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Pathology

Evaluation of Ki67 and P53 Expression in Oral and Oropharyngeal Squamous Cell Carcinoma (SCC) and Its Correlation with Histological Grade

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Abstract **Original Research Article**

Carcinoma of the oral cavity is one of the most common malignant tumors worldwide, with major predominance in South-East Asia and India. In India, cancer of the oral cavity is one of the five leading sites of cancer. ***Objective***– 1) To evaluate the expression of Ki67 & p53 in oral cavity & oropharyngeal Squamous Cell Carcinoma (SCC). 2) To correlate the result of IHC with tumor histological grade. ***Materials and methods***: This was the prospective study carried out in Department of Pathology MAMC, Agroha on 50 cases of Oral Squamous Cell Carcinoma from period of February 2017 to March 2018. Bryne's histological scoring was done and IHC with Ki67 and p53 was done on diagnosed cases. ***Result:*** Ki67 LI (Labelling Index) between the three histological grades showed that poorly differentiated group had the highest value and well differentiated had the least value. Ki67 LI increased with increase in histological grade of tumor. P53 LI between the three histological grades showed that poorly differentiated group had the highest value and well differentiated had the least value. The correlation between the parameters KI67 & P53 showed a good positive correlation, and was significant with a p value of <0.001. ***Conclusion***: In conclusion, our result emphasized the importance of preliminary knowledge of the expression of P53 and Ki67 as a prognostic marker of survival in order to obtain the best chance for a cure for these tumors in terms of deciding the treatment option for these patients.

Keywords: Oral Squamous Cell Carcinoma (OSCC), Ki67 & P53 Labelling Index.

INTRODUTION

Carcinoma of the oral cavity is one of the most common malignant tumors worldwide, with major predominance in South-East Asia and India. In India, cancer of the oral cavity is one of the five leading sites of cancer and accounts for 19% of the total cancer cases in men and 7% of that in women. Among the oral tumors, 90% are squamous cell carcinoma [1]. High mortality rate give rise to a considerable global health burden. Despite the currently available therapeutic strategies, comprising the surgical excision of malignant tissue and a combination of radiotherapy and chemotherapy, the 5-year survival rate is still poor. The high mortality rate is usually attributed to late diagnosis but some cases of OSCC surgically treated at an early stage still present with aggressive behaviour and disease progression [2]. Therefore, current research efforts focus on the discoveries of new predictive biomarkers to determine the risk of OSCC occurrence, progression and metastatic spread and thereby to reduce mortality rates. Among all the new techniques, immunohistochemistry has become a powerful tool for the pathologists, as it provides insight into tumor histopathogenesis and has contributed to more accurate determination of patient's prognosis.

Genetically altered cells may escape macroscopic or routine histopathological examination and may require sophisticated approaches. Immunohistochemistry is a simple, low-cost procedure that is frequently used to improve histological diagnosis. The Ki-67 protein is expressed in all proliferating cells and is detectable during the active phases of the cell cycle (G1, S, G2 and mitosis). Resting cells in G0 phase do not express Ki-67. During interphase, the Ki-67 antigen can be exclusively detected within the cell's nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Since it is strictly associated with cell proliferation it is widely used as a proliferation marker to determine the growth rate of specific cells/diseases [3].

The fraction of Ki-67 positive tumor cells i.e. the Ki-67 labelling index, is often associated with the progression of a disease. Numerous studies, among

them multivariate analysis covering more than 4000 cases conclude Ki-67 to be an independent prognostic factor in both prostate and breast cancer. Studies performed on oral cancer have been incongruous but support in general that a high proliferative activity is correlated with poor prognosis [4].

The monoclonal antibody Ki-67 was first described in 1983 by Johannes Gerdes *et al.* who suggested that it might be used as a marker for proliferating cells. Immunostaining with antibodies to Ki-67 antigen is well established as a quick and efficient method for evaluating growth fractions of various tumor types because of its distinctive reaction patterns that exclusively involves the proliferating cells [5]. The Ki-67 antibody was first isolated during attempts to raise monoclonal antibodies to antigens specific for Hodgkin and Reed-Sternberg cells. The Ki-67 antigen was named after its place of characterization in Kiel, Germany and because the clone producing the antibody was grown in the 67th well of tissue culture plate [6].

