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Original Research Article

Expression of P53 and Ki67 in Primary Invasive Squamous Cell Carcinoma of Head and Neck in the Southern Part of Assam

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Conflict of interest: Nil

Abstract:

Head and neck squamous cell carcinomas (HNSCCs) is the most common cancer of head and neck region and it develops from the mucosal epithelium in the oral cavity, pharynx and larynx. Every year, over 650,000 persons worldwide are diagnosed with head and neck cancer, with 350,000 dying as a result of the condition. The prevalence of HNSCC is different in different countries/regions and has been linked to tobacco-derived carcinogens, excessive alcohol intake, or a combination of both. Prior infection with oncogenic strains of human papillomavirus (HPV), especially HPV-16, and to a lesser extent, HPV-18 and other strains, is increasingly being associated to oropharyngeal tumours. Among the oral squamous cell carcinomas (OSCCs), squamous cell carcinoma of the tonsil had the highest incidence of HPV-16 DNA, implying a higher risk of HPV infection in this region. Common premalignant lesions of the oral cavity are leukoplakia with associated dysplasia, erythroplakia and oral submucous fibrosis. In addition, genetic factors play a role in the development of HNSCC. Individuals with Fanconi anaemia, a rare inherited genetic illness characterised by impaired DNA repair (due to mutations in any of the 22 FANC genes), have increased risk of HNSCC, mainly oral malignancies. Malignant transformation of a normal cell is a multistep process, where accumulation of genetic mutation is reflected by molecular changes, followed by clonal selection and expansion of the mutated cells, which will finally progress to occurrence of an overt carcinoma. The p53 gene product, which is able to block the cell cycle at the G1 phase after encountering genotoxic stress, plays an important role in the Gap1 phase of the cell cycle. It is a tumor suppressor gene which monitors the integrity of the genome. It inhibits the cell cycle progression and promotes DNA repair process by activation of p53 gene through DNA protein kinase during DNA damage. Mechanism of inactivation or loss of wild type of p53 is by the following processes-mutation within the genome, mutant p53 can form an oligomeric complex with wild type of p53 which turns out to be functionless and mutant p53 gains a new oncogenic function that overcomes the negative regulation by small quantities of wild type of p53. Ki67 is a nuclear protein. It is associated with cell proliferation and was demonstrated in 1990, that MIB-1 antigen detects Ki-67 antigen in G1, S, G2 and M phase, but it is absent in G0 phase. This antibody is therefore a useful marker of proliferation in dysplastic lesions and can be of diagnostic and prognostic value. It is also used as a potential prognostic biomarker for a variety of malignancies, including laryngeal neoplasms. The aim of this study is to assess the expression of p53 and Ki67 in the primary invasive head and neck squamous cell carcinoma and to study the clinicopathological findings in primary invasive head and neck squamous cell carcinoma.

Keywords: Squamous Cell Carcinoma of Head and Neck, p53, Ki67.

Introduction

Head and neck squamous cell carcinomas (HNSCCs) are the most common cancer of head and neck region and it develops from the mucosal epithelium in the oral cavity, pharynx and larynx. Every year, over 650,000 persons worldwide are diagnosed with head and neck cancer, with 350,000 dying as a result of the condition. [1]

The prevalence of HNSCC is different in different countries/regions and has been linked to tobacco-derived carcinogens, excessive alcohol intake, or a combination of both. Prior infection with oncogenic strains of human papillomavirus (HPV), especially HPV-16, and to a lesser extent, HPV-18 and other strains, is increasingly being associated to oropharyngeal tumours. Vegetable and fruit consumption may help to mitigate the carcinogenic effects of cigarettes and alcohol, however a low BMI raises the risk of oral cancer. Among the oral squamous cell carcinomas (OSCCs), squamous cell

carcinoma of the tonsil had the highest incidence of HPV-16 DNA, implying a higher risk of HPV infection in this region. [2,3,4] Many oral carcinomas arise in the areas that was previously affected by a premalignant lesion. Common premalignant lesions of the oral cavity are leukoplakia with associated dysplasia, erythroplakia and oral submucous fibrosis. In addition, genetic factors play a role in the development of HNSCC. Individuals with Fanconi anaemia, a rare inherited genetic illness characterised by impaired DNA repair (due to mutations in any of the 22 FANC genes), have increased risk of HNSCC, mainly oral malignancies.

