy 17 (Table 1). Within the test group, only the cells with polysomy 17 and disomy 17 were significantly different from the normal controls ($p < 0.05$). Our study demonstrated that numerical aberration of chromosome 17 is frequent in oral SCC (17/20 cases, 85%) (Figure 2). Only 2 cases showed monosomy and one case was a disomy (Table 3, Figure 3).

<<Table 3>>

<<Figure 2>>

<<Figure 3>>

In the present study, we applied DNA probes specific human chromosome 17 to both 5 cases of normal control, and specimen tissues collected from 20 archival blocks of oral SCC. FISH was performed on the normal buccal mucosa to ascertain whether the hybridization technique was successful and also to obtain baseline values of the distribution of hybridization signals within the nuclei of cells in the control sample. We found that in chromosome 17 FISH assay, most of the cells (mean, 67.8±11.0%; range, 38-89%) in the normal control had two hybridization signals, indicative of chromosome 17 disomy (Figure 3), followed by cells (mean, 29±12.8%; range, 5-61%) with single hybridized signal (chromosome 17 monosomy) (Figure 3) and the least common were cells (mean, 3.2±1.79%; range 0-7%) with more 3 or more signals (chromosome 17 polysomy) (Figure 3). These findings are not unexpected as in a normal sample cells tend to yield disomic signals and the cutoff value for disomy is 61%. These results suggest that formalin-fixed, paraffin-embedded materials are suitable for FISH analysis. In addition, the result also demonstrated that the FISH technique allows the evaluation and detection of chromosome aneusomy in interphase cells in individual cases of oral SCC.

We detected that 17 cases of oral SCC yielded more than three hybridized signals of chromosome 17, indicative of chromosome 17 polysomy. An extensive amount of chromosomal abnormalities has been previously described also in head and neck squamous cell carcinoma (HNSCC) by various methods, including classical and molecular cytogenetics (CGH and interphase FISH) and loss of heterozygosity (LOH) assays. (Jin et al., 1995; Komyiama et al., 1997; Bhuvanesh, 2002) To some extent, all these techniques disclosed similar results, mainly represented by an extensive genomic imbalance, which was also confirmed in our study.

Genetic instability is putatively involved in the multistep process of carcinogenesis of most cancers. Current evidence suggests that this genomic instability occurs at two levels: the nucleotide level and the chromosome level. (Lengauer et al., 1998) Gains or losses of whole or large portion of human chromosome in tumor cell (aneuploidy) are found in most cancers. (Mitelman et al., 1994) This has been proposed as a major driving force for determining the rate of accumulation of specific genetic hits in several human cancers. (Kahlenberg et al., 1996; Limoli et al., 1997) 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. There were 17 cases of chromosome 17 polysomy and only 2 cases of chromosome 17 monosomy detected in this study, and this implies that chromosome gains was more frequently encountered than chromosome loss in the series of oral SCCs studied here. It has been observed that the frequency of cells with polysomy increased with histological progression. (Charlotte et al., 2002) Within each histological grade, there was an intersubject variation in the levels of chromosome polysomy present, suggesting that the biological factors might influence the rate of accumulation of genetic hits. Genomic instability may also lead to chromosome non-disjunction and the generation of cells with zero, one, two, and three or more chromosome copies. Hence, the presence of cells exhibiting three or more chromosome copies (chromosome polysomy) might be considered a quantitative marker of ongoing or accumulated genomic instability in tumors. (Kanekawa et al., 1999) Although there were only 2 cases of WDSCC showing an increase number of cells with monosomy 17, this seems to suggest that the loss of chromosome 17 may have occurred as an early event before its transformation to oral SCC.

Interestingly, in our study the chromosomal imbalance appeared in low frequencies in cells within apparently normal cell populations. This finding support the field cancerization hypothesis originally postulated by Slaughter et al in his study on multi-centric primary tumors of the oral cavity. Subsequently, others also demonstrated this chromosomal imbalance in a variety of HNSCC. (Choi and Chung, 1996; Papadimitrakopoulou et al., 1996; Scoles et al., 1998; Shin et al., 2001; Poh et al., 2006) Although the frequency of polysomic cells was relatively low in this area, it must be remember that nuclear truncation happened during sectioning, and this may lead to under representation of the chromosomal frequency. Furthermore, altered genomes presenting in a low frequency may also be masked by majority of normal cells.

4. Conclusion

This study conducted in two referred Malaysian oral cancer center, demonstrated that the most common histological grading of oral SCC was WD, and the most common numerical abnormalities of chromosome 17 were trisomy and tetrasomy, which considered as polysomy 17 groups. Our results showed frequent numerical aberrations of chromosome 17 in oral SCC. Cells with polysomy 17 significantly increased ($p=0.0005$) while cells with disomy 17 significantly decreased ($p=0.0005$) in oral SCC. This demonstrated than numerical chromosome 17 abnormality, was associated with carcinogenesis of oral SCC. Although there were only 2 cases of monosomy 17 found in our study, it might be an early event of oral SCC. Furthermore, the degree of numerical abnormality of chromosome 17 varied from case to case. This finding suggests that numerical chromosome 17 abnormality is involved in the process of carcinogenesis and development of oral SCC as observed by FISH. Therefore, FISH can be used as a marker for evaluating neoplastic activity at the surgical margin of oral SCC in Malaysia.