P53 is the name of the tumor suppressor gene located on the short arm (p) of chromosome 17, as well as the protein encoded by this gene. The TP53 gene consists of 11 exons, of which the first one is noncoding. In turn, the p53 protein consists of 393 amino acids and comprises four regions with different functions. Once activated, the p53 protein can induce growth arrest as well as cell death. The p53 protein has a very short half-life and therefore can be hard to detect in normal tissue. However, the protein can remain in the tissue longer for certain reasons, such as a mutation, a defect in the degradation pathway, or by binding to other proteins. Mutation affecting p53 gene will result in structural changes, which increases the stability and hence accumulation of mutant p53 protein, which can be detected immunohistochemically. This gene gets commonly mutated in oral cancer and hence, has been extensively studied in OSCC with different results in various studies [7, 8].

This study was undertaken to study the immunohistochemical expression of Ki67 and p53 and their correlation with histological grade in oral and oropharyngeal carcinomas.

MATERIALS AND METHODS

This study was carried out in the Department of Pathology, Maharaja Agrasen Medical College from period of February 2017 to March 2018. Fifty prospective cases of oral and oropharyngeal carcinoma were included in the study. A complete clinical profile of the patient including age, gender, anatomic location and nodal metastasis was recorded. The entire biopsy specimen submitted with clinical diagnosis of Squamous cell carcinoma from oral cavity were grossly examined and fixed. The tissue was processed and paraffin embedding was done by routine histological technique [9]. Cases with histological diagnosis of squamous cell carcinoma were further submitted to immunohistochemical staining for Ki67 and p53.

Immunohistochemical staining

All specimens were fixed in 10% formalin and routine histologic paraffin blocks were made. Sections were cut to 3–4-µm thickness and mounted on poly-L-lysine-coated slides. The sections were deparaffinized in xylene and rehydrated in alcohol. Antigen retrieval was done manually using - Dako PT Link. This system requires pre-treatment with heat induced epitope retrieval. Endogenous peroxidase was blocked with 3% hydrogen peroxide. Application of ready to use primary antibody for 40 minutes and washing in TBS (Tris buffered saline). Then the sections were incubated with secondary antibody and HRP block for 20 minutes followed by washing in TBS. Incubation with 3, 3-diaminobenzidine tetrahydrochloride was performed for 10 min as a substrate chromogen solution to produce a brown color. Finally, the sections were counterstained with Mayer's hematoxylin [10].

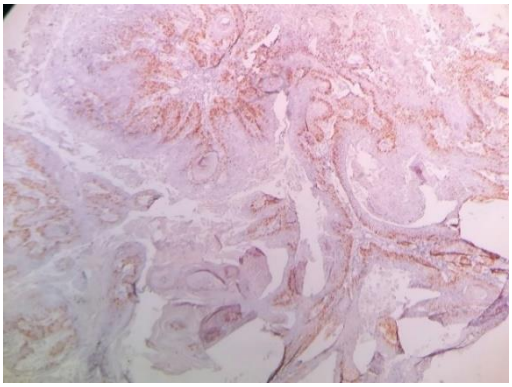

Fig-1: Ki67 positivity in well differentiated SCC in basal layers only (100x)

Positive control - A breast carcinoma with p53 & Ki67 expression was taken as positive control.

Negative control - Negative control for both markers was processed in same manner except that TBS will be used instead of primary antibody.

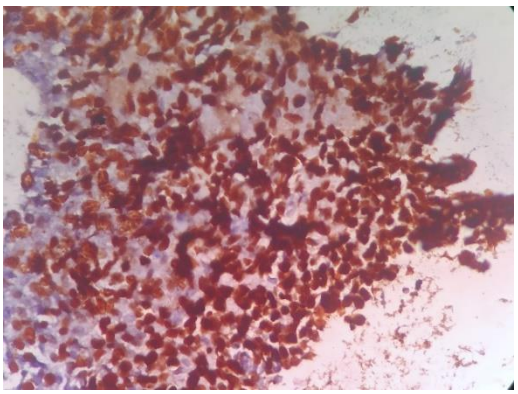

Fig-2: Ki67 positivity in moderately differentiated SCC (400x)

Quantitative analysis

Cells were considered positive for Ki67 & p53 with staining of nucleoplasm or nucleoli regardless of the staining intensity. Labeling Indices (LI) of Ki67 & p53 were determined by number of positive nuclear profile /mm2 of epithelial cells. The p53 LI & Ki67 LI in the tissue of OSCC per 5 fields at the magnification of 400X (0.196 mm2 in size) were counted under light microscope and mean was calculated. The expression of p53 was semi-quantitatively determined by using the criteria that tumor having more than 10% of positively stained cells were considered positive [11].