Malignant transformation of a normal cell is a multistep process, where accumulation of genetic mutation is reflected by molecular changes, followed by clonal selection and expansion of the mutated cells, which will finally progress to occurrence of an overt carcinoma. [5] The p53 gene product, which is able to block the cell cycle at the G1 phase after encountering genotoxic stress, plays an important role in the Gap1 phase of the cell cycle. It is a tumor suppressor gene which monitors the integrity of the genome. It inhibits the cell cycle progression and promotes DNA repair process by activation of p53 gene through DNA protein kinase during DNA damage. Mechanism of inactivation or loss of wild type of p53 is by the following processes-mutation within the genome, mutant p53 can form an oligomeric complex with wild type of p53 which turns out to be functionless and mutant p53 gains a new oncogenic function that overcomes the negative regulation by small quantities of wild type of p53. [6]

Ki67 is a nuclear protein. It is associated with cell proliferation and was demonstrated in 1990, that MIB-1 antigen detects Ki-67 antigen in G1, S, G2 and M phase, but it is absent in G0 phase. This antibody is therefore a useful marker of proliferation in dysplastic lesions and can be of diagnostic and prognostic value. [15] It is also used as a potential prognostic biomarker for a variety of malignancies, including laryngeal neoplasms. [7] The aim of this study is to assess the expression of p53 and Ki67 in the primary invasive head and neck squamous cell carcinoma and to study the clinicopathological findings in primary invasive head and neck squamous cell carcinoma.

Materials and Methods

The present study is a hospital based prospective cross-sectional study, undertaken in the Department of Pathology, Silchar Medical College and Hospital, Silchar from June 2020 to May 2021. There was total 62 head and neck squamous cell carcinoma biopsy/resection specimens submitted.

Detailed clinical history is taken and all routine investigations done after taking consent from the patients. Immunohistochemistry with p53 and Ki67 antibody was done on these specimens as per IHC protocol after staining with H&E initially.
Any secondary cases, primary squamous cell carcinomas in other sites than head and neck and all carcinoma in situ cases were excluded from the study.

Ki67 grading[8]: To determine the grade of Ki-67 expression, nuclei of 200 epithelial cells located across the whole epithelial layer were examined in a high-power field (× 400). Percentage of Ki-67 positive cells is Ki67 index:

Negative: when the Ki-67 index was <10%,
1+ : when the Ki-67 index was 10-30%,
2+ : when the Ki-67 index was 30%-50%
3+ : when the Ki-67 index greater than 50%

Assessment of expression of p53: [9]

Strong nuclear staining was regarded to denote p53 positivity. 100 cells were evaluated in representative high power fields to obtain the percentage of cell positivity.
Grading of p53 expression92:
Grade 0- 0-10% cells showing positivity (Negative)
Grade 1- 10-30% of cells showing positivity (Mild expression)
Grade 2- 31-50% expression (Moderate expression)
Grade-3- Greater than 50% expression (Intense expression)

The percentage of p53 and Ki67 protein staining for squamous cell carcinoma of head and neck region were evaluated using the chi-square test. Any 'p' value <0.05 was considered to be statistically significant.

Results and Observations:

The present study was a hospital based cross-sectional observational study. A total of 62 cases of punch biopsy/incisional biopsy/excisional biopsy/resection specimens were submitted to the Department of Pathology. Age, sex distribution and clinical profile were documented in all cases.
Immunohistochemistry with p53 and ki67 in these 62 cases were reported as the extent and intensity of nuclear immunoexpression.

