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Pathology

ROLE OF Ki67 IN ORAL PREMALIGNANT AND MALIGNANT SQUAMOUS CELL LESION

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ABSTRACT

Aims-To study Ki67 expression in oral premalignant lesion & its comparison with malignant lesion.

Material and methods- The standard immunohistochemical method along with MIB-1 DO-7;DAKO antibodies was use to study the expression of Ki67 in paraffin embedded tissue specimens.

Results- all sample study showed positive staining for ki67, only 9% case from oral squamous cell carcinoma(OSCC) group showed negative staining for ki67. The staining was confined to basal layer in most of the cases except OSCC in which it was seen in all layers. The intensity of staining was moderate to intense. The percentage of ki67 positive cells in malignant mucosa was 91%. The statistical analysis revealed that the expression of ki67 increases from dysplasia to malignancy.

Conclusion- these results emphasize the potential use of ki67 antigen as marker of malignant transformation and carcinogenesis in oral premalignant lesions, conditions and OSCC, respectively; and in future they may serve as prognostic tools in the early detection of malignant transformation in oral premalignant lesions and conditions.

KEYWORDS :

INTRODUCTION-

Oral cancer is the sixth most common cancer world wide and the third most common cancer in the developing countries, accounting for approximately 4% of all cancers and 2% of all cancer deaths.Oral malignancies occur in about twice as many men as women and 95% are found in persons more than 40 years of age(Silvermann, 1988)[1], More than 3 lac cases are diagnosed every year**(Tanaka T et al2011)[2](sudbo, 2004).** The vast majority of head and neck cancers are oral squamous cell carcinomas (OSCCs) that arise from the epithelial lining of oral cavity, including tongue and lips. More than 95% of oral cancers are Squamous cell carcinomas. Etiology of oral cancers like most other malignancies is multifactorial involving both chemical carcinogens and genetic factors. In the western countries most are attributable to separate and combined habits of tobacco use and alcohol consumption. In the developing Asian countries chewing habits of betel quid, betel quid substitutes along with smoked and chewed tobacco are more prevalent. Tobacco or gutkha and alcohol are the most important risk factor for oral cancer. Among the premalignant lesions are leukoplakia, erythroplakia and submucous oral fibrosis [Shafer, 1995][3].Around 90% of the oral cancers are squamous cell carcinomas, originating in the tissues that line the mouth and lips. Oral and mouth cancers most commonly involve the tongue. A significant proportion of OSCC develop from premalignant lesions such as leucoplakia and oral submucous fibrosis (OSMF). Histological examination remains the gold standard for diagnosis and identification of malignant oral lesions.

Ki67 antigen Ki67 is one of the most important cell proliferative marker(Scholzen T et al 2000)[4]. Ki67 is nuclear protein that is present in all cell cycle phase except the G0 and early G1 phase(Bruno S et al1992)[5]and making it is a good marker for cell cycling. Ki67 has a prognostic and predictive value in different tumor type. The use of antibody to Ki67 is a reliable marker and accurately assesses the growth fraction of neoplasm.

Antigen KI-67 also known as Ki-67 or MKI67 is a protein that in humans is encoded by the MKI67 gene (antigen identified by monoclonal antibody Ki-67)(Schonk DM et al 1989)[6],(Bullwinkel J et al 2006)[7] Inactivation of antigen MKI67 leads to inhibition of ribosomal RNAsynthesis(R.Rahmanzadeh et al 2007)[8].MKI67 was not found to be predictive for long-term follow-up after chemotherapy. MKI67 could be considered as a prognostic biomarker for therapeutic decision(Scholzen&Gerdes 2000).

MATERIALS AND METHODS

Study duration- duration of study was from January 2018 to September 2019.A total of 200 cases diagnosed in the Department of pathology in collaboration with department of surgery G.S.V.M Medical college Kanpur and associated hospital Kanpur, India.All patients with suspicious premalignant and malignant lesions of oral cavity and oropharynx were worked up with histopathological examination after detailed clinical examination. Premalignant lesions were selected based on the absence of any invasive squamous cell carcinoma.

The study group includes patients of different age groups, sex, symptoms, duration of illness, adverse oral habits like smoking or chewing of tobacco, alcohol consumption, clinical details of the lesion and clinical staging of patients.

