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Assessment of Immunohistochemical Expression of P16 and EGFR in Head and Neck Squamous Cell Carcinoma and Their Correlation with Clinicopathological Parameters

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Abstract
Introduction: *Oral squamous cell carcinoma is a major cause of death throughout the developed world. It is associated with tobacco chewing, paan chewing and alcohol consumption. Human papillomavirus (HPV) type 16 has also been suggested to play a role in etiology of head and neck squamous cell carcinoma (HNSCC). p16 expression is now being used as a surrogate marker for HPV infection in squamous cell carcinoma. Overexpression or mutation of EGFR is found in 80-100% of the patients with HNSCC, and is associated with poor prognosis and decreased survival.*
Materials and Methods: *In this Cross-sectional observation study, total of 100 cases of HNSCC were taken. p16and EGFR expression was determined by immunohistochemical staining and correlated with clinicopathological parameters. p16 expression was also correlated with expression of EGFR. The obtained results were analysed and evaluated using Chi ‑square test, value of p < 0.05 was taken significant.*
Results: *p16 and EGFR were positive in 60% and 58% cases respectively. A statistically significant direct association was observed between p16 with age, site of the tumour, abnormal sexual habits and lymph node involvement. Statistically significant correlation was also found betweenimmunohistochemical expression of p16 with EGFR (p=0001).*
Conclusion:*Immunohistochemical expression of p16 can be used as a surrogate marker of HPV. Study of p16 and EGFR expression may provide clinicians with more exact information in order to evaluate tumour aggressiveness, treatment modalities and can provide support for vaccination program in high risk group.*
Key Words:*Head and neck squamous cell carcinoma, human papillomavirus, immunohistochemistry, p16, EGFR.*

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I. Introduction

Head and neck cancer (HNC) is the tenth most common malignancy globally.[1] In India, it ranks among the top three types of cancer.[2] More than 90% of head and neck cancers are squamous cell carcinomas (HNSCC). Others are quite uncommon types which include lymphoma and adenoid cystic carcinoma.[3] Squamous cell carcinoma of the upper aerodigestive tract typically occurs in older patients in their fifth to seventh decades of life and older. Commonly, there is a prolonged history of tobacco exposure and alcohol abuse.[4]

According to the current literature, the risk factors of HNSCC are surprisingly similar to those of cervical cancer and cervical intraepithelial neoplasia (CIN), including the number of sexual partners, younger age at first sexual intercourse, practice of oral sex, history of genital warts and younger age.[5] HPV status is associated with p16 expression, and HPV positive tumor are less likely to harbor p53 mutations.[6]The prognosis for patients with HNSCC is determined by the stage at presentation.Early-stage tumors are treated with surgery or radiotherapy and have a favorable prognosis.[7]

The loss of expression of p16 has been observed in oral premalignant lesions and primary tumors of the oral cavity. Mechanisms of inactivation include homozygous gene deletion, gene mutation and hypermethylation of upstream CpG island regions.[8] HPV associated cancers are caused by expression of HPV's E6 and E7 proteins that bind to and inactivate tumor suppressor proteins p53 and retinoblastoma protein (pRb) respectively, leading to malignant transformation of HPV infected cells.[9] As with female genital (or cervical) carcinogenesis, the immunohistochemical detection of p16 protein (p16 IHC) has been proposed as surrogate marker of HPV infection in head and neck cancer.[10]

Binding of natural ligands to EGFR promotes homo- or heterodimerization of EGFR with other ErbB/HER family of receptors with subsequent autophosphorylation and activation of the tyrosine kinase. This

activation of EGFR leads to the initiation of intracellular signaling pathways which regulate the activation of cell proliferation, invasion, angiogenesis, and metastasis.Overexpression or mutation of EGFR is found in 80-100% of the patients with HNSCC, and are associated with poor prognosis and decreased survival.[11]

Aim of this study was to study p16 and EGFR expression in head and neck squamous cell carcinoma on immunohistochemistry and correlate expression of IHC markers with clinicopathological parameters as well as correlation of p16 expression with expression ofEGFR.