1. Age distribution of patients:

In the present study, the age of patients of head and neck squamous cell carcinoma ranged from 30 to 89 years. The mean age was 53.37 years with a standard deviation of 11.79 years and median age is 50.5 years and mode is 40 and 45 years . The peak incidence of head and neck squamous cell carcinoma was seen in the fifth decade followed by sixth decade.

2. Distribution of patients according to gender:

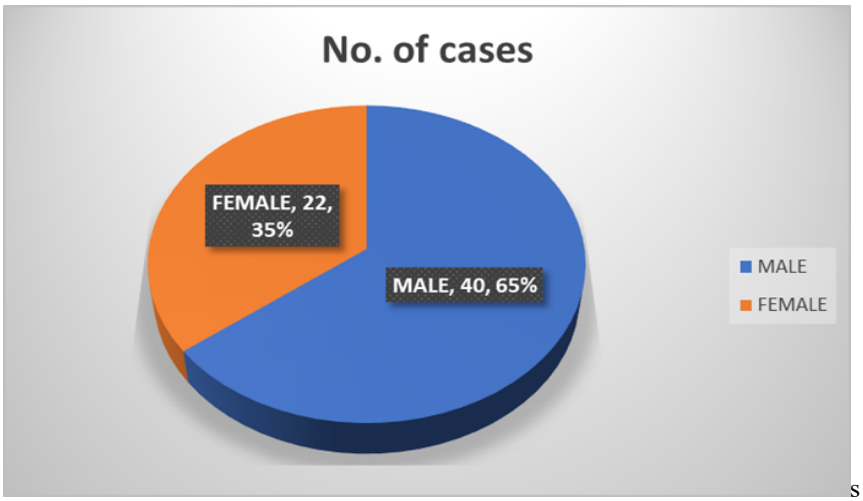


Figure 1: Pie diagram showing the distribution of patients according to gender

From the above table and pie diagram, it is seen that out of the 62 cases, 40 cases (65%) were male and 22 cases (35%) were female. The male- female ratio is 1.8:1.

3. Site wise Distribution of Cases:

Table 1: Distribution of cases according to site of the lesion

Site of the lesion	No. of Cases	Percentage
Laryngopharynx	1	2
Larynx	4	6
Nasopharynx	0	0
Oropharynx	7	11
Oral Cavity	50	81
TOTAL	62	100

Majority of the patients (50 numbers or 81%) hadtumor in the oral cavity followed by 7/62 (11%) in the oropharynx, 4/62 (6%) in the larynx and 1/62 (2%) in the laryngopharynx**.**