Morphometry

Magnus pro analyzer 4.1 will be used. The sequence of steps involved in the analysis of microscopic image is Microscopic image →CCD video camera →Video frame grabber→Image processing→Image segmentation→Interactive measurement →Data recording→Data analysis. The whole process can be done semi-automatically and automatically. The advantage is that it can remove human bias and error from measurement and make the accumulation of morphometric data more rapid [12].

Statistical analysis

A descriptive study was carried out for all the variables included in the study using Pearson's correlation and independent t test in SPSS (Statistical Package for Social Sciences) version 20.0. P-value <0.05 was accepted as statistically significant

OBSRVATIONS AND RESULTS

Out of 50 cases, 4 (8%) cases were females and rest 46 (92%) were males. Youngest patient was 28 year of age and oldest was 85 years, with mean age of 56.6 years. Lymphadenopathy was present in only 6 (12%) cases. The duration of seeking medical help in our institution was from 1 month to 6 months. Various risk factors evaluated for the study were smoking, alcohol intake and others which included snuff and chewing tobacco. The history of chronic smoking was present in 34 (68%) cases. In comparison to Smoking there were fewer cases 18 (36%) who had regular drinking habit. Other form of tobacco intake included snuffed and Chewing tobacco, only 10 (20%) cases gave history of other form of tobacco intake. All the patients with tobacco chewing habit had bad oral hygiene. On categorising cases according to location, out of total 50 cases, maximum number of cases 20 (40%) were located on tongue, followed by 16 (32%) cases from tonsils. Rest of the cases included Floor of the mouth 7 (14%) cases, Buccal Mucosa 5 (10%) cases and 1 (2%) cases of each from soft palate and pyriform fossa.

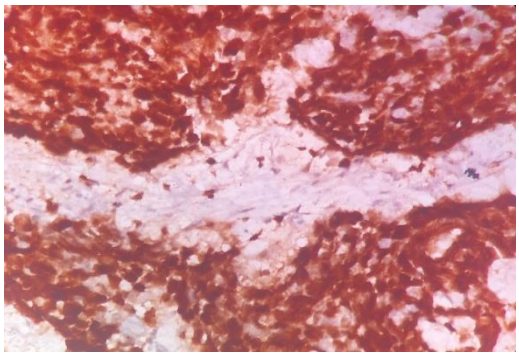

Fig-3: Ki67 staining in poorly differentiated SCC. Note that only intervening stroma is negative for stain (400x)

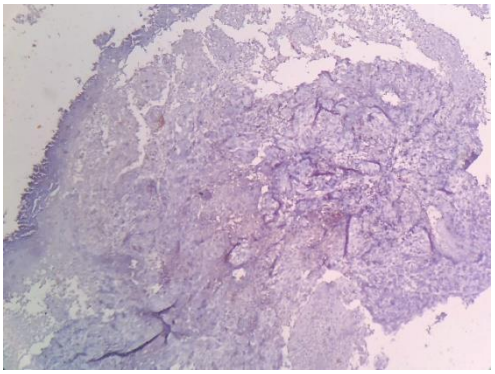

Fig-4: P53 staining absent in well differentiated SCC (100x)

Bryne's histopathological grading [13] of the biopsy was done. Out of 50 cases 14 (28%) were Well differentiated, 34 (68%) cases Moderately differentiated and 2 (4%) cases were categorized as Poorly differentiated Squamous Cell Carcinoma.

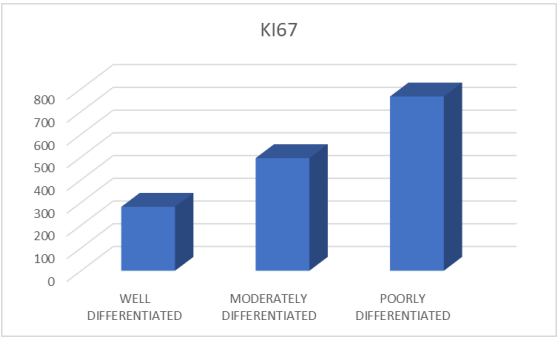

Fig-5: Ki67 Labelling index in different histological grade

On comparison of Ki67 between the three groups showed that poorly differentiated group had the highest value of 767 and well differentiated had the least value of 281.43 as shown in Figure 1. This difference was statistically significant with a test value of 21.544 and p value of <0.001.