II. Materials And Methods

This was a cross-sectional observation study in duration of January 2017 to June 2018 done in Department of Pathology. Hundred histologically diagnosed cases of head and neck squamous cell carcinoma were studied. Patients with other than HNSCC such as adenocarcinoma, melanoma, sarcoma, metastasis, etc., were excluded. Data obtained were analyzed with other clinicopathological parameters including age and sex of patient, history of tobacco use, paan chewing, alcohol abuse, abnormal sexual habits, site of lesion, grade of tumor, and lymph node metastasis.

The tissue was fixed in buffered formalin (pH = 7.0), and embedded in paraffin. The tissue block was sectioned at 4–5 μm and the sections were stained for hematoxylin and eosin as per standredguidelines and examined. Histopathologic grading was done according to World Health Organization criteria based on three parameters (1) flattened polyhedral, round, or ovoid epithelial cells; (2) intracellular or extracellular keratinization; and (3) intercellular bridges.[7]

Grade I: Well‑differentiated

Grade II: Moderately differentiated

Grade III: Poorly‑differentiated or anaplastic

Immunohistochemical (IHC) profile of the tumor was assessed by subjecting one representative section from tumor block to p16and EGFR each. Immunohistochemistry was performed on 4 μm thick sections from 10% formalin‑fixed paraffin‑embedded specimens, according to the streptavidin‑biotin immunoperoxidase technique. Positive and negative controls were run simultaneously for each IHC marker.

Interpretation of results

The IHC expression of p16 was classified according to nuclear and cytoplasmic positivity. These were scored as positive when more than 5% cells (cut‑off) stain positive. Biopsies with diffuse pattern(>30–85% of labeled cells with strong positivity, spreading in several tissue areas) were considered to have high IHC expression of p16 (Grade III). Focal distribution (>10–30% of labeled nuclei and cytoplasm strongly positive, spreading in one tissue area) was considered as moderate expression (Grade II) and sporadic positivity (5–10% of nuclei and cytoplasm with weak and scattered positivity as low expression (Grade I).[12]

For EGFR, predominantly membranous expression was considered and scored based on following criteria. Score 0 = no staining, 1+=weak (light brown color), 2+=moderate (dark brown color), 3+=strong staining (very dark brown color). Score 0 and 1+ was considered negative and 2+ and 3+ was considered positive.[11]

A descriptive study was carried out for all the variables included in the study. The whole data was entered in Microsoft Excel master sheet and analysed using Statistical Package for Social Sciences (SPSS) 15.0 software. As the data was qualitative, Chi‑square test was used to assess the association between these parameters. A value of $p< 0.05$ was taken as significant and <0.01 as highly significant; whereas, $p> 0.05$ was taken as nonsignificant.

III. Observations

In this study range of the age of the patients was 17 years to 92 years, with the mean age of 46.56 ± 14.40 years and most common age group was 31-40 years. The mean age of the males and females were 46.03 ± 14.33 years and 50.08 ± 14.91 years respectively. Eighty seven (87%) patients were male and 13 (13%) were female with a sex ratio of approximately 6.7:1.

Eighty six (86%) patients were tobacco users, intake being in the form of gutka, 24 (24%) were alcoholics, 60 (60%) were paan chewers and 24(24%) were having history of abnormal sexual habits. The most common site of involvement was oral cavity (79%) followed by oropharynx (12%), hypopharynx (3%) and the larynx (2%). Sixty one (61%) cases were histologic grade I, thirty five (35%) were histologic grade II and 4(4%) were histologic grade III tumours.In 74% cases lymphadenopathy was absent.

Sixty cases (60%) were positive for p16, including 23 (23%) grade 1, 18 (18%) grade 2 and 19 (19%) cases with grade 3 p16 expression.Fifty eight (58%) cases were positive for EGFR, out of which 27 cases were EGFR score 2+ while 31 cases were EGFR score 3+ positive.

