www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v6i8.06

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Original Article

A study on the expressions of Ki-67 and Cyclin-D1 in oral pre malignant lesions

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Abstract
Objective: *To evaluate the expressions of Ki-67 and Cyclin-D1 in oral pre malignant lesions.*
Methods: *This was a prospective observational study. Clinical history was taken from all the cases with more stress on probable risk factors of lesions. Patients were questioned about habits of smoking, alcohol consumption and tobacco chewing. Patients were also questioned about their socioeconomic status, dietary habits and were examined for oral hygiene. Stained sections from all the formalin-fixed, paraffin embedded tissue specimens (n=44), serial sections (4-5 Im thick), were prepared and processed for routine histopathological and subsequent immunohistochemical studies.*
Results: *More than one third of patients were between 41-50 years (47.7%). More than one half of patients were males (65.9%).Severe dysplasias was among 34.1% patients and moderate dysplasias was in 20.5% patients. Mild dysplasias was found in 18.2% and lichen planus was in 15.9% patients. Hyperplasias was found to be in 11.4% patients. Over expression of cyclin D1 (≥10%) was found among 79.5% patients. Cyclin D1 level was <10% in 13.6% of the patients. Overexpression of Ki67 (≥10%) was found among 70.5% patients. Ki67 level was <10% in 20.5% of the patients.*
Conclusion: *expression of the Cyclin-D1 and Ki67 with increasing severity of dysplasia may be a prognostic indicator of any preceding malignant transformation.*
Keywords: *Malignant lesions,Cyclin-D1, Ki67.*

Introduction

Oral cancer constitutes a major health problem in developing countries and represents leading cause of death. Squamous carcinomas represent about 3% of human cancers and over 90% of malignant tumors at oral location, being diagnosed worldwide each year in over 350,000 new cases (Dragomir et al, 2012).

Oral submucous fibrosis (OSMF) is a potentially malignant disorder which has risen rapidly in India reaching the count more than two millions in the last decade. The reported rate of malignant transformation in OSMF ranges from 3% to 19% (Ranganathan and Kavitha, 2011).

The etiology of premalignant lesions is generally accepted to be multifactorial, with tobacco and alcohol being reported as important cofactors in transition from premalignancy to malignancy. Consumption of tobacco and betel quid (betel leaf-coated with slaked lime and wrapped around areca nut plus some spicy ingredients) can cause genetic and molecular alterations in clinically distinct oral premalignant lesions and conditions (Win et al, 2005).

Ki-67 is a cell cycle associated human nuclear protein present in peri-chromosomal region, the expression of which strictly associated with cell proliferation and which is widely used in pathology as a proliferation marker to measure the growth fraction of cells in human tumors (Schlüter et al, 1993).

Cyclin D1 gene is the key regulator of the G1 phase of cell cycle located on chromosome 11q13. A significant proportion of dysplasias contain molecular abnormalities that may result in cyclin D1 overexpression (Ramasubramanian et al, 2013).

The objective of this study was to evaluate the expressions of Ki-67 and Cyclin-D1 in oral pre malignant lesions.

Material and Methods

This was a prospective observational study conducted in the Department of Pathology, HIMS, Barabanki on oral pre malignant lesions. The Ethical Approval from the Ethical Committee of the Institute was obtained before starting the study.The result of the biopsy was sent for oral pre malignant lesions from the Laboratory was extracted.

Methods: Clinical history was taken from all the cases with more stress on probable risk factors of lesions. Patients were questioned about habits of smoking, alcohol consumption and tobacco chewing. Patients were also questioned about their socioeconomic status, dietary habits and were examined for oral hygiene.

Stained sections from all the formalin-fixed, paraffin embedded tissue specimens (n=44), serial sections (4-5 Im thick), were prepared and processed for routine histopathological and subsequent immunohistochemical studies. H&E-stained sections were examined under a light microscope and diagnosed according to WHO criteria for histological typing of cancers and pre-cancers of the oral cavity (Pindborg et al, 1997). When present, epithelial dysplasias were graded as mild, moderate or severe.

Immunohistochemistry

All the cases were used for the expressions of cyclin D1 and Ki-67 by immunohistochemistry. Sections (on silane-coated slides) were deparaffinized in xylene, rehydrated in graded ethanol and washed in Tris-buffered saline (TBS; pH 7.6). Epitopes were retrieved by heating sections in a microwave oven at high power setting (900W, for a period of 7-9 min) and low power setting (300W for 15 min) using Tris/EDTA (pH 9.0) solution. After cooling (20 min) and washing in TBS (10 min), the sections were incubated with the Dako peroxidase block, 0.03% hydrogen peroxide (H2O2) containing sodium azide (Code K4007), for 5 min to eliminate endogenous peroxidase activity. After washing in TBS for 10 min, the sections were incubated with the corresponding primary antibodies for 60 min on the DakoAutostainer Universal Staining System (Dako A/S, Copenhagen, Denmark) using antibodies against Ki-67 (MIB-1; dilution 1:100 in Dako Antibody Diluent, Cat No, M724001-2) (both from Dako A/S) and cyclin D1(NCL-CYCLIN D1, Clone DCS-6, dilution 1:100 in Dako Antibody Diluent; Novocastra Laboratories Ltd., Newcastle-Upon-Tyne, UK). After washing for 10 min in TBS, the sections were incubated with the EnVision Horseradish Peroxidase (DAB) for 30 min,

washed twice in TBS for 5 min each, and were further developed twice with the DAB+ chromogen for 5 min each. The sections were counterstained with haematoxylin, rinsed in tap water for 10 min, rehydrated and mounted using the Eukitt mounting medium. Cases in which the primary antibody was omitted and substituted with the diluent TBS served as internal negative controls.