Comparison of P53 between the three groups shows that poorly differentiated group has the highest

value of 1189 and well differentiated has the least value of 305.93 as shown in Figure 2. This difference is statistically significant with a test value of 11.422 and p value of <0.001.

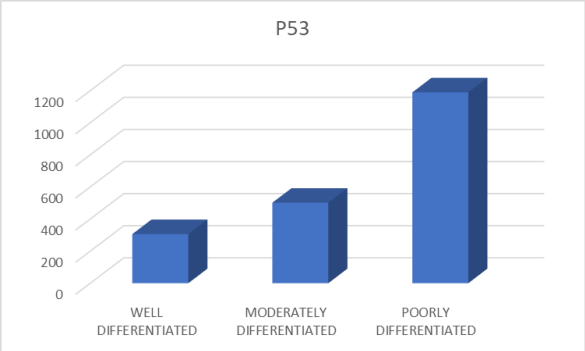

Fig-6: p53 labelling index in different histological grades of tumor.

The correlation between the parameters Ki67 & P53 shows a good positive correlation, and is significant with a p value of <0.001.

The correlation between the parameters Bryne's score & Ki67 showed a very good positive correlation, and is significant with a p value of <0.001.

The correlation between the parameters Bryne's score & P53 showed a good positive correlation, and is significant with a p value of <0.001.

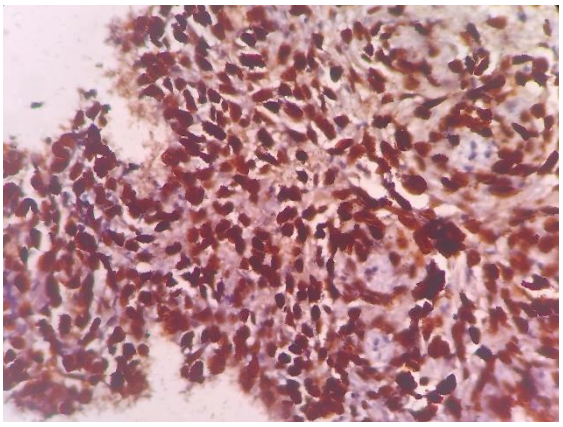

Fig-7: Strong p53 positivity in poorly differentiated SCC (400x)

P53 positivity was higher in smokers with a t value of -3.722 as compared to non-smokers P53. This value was statistically significant with a p value of 0.001.

However there was no correlation seen between alcohol intake and Ki67 LI; alcohol intake and p53 LI; smoking and Ki67 LI and Ki67, p53 LI and metastasis.

DISCUSSION

Worldwide oral cancer (when oropharyngeal sites are included) is the sixth most common cancer in the world. The GLOBOCAN project estimated 300373 new cases in 2012, with a global age-standardized incidence rate of 4.0 cases per 100000 populations per year and a global mortality rate of 1.9 deaths per 100000 populations per year. High incidence of oral cancer is found in southern Asia with age-standardized incidence rates of > 10 cases per 100 000 population per year in parts of India and Pakistan. Most oral cancers occur in patients aged 50-70 years. In India, cancer of the oral cavity is one of the five leading sites of cancer and accounts for 19% of the total cancer cases in men and 7% of that in women. Among the oral tumors, 90% are squamous cell carcinoma [14, 15].

The age of patients in present study ranged from 28 to 85 years with mean age of 56.6 years. Among the various risk factors mainly exogenous factors tobacco and alcohol appear particularly important [16]. In our study smoking was the most common risk factor with history of smoking present in 68% of cases.

Early diagnosis and treatment are the goals. Since the conventional oral examination has undetermined sensitivity and specificity there is a need for more accurate diagnostic tools that can detect early lesions and determine either the potentially malignant or the benign nature of lesions. Newer techniques have been developed, current research efforts focus on the discoveries of new predictive biomarkers to determine the risk of OSCC occurrence, progression and metastatic spread and thereby to reduce mortality rates. Among all the new techniques, immunohistochemistry has become a powerful tool for the pathologists, as it provides insight into tumor histopathogenesis and has contributed to more accurate determination of patient's prognosis [18].

The use of biomarkers as adjuncts to routine histopathological examination may help prognostication and effective management of the patient. The most predictive of the molecular markers thus far available and assessed in OSCC development include the p53 protein expression and Ki67 antigen [18, 19].