IMMUNOHISTOCHEMISTRY

1. Keep the slides on hot plate at 60 degree for 30 minutes or incubate at 90 degree for 10 minutes.
2. Keep the slides in
a. xylene I for 10 minutes
b. xylene II for 10 minutes
c. absolute alcohol for 5 minutes
d. 85% alcohol for 5 minutes
e. 70% alcohol for 5 minutes
f. running tap water for 10 minutes
3. Antigen Retrieval in microwave oven.Keep the slides in citrate buffer- working solution(keep it in an oven)

a. Power 450 for 5 times- 3 minutes(check the buffer in coplin jar in between)
b. Keep adding buffer
c. Then keep the slides in room temperature for 30 minutes
d. Wash the slides with PBS for 3 times 3 minutes each.

4. Starting procedure: Wipe the slides with tissue paper (add peroxide block for 15 minutes in moist chamber)
5. Wash the slides with PBS for 3 times 3 minutes each.
6. Add power block, incubate for 15 minutes.
7. No washing
8. Add primary antibody for 1 hour in moist chamber at room temperature or overnight in refrigerator at 4-8 degree celcius.
9. Wash with PBS for 3 times 3 minutes each.
10. Add super enhancer and incubate in moist chamber for 25 minutes.
11. Wash with PBS for 3 times 3 minutes each.
12. Add secondary antibody (SS label); incubate for 30 minutes.
13. Wash with PBS for 3 times 3 minutes each.
14. Add DAB chromogen for 30 seconds to 3 minutes (2 drops chromogen DAB+1 ml of DAB buffer)
15. Wash with distilled water for 15 minutes.
16. Dip the slides in Haematoxylin for 30 seconds.
17. Keep the slides in running tap water for 5 minutes.
18. Keep the slides in 70% alcohol for 2 minutes.
a. 80% alcohol for 2 minutes

b. 100% alcohol for 2 minutes
c. xylene for 2 minutes
d. xylene for 1 minute
19. Mount with DPX.
20. Microscopic examination.

Interpretation–Nuclear staining was taken as positive in epithelium.
According to Jang Jaer et al 2005, p.475.
Positive staining: >5% cells are stained.
Negative staining: <5% cells or not at all.
Intensity – Accordingto Alison, Duangporn & Petre 1999, p.104.
The fraction of stained cells was scored according to following criteria.
Weak staining: + (6-25% cells take staining).
Moderate staining: ++ (26-50% cells take staining).
Strong staining: +++(51-100% cells take staining).

RESULTS-

The study include 200 cases of oral lesion. Maximum number of cases were in their 3rd and 4th decades of life.

sex wise distribution of cases analysed. There were 151 males(75.5%) and 49 females(24.5%). Males predominated over females with male:female ratio of 3:1.

In present study the most common site of oral mucosal lesions was buccal mucosa which accounted for 37.5% of total cases. Tongue is the next common site of oral lesions (20%), base of tongue & others were less common sites.

Expression of Ki67 was found in 66.7%(60/90) of the pre-malignant cases and 90.9%(100/110) of the malignant lesions of the oral cavity.

Expression of Ki67 increases from premalignant to malignant oral lesions. In all cases of OSCC showed staining in basal and supra basal layers upto 93.7% only 6.3% cases were negative.

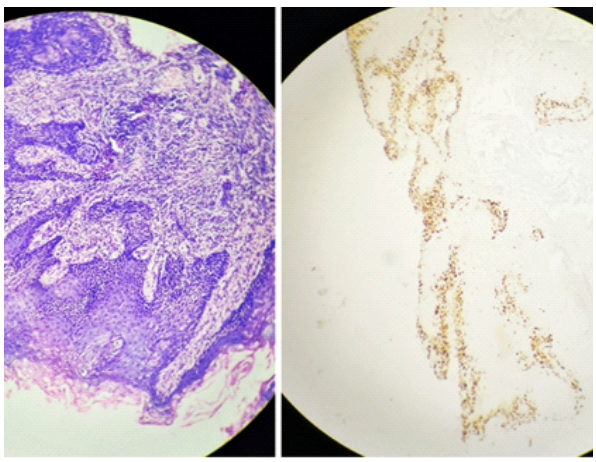

Figure1. a) severe dysplasia (H&E)x100, b) Strong basal &supral basal positivity by Ki67 (x100).