4. Lymph Node Metastasis (At the time of Presentation)

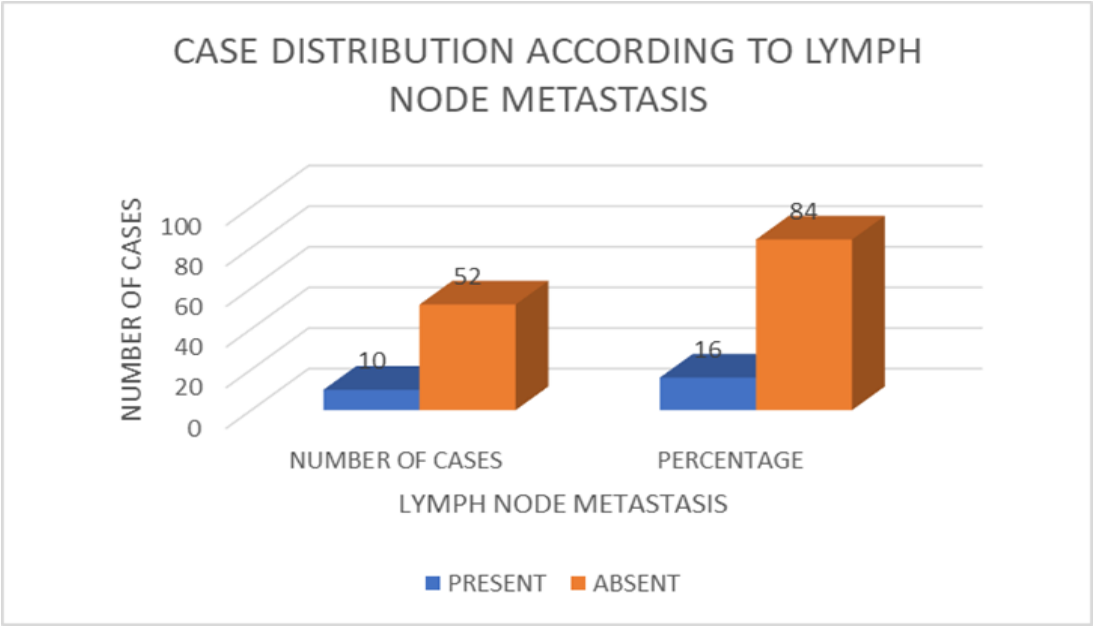


Figure 2: Bar diagram showing the distribution of patients according to lymph node metastasis

Most of the cases (52 out of 62 or 84%) did not have lymph node metastasis. Only 10/62 (16%) cases showed lymph node metastasis.

5. Case Distribution According to Risk Factors or Habits:

Table 2: Distribution of cases according to risk factors or habits:

Habits	Number Of Cases	Percentage
Smoking	32	52
Tobacco Chewing	4	7
Pan/Gutkha	7	11
Smoking+Pan/Gutkha	2	3
Alcohol+Smoking	12	19
No Associated Risk Factors Or Habits	5	8

Among the 62 cases, 32 (52%) had a positive history of smoking, followed by 12/62 (19%) consuming of alcohol along with smoking. 7 patients (11%) had a history of pan or gutkha consumption, 4 patients chewed tobacco whereas 2 patients had a history of smoking+pan. 5/62 (8%) patients did not have any risk factor or any habits.

6. Distribution of Cases According To Histologic Differentiation:

Table 3: Distribution of cases according to histological differentiation

Differentiation Of Tumors	Number Of Cases	Percentage
Well Differentiated	35	57
Moderately Differentiated	17	27
Poorly Differentiated	10	16
Total	62	100

According to histological differentiation, 35/62 (57%) cases were well differentiated carcinoma, 17/62 (27%) cases were moderately differentiated carcinoma and 10/62 (16%) cases were poorly differentiated carcinoma.

7. Distribution of Cases According To P53 Positivity:

Table 4: Distribution of cases according to p53 positivity:

% of tumor cell showing p53 expression	Number of cases	Percentage
Negative (<10%)	24	39
Positive (>10%)	38	61
Total	62	100

We found that 38 out of 62 cases (61%) showed p53 positive expression whereas 24 cases (39%) had negative p53 expression.

8. Distribution of Cases According to Grading Of p53 Expression: Grading of p53 expression:

Strong nuclear staining was regarded to denote p53 positivity

- Grade 0- 0-10% cells showing positivity (Negative)
- Grade 1- 10-30% of cells showing positivity (Mild expression)
- Grade 2- 31-50% expression (Moderate expression)
- Grade-3- Greater than 50% expression (Intense expression)

Table 5: Table showing p53 grading of cases:

Grade of P53 Staining	No. of Cases	Percentage of Cases
Negative	24	39
Grade 1+	4	6
Grade 2+	15	24
Grade3+	19	31

According to percentage of p53 nuclear staining, 24/62 cases showed negative staining (0-10% cells showing positivity), 4/62 cases showed grade 1+ (10-30% of cells showing positivity), 15/62 cases had grade 2+ score (31-50% expression) and 19/62 cases showed grade 3+ (Greater than 50% expression).