Correlation of p16 expression with sex, site of primary tumour and abnormal sexual habits (p value=0.004, 0.03 and 0.02 respectively), was found to be statistically significant (table 1).

Correlation of expression ofEGFR with lymphadenopathy was statistically insignificant (p= 0.67) while p16 expression with lymphadenopathy was statistically significant (p=0.009).

Correlation of p16 expression with EGFR was found to be statistically significant (p=0.001) (table 2).

While correlation of both p16 and EGFR expression with age (p=0.224 and 0.18 respectively), tobacco abuse(p=0.16and 0.22 respectively), alcohol abuse(p=0.44and 3.62 respectively), paan chewing(p=0.09and 0.19 respectively) and histologic grading (p value=0.36 and 0.09 respectively) (table1 and 3)was not found to be statistically significant. EGFR expression with sex (p= 0.13), site of primary tumour (p= 0.48) and abnormal sexual habits (p=0.14) was also statistically insignificant(table 1).

IV. Discussion

In our study, sixty percent HNSCC cases positively expressed p16 which was concordant with study of Fregonesiet al[12] and Yuen et al.[13]Fifty eight percent cases positively expressed EGFR which was concordant with Afriyie et al[14] and other studies**.** Whereas wide variability and discordance was observed in expression of these markers with other studies when the criteria's used were different.

The age and sex distribution of present as well as previous studies indicate that the incidence of head and neck malignancies is higher in older age group and in males. This can be attributed to habit of tobacco chewing or smoking or alcohol consumption being more common amongst males in our part of the world which play an important role in the etiopathogenesis of HNSCC. The age and sex distribution in our study was in concordance with the study conducted byRalli et al and other studies.[15]

Significant association was seen between p16 and tumour site (p=0.03) which was in concordance with the study of Yuen et al[13]and other studies,while discordant with Shinhora et al.[16] This was because of more use of smokeless tobacco by lower and middle class socioeconomic group males in Saifai. In study by Shinhoraet al[16], tonsil was the most common site of primary tumour and it was statistically significant with p16 expression (p<0.01).No significant association was seen between EGFR and tumour site (p=0.48). This is in concordance with Sweeny et al[17] and Dalal et al.[18]

Most of the patients in our study had history of tobacco and paan chewing but no statistically significant association found between p16 expression and tobacco chewing (p=0.16), paan chewing (p=0.09) and alcohol consumption (p=0.44). This was concordant with Dragomiret al[19] and other studies.Ralli et al[15]found a statistically significant association of p16 expression with paan chewing (p=0.03).

A significant association was seen between p16 expression and history of abnormal sexual habits (p=0.02)like practice of oral sex and history of multiple sexual partners in our study. According to study by Ralli et al,[15] significant association was seen between p16 expression and history of multiple sexual partners (p=0.003). According to studies conducted by Pannone et al, Fregonesi et al and other studies p16 expression was strongly associated with HPV infected HNSCC.[10,12]This is because p16 expression is strongly associated with HPV infected HNSCC.

In our study there was no statistically significant association of EGFR expression with tobacco chewing (p=0.22), alcohol consumption (p=3.62), paan chewing (p=0.19) and abnormal sexual habits (p=0.14) which was concordant with Sweeny et al and Dalal et al.[17,18]

In the present study p16 expression had no significant correlation with histological grade of the tumour (p=0.36) which was concordant with Yuen et al and Dragomir et al[13,19] and discordant with the study of Ralli et al (p=0.045) who observed that p16 overexpression was more likely to be detected with later stage and higher grade.[15]

Some authors hypothesized that tumors that exhibited p16 expression had an effect on cell differentiation. The cells are probably arrested at a stage within the process of differentiation leading to tumour comprised predominantly of poorly‑differentiated nonkeratinizing areas.