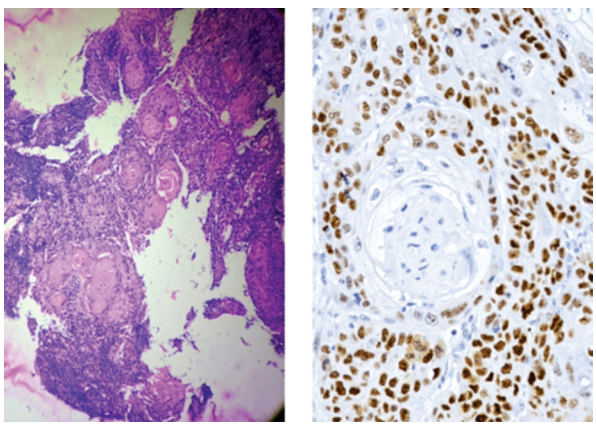

Figure2. a) Well differentiated squamous cell carcinoma (H&E, x100), b) Strong suprabasal Ki67 positivity with negative staining in central keratinized areas (Ki67, x100).

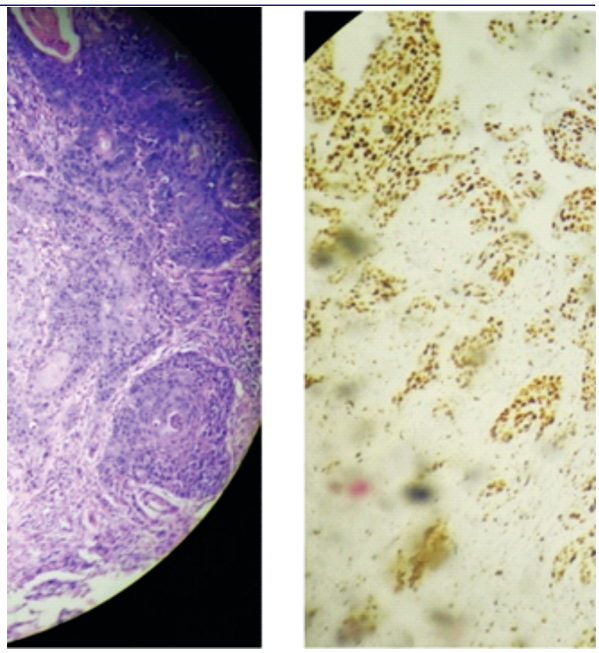

Figure3. a) Moderately differentiated squamous cell carcinoma (H&E, x100), b) Strong diffuse Ki67 positivity (Ki67, x100)

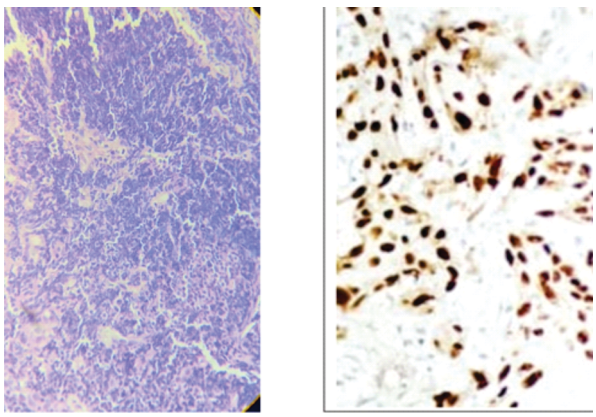

Figure 4. a) shows Poorly differentiated squamous cell carcinoma, (H & E x 100) b. Ki67 positivity in poorly differentiated squamous cell carcinoma (x100).

DISCUSSION-

Amongst head and neck cancer squamous cell carcinoma, oral cavity and oropharyngeal carcinoma predominate (Elargo et al, Mehrotra et al[9], Yeole et at, 2006). Among the common premalignant lesions are leukoplakia and submocous fibrosis.

In present study the relative incidence of oral lesions was 57.14% of all head and neck malignancy. Our findings were in accordance with Gangadharan (1979) who reported a relative incidence of 63.5% of all cases of oral cavity, oropharynx and hypopharynx.

Male preponderance was noted with male female ratio of 3:1, which is in accordance with Oliver et al,(1996) and Agarwal et al, (2001). This is may be primarily due to their addiction to tobacco chewing and smoking, which is the most important risk factor for oral cancer and presence resultant precancerous lesions, secondarily low incidence in females can also be attributed to lack of awareness in them.Hindle et al (1996) and Parket al (2006) have also described male predominance in oral cancer patients in western countries as well as in India. Mehrotra et al (2003) found a male to female ratio of 3.27:1 in their study. Wahi et al (1968) found male to female ratio 2.3:1 an analysis of cancer registry data all over India in 1965.In accordance by Pinholt et al(1997) the male and female ratio of 1.19:1. However, in studies of Greek and Brazilian population show quiet a higher ratio of 9.2 : 1 to 4.8 : 1 respectively.