9. Positive and Negative Expression of Staining of Tumor Cells By p53 With Histological Differentiation:

Table 6: Table showing p53 grading of cases according to histologic grade

Histologic Grade	Total Case	Number Of P53 Positive Cases	Number Of P53 Negative Cases	Percentage Of P53 Positive Cases According To Histologic Grade
Well Differentiated	35	16	19	46
Moderately Differentiated	17	13	4	76
Poorly Differentiated	10	9	1	90

P value is 0.012, this is significant at $p<0.05$. When we compared the p53 staining of the tumor cells with histological grade or differentiation of the tumor, it was observed that there was a significant statistical correlation between histologic grade and p53 expression. 16 cases out of 35 well differentiated carcinoma (46% of all well differentiated carcinoma cases), 13 cases out of 17 moderately differentiated carcinoma (76% of all moderately differentiated carcinoma cases) and 9 cases out of 10 poorly differentiated carcinoma (90% of all poorly differentiated carcinoma cases) are positive for p53 expression.

This implies that there is increased p53 expression in higher grade tumors.

10. Distribution Of Case According To Ki67 Expression:

Table 7: Table showing distribution of cases according to Ki67 expression in tumour cells:

% Of Tumor Cells Showing Ki67 Expression	Number Of Cases	Percentage
Negative (<10%)	18	29
Positive (>10%)	44	71
Total	62	100

We found that 44 out of 62 cases (71%) showed Ki67 positive expression whereas 18 cases (29%) had negative Ki67 expression.

11. Distribution of cases according to Ki67 grading of head and neck squamous cell carcinoma cases: To find the grade of Ki-67 expression, nuclei of 200 epithelial cells over the whole epithelial layer were examined in a high-power field. Ki-67 index has been defined as the percentage of Ki-67 positive cells:

Negative: when the Ki-67 index was <10%,

1+ : when the Ki-67 index was 10-30%,
2+ : when the Ki-67 index was 30%-50%
3+ : when the Ki-67 index greater than 50%91

Table 8: Table showing Ki67 grading of cases:

Grade of Ki67 Staining	No. of Cases	Percentage of Cases
Negative	18	29
Grade 1+	11	18
Grade 2+	19	31
Grade3+	14	22
Total	62	100

According to percentage of Ki67 nuclear staining, 18/62 cases showed negative staining (0-10%cells showing positivity), 11/62 cases showed grade 1+ (10-30% of cells showing positivity), 19/62 cases had grade 2+ score (30-50% expression) and 14/62 cases showed grade 3+ (Greater than 50% expression).

12. Ki67 Expression with Histologic Grade:

Table 9: Table showing Ki67 expression according to histologic grade or differentiation

Differentiation Of Tumors	Number Of Cases	Positive Ki67 Expression	Negative Ki67 Expression	Percentage Of Ki67 Positivity According To Histologic Grade
Well Differentiated	35	18	17	51
Moderately Differentiated	17	16	1	94
Poorly Differentiated	10	10	0	100

p value is 0.0005. This is significant since $p<0.05$. When we compared the Ki67 staining of the tumour cells with histological grade or differentiation of the tumour, it was observed that there was a significant statistical correlation between histologic grade and Ki67 expression. 18 cases out of 35 well differentiated carcinoma (51% of all well differentiated carcinoma cases), 16 cases out of 17 moderately differentiated carcinoma (94% of all moderately differentiated carcinoma cases) and 10 cases out of 10 poorly differentiated carcinoma (100% of all poorly differentiated carcinoma cases) are positive for Ki67 expression. This implies that there is increased Ki67 expression in higher grade tumours.

Figures:

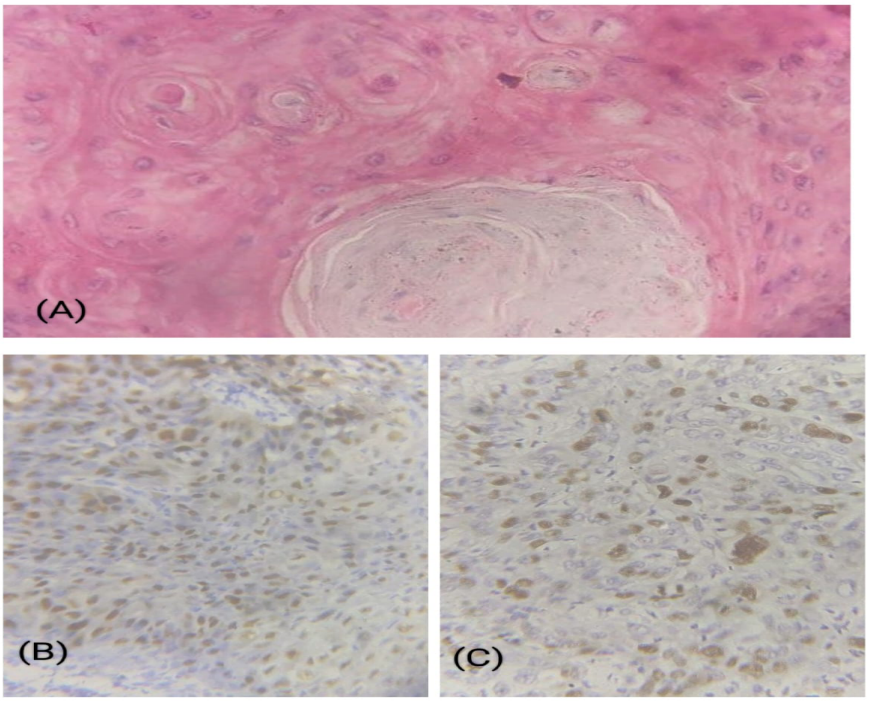


Figure 3: (A) H&E Section Of Well Differentiated Squamous Cell Carcinoma, (B) P53 Immunohistochemistry, (C) Ki67 Immunohistochemistry

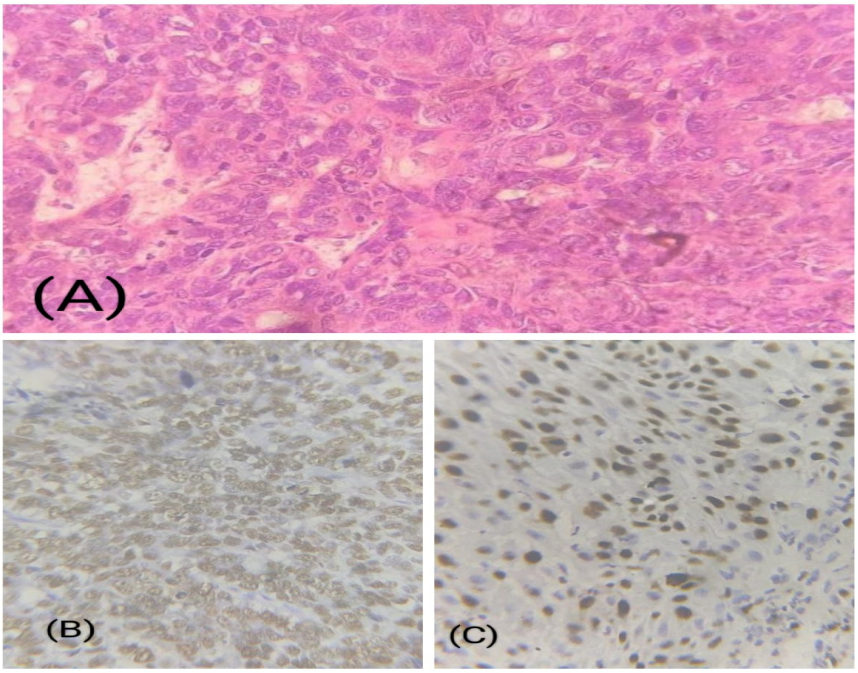
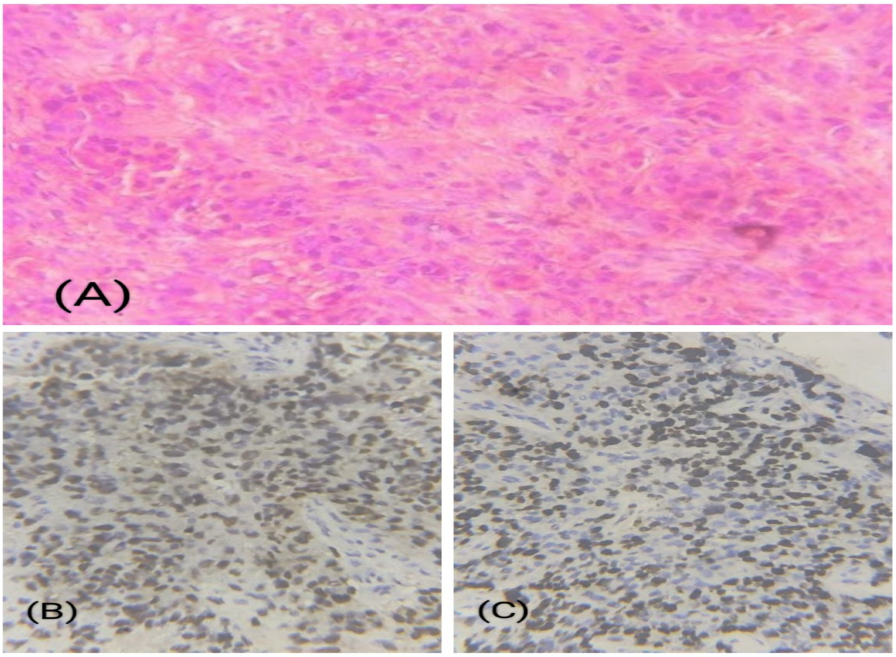