These differences could also be attributed to difference in geographic distribution of tumour, difference in sample size, difference in scoring criteria and different type of antibodies used by different authors.

p16 expression was seen in 50 out of the 74 (67.56%) lymph node negative cases which was statistically significant (p=0.009). This was discordant with Yuen et al,[13] while in accordance with other studies.

The comparison of EGFR with lymph node involvement reveal no statistically significant association (p=0.67) which was concordant with study of Sweeny et al[17] but discordant to Dalal et al.[18]

Correlation between p16 and EGFR expression was statistically significant which was discordant with study by Afriyie et al,[14] Husain et al[20] and other studies. According to study by Husain et al[20] expression of total and nuclear EGFR was higher in p16-negative tumours compared to p16-positive tumours.

V. Conclusion

As HPV integration with transcription of viral oncoprotein induces overexpression of p16, we can use p16 immunohistochemistry as a surrogate marker of HPV. Significant expression of p16 in node negative

patients may guide the type and intensity of the therapy in patients with head and neck squamous cell carcinoma.

Overexpression of p16 has been significantly seen in male patients of HNSCC who had tumor in oral cavity and had the history of abnormal sexual habits. Vaccination programme may provide prevention from HPV infection in high risk population.

A significant association was found between p16 and EGFR. Thus associations of these markers with other well established prognostic markers needs to be assessed for any variable outcome.

Hence study of p16and EGFR expression may provide clinicians with more exact information in order to evaluate tumour aggressiveness and treatment modalities.

References

[1]. Siddiqui MS, Chandra R, Aziz A, Suman S. Epidemiology and histopathological spectrum of head and neck cancers in Bihar, a state of eastern India.Asian Pac J Cancer Prev2012;13:3949-53.

[2]. Elango JK, Gangadharan P, Sumithra S, Kuriakose MA. Trends of head and neck cancers in urban and rural India. Asian Pac J Cancer Prev 2006;7:108–12.

[3]. Pena JC, Thompson CB, Recant W, Vokes EE, Rudin CM. BCL-x and bcl2, p53 expression in squamous cell carcinoma of the head and neck cancer. Cancer 1999;85:164-70.

[4]. El-Mofty SK, Lu DW. Prevalence of human papillomavirus type 16 DNA in squamous cell carcinoma of the palatine tonsil, and not the oral cavity, in young patients: a distinct clinicopathologic and molecular disease entity. Am J SurgPathol 2003;27:1463-70.

[5]. Hennessey PT, Westra WH, Califano JA. Human papillomavirus and head and neck squamous cell carcinoma: recent evidence and clinical implications. J Dent Res 2009;88:300–6.

[6]. Syrajanen S. The role of human papillomavirus infection in head and neck cancers. Ann Oncol 2010;21:243-5.

[7]. Barnes L, Eveson JW, Reichart P, Sidransky D, editors. World Health Organization classification of tumours. Pathology and genetics of head and neck tumours. France.Lyon: IARC Press 2005;11:1-15.

[8]. Vairaktaris E, Yapijakis C, Psyrri A, Spyridonidou S, Yannopoulos A, Lazaris A et al. Loss of tumor suppressor p16 expression in initial stages of oral oncogenesis. Anticancer Res 2007;27:979-84.

[9]. Smeets SJ, Plas M, Schaaij-Visser T, Veen E, Meerloo J, Braakhuis B et al. Immortalisation of oral keratinocytes by functional inactivation of p53 and pRb pathways. Int J Cancer 2011;128:1596-605.

[10]. Pannone G, Rodolico V, Santoro A, Muzio L, Franco R, Botti G et al. Evaluation of combined triple method to detect causative HPV in oral and oropharyngeal carcinomas: p16 immunohistochemistry, consensus PCR HPV-DNA, and in situ hybridization. Infectious Agents and Cancer 2012;7:4-17.

[11]. Abusail MS, Dirweesh AMA, Salih RAA, Gadelkarim AH. Expression of EGFR and p53 in Head and Neck Tumors among Sudanese Patients. Asian Pac J Cancer Prev 2013;14:6415-18.