In the present study we have studied expression of ki67 and p53 and their correlation with histopathological grading of tumor

In our study comparison of Ki67 expression between the three groups showed that poorly differentiated group had the highest value and well differentiated had the least value. This difference was statistically significant. This finding was in concordance with studies done by Tumuluri *et al*. [20].

In the study done by Dwivedi *et al*. [21] the expression of ki67 positivity was more at the periphery

of tumor Cell Island in WDSCC when compared to MDSCC where the staining was present in tumor islands and other areas. In PDSCC staining was more intense and diffuse. We have the similar finding in our study.

The very good correlation was found between the Bryne score Ki67 LI and is significant with a p value of <0.001. This finding of our study was similar to the finding of study of Kurokawa *et al*. 22 who concluded that cases with higher bryne score had higher Ki67 LI expression with p value <0.0001. Our study was in consistent with meta-analysis done by Xie *et al*. [23].

However our study result was in contrast to the findings of Roland *et al*. [24], Piffko *et al*. [25], Bettendorf O *et al*. [26] and Motta *et al*. Their study showed no significant correlation between Ki67 expression & histological grade of tumor.

We studied the expression of p53 and its correlation with histological grade and presence of metastasis. Comparison of P53 between the three histological groups showed that poorly differentiated group has the highest value and well differentiated has the least value. This difference was statistically significant with p value of <0.001.

Kurokawa *et al*. [22] concluded in their study that expression of P53 was higher in the patient with high Bryne's score with a significant P value of <0.0054. A similar result was seen in our study with good positive correlation, and was statistically significant with a p value of <0.001.

The result of our study was in concordance with Jayade *et al*. [28]. Their study concluded that expression of P53 increased with increase in histological grade with a significant P value of < 0.001.

In the study of Dave *et al*. [29] the positive expression of p53 was found in 62% of carcinomas studied. In general, in well-differentiated cases p53 staining was seen in peripheral cells, whereas in poorly differentiated carcinoma staining was observed consistently which was also seen in our study. Nuclear accumulation of p53 was related to the histological grade of malignancy with P value <0.05.

Our study result was in contrast to Motta *et al*. [27] and Abbas *et al*. [7], their study showed no statistical significance between P53 expression with histological grade of tumor.

In various studies comparison between the expression of Ki67 and P53 with smoking and alcohol intake had been done. In our study we also compared these variables and found that comparison of Ki67 expression between smokers and non-smokers showed that KI67 LI was higher in Smokers with a t value of -1.186 and is statistically non-significant with a p value of 0.241.

Our results were in contrast to the study result of Tumuluri *et al*. [20] and Mondal *et al*. [30]. Tumuluri *et al*. showed that cases with history of smoking and alcohol consumption showed significantly higher Ki67 expression than cases without these risk factors. Mondal *et al*. showed positive correlation of Ki67 LI with smoking with a P value of P – 0.0028.

However, we found positive correlation between p53 expression and smoking; P53 expression was higher in smokers with a t value of -3.722 and was statistically significant with a p value of 0.001. Our result was similar to the result of the study done by Dave *et al*. [29] and Takeda *et al*. [31].They found positive association between p53 expression and smoking. On the other hand, Nylander *et al*. [32] showed no positive correlation between P53 expression and smoking which is in contrast to our study.

CONCLUSION

Oral Squamous Cell Carcinomas are more common in males with smoking as major risk factor. Our study showed that tumor cell proliferation has a positive association with histologic grading in human OSCC. Various studies have shown that high Ki67 expression in patients with OSCC results in poor prognosis. This finding ascertains that Ki67 antigen can be used to determine the tumor behavior and prognosis in incisional biopsy material. Further studies considering a greater sample size with follow up should be done to explore differences in the biological behavior and studies correlating the clinical course of the different histological grade of OSCC with their mean Ki67 LI would be useful as prognostic indicators.TP53 mutation is associated with short survival in squamous carcinoma of head and neck irrespective of tumor stage and tumor site and decrease sensitivity of tumor to radiotherapy and certain chemotherapeutic drugs. IHC detects TP53 mutation indirectly and is relatively a cheaper diagnostic technique then PCR. Positive cases then can be further evaluated for TP53 mutation by PCR and gene analysis to know the outcome of disease and for further planning of the treatment of patient.

In conclusion our result emphasizes the importance of preliminary knowledge of the expression of P53 and Ki67 as a prognostic marker of survival in order to obtain the best chance for a cure for this tumor in terms of deciding the treatment option for these patients.

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