Figure 4: (A) H&E Section Of Moderately Differentiated Squamous Cell Carcinoma, (B) P53 Immunohistochemistry, (C) Ki67 Immunohistochemistry



Figures 5: (A) H&E Section of Poorly Differentiated Squamous Cell Carcinoma, (B) P53 Immunohistochemistry, (C) Ki67 Immunohistochemistry

Discussion:

The present study was undertaken in the Department of Pathology, Silchar Medical College & Hospital, Silchar during the study period of June, 2020 to May, 2021.A total of 62 punch biopsy/incisional biopsy/excisional biopsy/ resection specimens were studied.

In the present study, the age of patients of head and neck squamous cell carcinoma ranged from 30 to 89 years. The mean age was 53.37 years with a standard deviation of 11.79 years and median age is 50.5 years and mode is 40 and 45 years. The peak incidence of head and neck squamous cell carcinoma was seen in the fifth decade followed by sixth decade. It is seen that out of the 62 cases, 40 cases (65%) were male and 22 cases (35%) were female. The male: female ratio is 1.8:1.Majority of the patients (50 numbers or 81%) had tumor in the oral cavity followed by 7/62 (11%) in the oropharynx, 4/62 (6%) in the larynx and 1/62 (2%) in the laryngopharynx.

There was no incidence of squamous cell carcinoma in the nasopharynx during this study period. Among the 62 cases, 32 (52%) had a positive history of smoking, followed by 12/62 (19%) consuming of alcohol along with smoking. Most of the cases (52 out of 62 or 84%) did not have lymph node metastasis. Only 10/62 (16%) cases showed lymph node metastasis. According to histological differentiation, 35/62 (57%) cases were well differentiated carcinoma, 17/62 (27%) cases were moderately differentiated carcinoma and 10/62 (16%) cases were poorly differentiated carcinoma. These observations are similar to the studies conducted by Ruchi Dhuria et al [10] (2020), K. Boslooper et al [11] (2008) and Juan Carlos de Vicente et al [12] (2003).