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Table 1. Mean number of hybridization signals for chromosome 17 in normal control and test sample

Type	Mean No. of Signals (%)		
	Monosomy	Disomy	Polysomy
Normal	29±12.8	67.8±11.0	3.2±1.8
Test	43.7±13.0	40.9±11.1	15.3±9.03

Table 2. Distribution of oral SCC according to their histological grades

Histological Grades	No of Cases	Percentage (%)
WDSCC	13	65
MDSCC	7	35

Table 3. Distribution of oral SCC according to their chromosomal aneusomy

Chromosomal Aneusomy	No of cases	Percentage (%)
Monosomy	2	10
Disomy	1	5
Polysomy	17	85

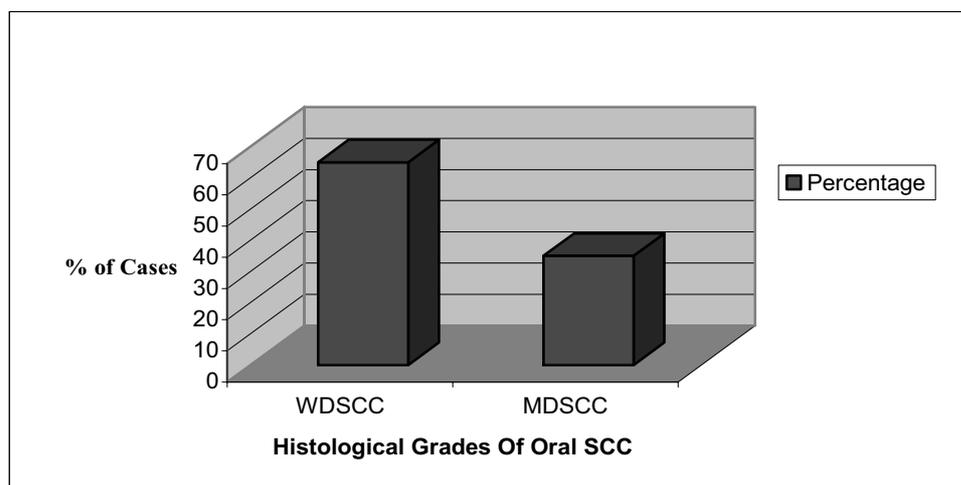


Figure 1. Percentage distribution (%) of 20 oral SCC cases according to their histological grades

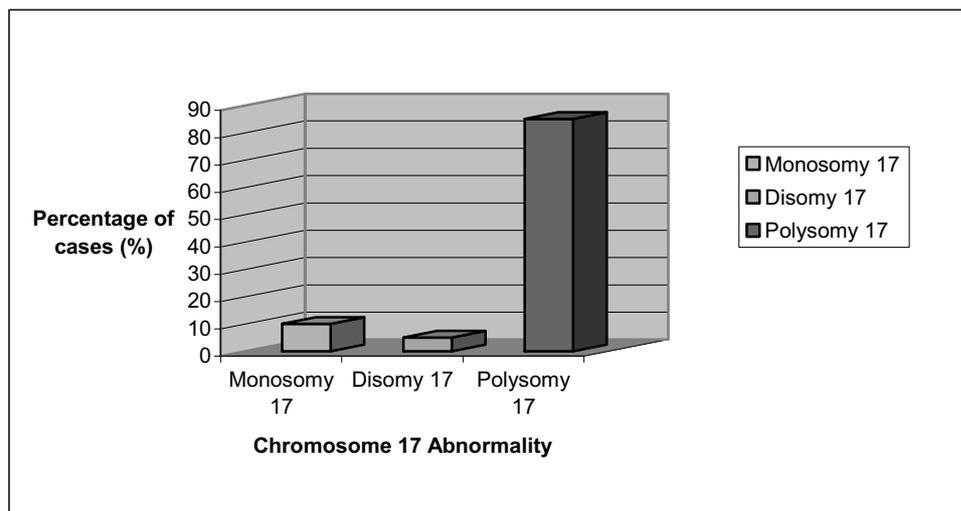


Figure 2. Percentage of Cases (%) of Chromosome 17 Abnormality

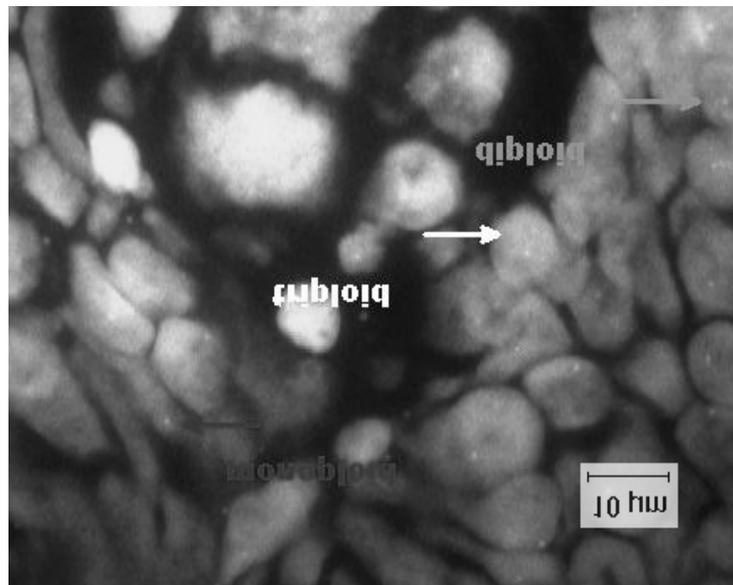


Figure 3. Various signal probe of Chromosome 17. Monoploid, diploid and triploid green signals were observed in the cells. (Original magnification x 400)