In present study it was found that the peak incidence of oral carcinoma

was in the fourth and fifth decade of life. Similar findings were reported by two other indian workers (Wahi et al 1965 and Padmanabhan and Vasudevan 1982). Whereas Landon et al 1997 reported higher mean age (63.6yrs). The reasons for earlier occurrence of carcinoma among Indian people might be due to habit of tobacco chewing and smoking started early in the life and prevailing poor socio-economic conditions which affect the general nutritional status of the individual.

In present study tobacco chewing alone is the most common habit accounting 49% of total cases. Tobacco chewing and smoking has been the next most common personal habit accounting for 35% of cases and pan masala accounts 11.5% of cases. Jussawala et al 1971 stressed similarly upon tobacco chewing and smoking as the important risk factors for development of oral cancer.

Most common site of oral lesion was buccal mucosa which accounted for 37.5% of cases. The next common site of oral mucosal lesions was tongue(20% of cases). Silverman et al 1981 has reported buccal mucosa as the commonest site for development of leukoplakia and OSCC. Balachandra reported buccal mucosa (57.5%) was the commonest site followed by tongue, whereas Kroll Hoffman reported lower lip was the most common site of OSCC.

In our study incidence of premalignant lesion was 45% and malignant lesion were 55%. Shafer 1975 who has reported similar incidence of various premalignant lesions in oral cancer.

Incidence of malignant lesions in our study was 55%. Common malignant lesions were observed in the form of well differentiated squamous cell carcinoma ,moderately differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma.

The Ki-67 expression was detected in all cases of normal oral epithelium (NOE) and was restricted to the basal and parabasal layers of the epithelium with parabasal layer showing intense staining which is in accordance with a previous report by **Takeda et al,2006.**

In our study of 200 cases, 80% were Ki67 positive. There were no normal tissues used in this study Expression of Ki67 immunostain were identified at a nuclear level in 66.7% of premalignant lesions and 90.9% of malignant lesions of oral cavity.This study indicated that Ki67 expression increases from hyperplasia to dysplasia to oral SCC. **Vandana et al (2010)[10], Francesca et al(2008)[11],** In our study all cases of OSCC showed staining in basal and supra basal layers upto 93.7% only 6.3% cases were negative.This study indicated that increased proliferating cell population in both basal and suprabasal layers of OED in this study suggest that proliferating cells might increase not only in a superficial direction but also downward to the basal layer in OED **Miguel et al(2000)[12] ,Dwivedi et al(2014)[13], Kumar et al(2015)**

Ki-67 positive cells in well differentiated SCC were located in the periphery of the tumor nests where frequent mitoses were observed than the central areas of squamous maturation which suggest that less differentiated cells are located in peripheral layer and the central cells are highly differentiated with an ability to keratinize, thus no expression of Ki-67 was observed in the central cells of the tumor island. **(SS Birajdar et al 2014)[14]**

In moderately differentiated SCC, Ki-67 expression observed in both peripheral and part of central layer as cells were less differentiated than WDSCC and correlates with the study done by **Roland *et al*., (1994)** and **Piffko *et al*., (1996)** on OSCC.

Poorly differentiated SCC Ki-67 expression was diffuse and more intense as the cells were less differentiated than WDSCC as well as MDSCC. More number of cells were in proliferative phase and hence showed an increase Ki-67 LI than WDSCC and MDSCC. These findings correlate with previously mentioned studies. The staining of Ki-67 positive cells was patchy in most of the PDSCC whereas it was granular and localized to the nuclei in cases of MDSCC and WDSCC **(SS Birajdar et al 2014)**

Present study observed that , expression of Ki67 increases from premalignant to malignant oral lesions, which showed staining in basal and supra basal layers upto 89%, only 11% cases were negative.In 90 premalignant cases, Ki67 positivity found in 83.3% cases, with an increased in suprabasal positivity as the grade of dysplasia increased, such pattern of staining also observed by **Miguel et al(2000).**

CONCLUSION-

these results emphasize the potential use of ki67 antigen as marker of malignant transformation and carcinogenesis in oral premalignant lesions, conditions and oral squamous cell carcinoma, respectively; and in future they may serve as prognostic tools in the early detection of malignant transformation in oral premalignant lesions and conditions

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