Statistical analysis

The results are presented in mean±SD and percentages. The Chi-square test was used to compare the categorical variables. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Results

More than one third of patients were between 41-50 years (47.7%) followed by 30-40 years (27.3%) and >50 (25%) years. More than one half of patients were males (65.9%). More than one third of patients were vegetarian (43.2%) followed by non-vegetarian (29.5%) and both (27.3%). More than half of patients were tobacco chewers (63.6%) and 68% were smokers. However, 40.9% were alcoholic. Gingiva was among 34.1% patients and lip/intra oral was in 31.8% patients. However, tongue and cheek site was in 20.5% and 13.6% respectively (Table-1).

Severe dysplasias was among 34.1% patients and moderate dysplasias was in 20.5% patients. Mild dysplasias was found in 18.2% and lichen planus was in 15.9% patients. Hyperplasias was found to be in 11.4% patients (Table-2).

Over expression of cyclin D1 (≥10%) was found among 79.5% patients. Cyclin D1 level was <10% in 13.6% of the patients. Over expression of Ki67 (≥10%) was found among 70.5% patients. Ki67 level was <10% in 20.5% of the patients (Table-3).

Table-1: Basic profile of patients

Basic profile	No. (n=44)	%
Age in years		
30-40	12	27.3
41-50	21	47.7
>50	11	25.0
Mean±SD	46.70±10.61	
Gender		
Male	29	65.9
Female	15	34.1
Dietary habit		
Vegetarian	19	43.2
Non-vegetarian	13	29.5
Both	12	27.3
Addiction habi		
Tobacco chewing		
Present	28	63.6
Absent	16	36.4
Smoking		
Present	30	68.2
Absent	14	31.8
Alcohol		
Present	18	40.9
Absent	26	59.1
Anatomical sites		
Tongue	9	20.5
Gingiva	15	34.1
Lip/Intraoral	14	31.8
Cheek	6	13.6

Table-2: Distribution of patients according to pre-malignant lesions

Pre-malignant lesions	No. (n=44)	%
Hyperplasias	5	11.4
Lichen planus	7	15.9
Mild dysplasias	8	18.2
Moderate dysplasias	9	20.5
Severe dysplasias	15	34.1

Table-3: Distribution of patients according to Cyclin D1 level and Ki-67

Cyclin D1 level and Ki67	No. (n=44)	%
Cyclin D1 level		
Negative	3	6.8
<10%	6	13.6
≥10%	35	79.5
Ki67 level		
Negative	4	9.1
<10%	9	20.5
≥10%	31	70.5

Discussion

Extensive studies related to the expressions and mutations of the cell cycle regulatory genes and cell proliferative activity have been done in pre-malignant and malignant oral mucosal lesions from the West (Schoelch et al, 1999). However, only a few studies have been carried out in India. Oral carcinogenesis is a multistep process in which occurrence of a series of genetic events may lead to dysregulation of the cell cycle (Regezi and Jordan, 2001).

In the present study, More than one third of patients were between 41-50 years (47.7%) followed by 30-40 years (27.3%) and >50 (25%) years. The mean age of patients was 46.70±10.61. In this study, More than one half of patients were males (65.9%). Raju et al (2005) mean age of pre-malignant patients 51.1 years with higher percentage of males than females.

In the present study, more than half of patients were tobacco chewers (63.6%) and 68% were smokers. However, 40.9% were alcoholic. In the study by Raju et al (2005), 83% were tobacco chewers and 46% smokers.

In the present study, gingiva was among 34.1% patients and lip/intra oral was in 31.8% patients. However, tongue and cheek site was in 20.5% and 13.6% respectively. Reju et al (2005) also reported gingiva being more affected site contributing 71%.

In the present study, severe dysplasias was among 34.1% patients and moderate dysplasias was in 20.5% patients. Mild dysplasias was found in 18.2% and lichen planus was in 15.9% patients. Hyperplasias was found to be in 11.4% patients. Raju et al (2005) reported that of the 29 pre-malignant lesions, there were 2 hyperplasias, 2 Lichen planus, 14 mild, 6 moderate and 5 severe dysplasias.

In this study, overexpression of cyclin D1 (≥10%) was found among 79.5% patients. Cyclin D1 level was <10% in 13.6% of the patients. Few studies have examined the expression of cyclin D1 in oral lesions and reported prevalence rates ranging from low (27%) to high (71%) (Michalides et al, 1997; Rousseau et al, 2001). This discrepancy may be attributed to difficulties in assessing cyclin D1 in formalin-fixed, paraffin-embedded tissue sections by immunohistochemistry (Liu and Zhang, 2001).

In the present study, over expression of Ki67 (≥10%) was found among 70.5% patients. Ki67 level was <10% in 20.5% of the patients. Raju et al (2005) reported that staining with Ki-67 was found to be quite high, with a stronger intensity, especially in the oral dysplasias. Kumar et al (2015) also reported in Lichen planus cases, the Ki-67 expression was less pronounced than compared to leukoplakia but the expression was more than the normal mucosa. Patel et al (2014) reported that strong association was found in expression of Ki-67 immunomarkers in premalignant oral lesions in compared to normal mucosa.

Conclusion

From this preliminary study, we can hypothesize that an increase in expression of the Cyclin-D1 and Ki67 with increasing severity of dysplasia may be a prognostic indicator of any preceding malignant transformation as proved by the previous studies performed using these markers and may hence serve as biomarkers for oral cancer progression.

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