Immunohistochemistry:

p53:

We found that 38 out of 62 cases (61%) showed p53 positive expression whereas 24 cases (39%) had negative p53 expression. According to percentage of p53 nuclear staining, 24/62 cases showed negative staining (0-10%cells showing positivity), 4/62 cases showed grade 1+ (10-30% of cells showing positivity), 15/62 cases had grade 2+ score (31-50% expression) and 19/62 cases showed grade 3+ (Greater than 50% expression).When we compared the p53 staining of the tumor cells with histological grade or differentiation of the tumor, it was observed that there was a significant statistical correlation between histologic grade and p53 expression.16 cases out of 35 well differentiated carcinoma (46% of all well differentiated carcinoma cases), 13 cases out of 17 moderately differentiated carcinoma (76% of all moderately differentiated carcinoma cases) and 9 cases out of 10 poorly differentiated carcinoma (90% of all poorly differentiated carcinoma cases) are positive for p53 expression. This implies that there is increased p53 expression in higher grade tumors.

The findings in the present study are similar to the following studies:

K. Boslooper et al (2008) found that p53 immunoreactivity was observed in 63% (73 out of 118) of the tumours, of which 29 cases were classified as grade 1+, 20 cases as grade 2+ and 24

cases as grade 3+. They also found that p53 immunoreactivity significantly correlated with the differentiation of the tumour. It is more often noted in higher grade lesions in head and neck squamous cell carcinoma. [11]

Jenni K Peltonen et al (2010)[13] showed that 52.2% patients of head and neck squamous cell carcinoma showed positivity for p53 immunohistochemistry and 50% of well differentiated tumors, 48.3% of moderately differentiated carcinoma and 71.4% of poorly diffrentiated carcinoma are p53 positive. [13]

Kanika Taneja et al (2016)[14] conducted a study on 50 cases. 30 cases out 50 were positive for p53 (60%) and a statistically significant association was observed between p53 and histologic grade. [14]

Erber et al [15] showed that strong association of p53 overexpression exists with histological grading (p=0.021). [15]

Ki67:

We found that 44 out of 62 cases (71%) showed Ki67 positive expression whereas 18 cases (29%) had negative Ki67 expression.

When we compared the Ki67 staining of the tumor cells with histological grade or differentiation of the tumor, it was observed that there was a significant statistical correlation between histologic grade and Ki67 expression. 18 cases out of 35 well differentiated carcinoma (51% of all well differentiated carcinoma cases), 16 cases out of 17 moderately differentiated carcinoma (94% of all moderately differentiated carcinoma cases) and 10 cases out of 10 poorly differentiated carcinoma (100% of all poorly differentiated carcinoma cases) are positive for Ki67 expression. This implies that there is increased Ki67 expression in higher grade tumours.18/62 cases showed negative staining (0-10%cells showing positivity), 11/62 cases showed grade 1+ (10-30% of cells showing positivity), 19/62 cases had grade 2+ score (30-50% expression) and 14/62 cases showed grade 3+ (Greater than 50% expression).

The findings in the present study are similar to the following studies:

Dragomir et al [16] (2012) showed that immunohistochemical expression of Ki67 was correlated to the degree of tumoral differentiation ($p<0.05$).

Mohamad Javad Ashraf et al [17] (2010) found that in laryngeal squamous cell carcinoma, there were significant correlations between tumoral Ki67 immunoexpression and tumor histologic grade ($P = 0.017$).

Nina Zidar et al [18] (2007) showed that a statistically significant correlation exists between its grade and the percentage of Ki-67-($p<0.01$) in squamous cell carcinoma.

Conclusion:

Head and neck squamous cell carcinoma is a major health problem in developing countries. We found majority of the cases being positive for p53 and Ki67 immunostaining and we also observed a significant correlation of the expression of p53 and Ki67 with histological differentiation.

Almost all of the higher grade lesions were positive for p53 and Ki67 whereas nearly half of the low grade lesions are positive for p53 and Ki67. This emphasizes the fact that increased p53 and Ki67 expression is present as cancer advances and it can be used in the evaluation of proliferative activity and progressive potential of the disease.

Head and neck carcinomas with advanced stages are challenging to treat and the outcome of patients with advanced stages remains still poor. Specific anticancer therapy targeting p53 and Ki67 coupled with radiotherapy can benefit the patients with advanced head and neck squamous cell carcinoma.

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