[12]. Fregonesi PA, Teresa DB, Duarte RA, Neto CB, Oliveira M, Soares CP. p16ink4a immunohistochemical overexpression in premalignant and malignant oral lesions infected with human papillomavirus. J HistochemCytochem. 2010;51:1291-7.

[13]. Yuen AP, Lam KY, Choy JT, Ho WK,Wong LY, Wei WI. Clinicopathologic significance of bcl-2 expression in the surgical treatment of oral squamous cell carcinoma. J SurgOncol. 2002;94:713-722.

[14]. Afriyie OO, Owiredu WKBA, Danquah KO, Reindorf RL. Expression of immunohistochemical markers in non-oropharyngeal head and neck squamous cell carcinoma in Ghana. Pone 2018;59:711-5.

[15]. Ralli M, Singh S, Yadav SPS, Sharma N, Verma R, Sen R. Assessment and clinicopathological correlation of p16 expression in head and neck squamous cell carcinoma. J Cancer Res Ther 2016;12:232-37.

[16]. Shinohara S, Kikuchi M, Kanazawa Y, Kishimoto I, Harada H, Imai Y et al. Prognostic Impact of p16 and p53 Expression in Oropharyngeal Squamous Cell Carcinomas. Japanese Journal of Clinical Oncology 2014;44: 232–240.

[17]. Sweeny L, Dean NR, Magnuson JS, Carroll WR, Helman EE, Hyde SO, et al. EGFR Expression in Advanced Head and Neck Cutaneous Squamous Cell Carcinoma. Head Neck. 2012; 34: 681–686.

[18]. Dalal V, Alam K, Maheshwari V, Sharma S C, Jain A, Khan R. Correlation of clinicopathological parameters with EGFR and Her-2 neu status in oral cancer patients in a tertiary care centre. International Journal of Biomedical Research 2015;6:897-902.

[19]. Dragomir LP, Simionescu C, Margaritescu Cl, Stepan A, Dragomir IM, Popescu MR. p53, p16 and Ki-67 immunoexpression in oral squamous carcinomas. Rom J MorpholEmbryol 2012;53:89-93.

[20]. Husain H, Psyrri A, Markovic A, Rampias T, Pectasides E, Wang H et al. Nuclear epidermal growth factor receptor and p16 expression in head and neck squamous cell carcinoma. Laryngoscope 2012 ; 122: 2762–2768.

Table 1: CORRELATION OF p16AND EGFR EXPRESSION WITH VARIOUS CLINICOPATHOLOGIC FACTORS

Clinicopathological parameters	**p16**	**EGFR**
A. Age	(n=60)	(n=58)
≤50 years	43	42
>50 years	17	16
Pearson's Chi Square Value (p value)	1.48(0.224)	1.83(0.18)
B. Sex	(n=60)	(n=58)
Male	47	53
Female	3	5
Pearson's Chi Square Value (p value)	8.49(0.004)*	2.34(0.13)
C. Site	(n=60)	(n=58)
Oral cavity	53	47
Oropharynx	4	7
Larynx	0	2
Hypopharynx	1	1
Face	2	1

Pearson's Chi Square Value (p value)	8.924(0.03)*	3.71(0.48)
D. Tobacco chewing	(n=60)	(n=58)
Positive	54	52
Negative	6	6
Pearson's Chi Square Value (p value)	1.993(0.16)	1.53(0.22)
E. Alcohol	(n=60)	(n=58)
Positive	16	12
Negative	44	46
Pearson's Chi Square Value (p value)	0.59(0.44)	0.83(3.62)
F. Paan chewing	(n=60)	(n=58)
Present	40	38
Negative	20	20
Pearson's Chi Square Value (p value)	2.78(0.09)	1.75(0.19)
G. Abnormal Sexual Habits	(n=60)	(n=58)
Present	19	17
Absent	41	41
Pearson's Chi Square Value (p value)	4.83(0.02)*	2.14(0.14)

***Statistically significant**

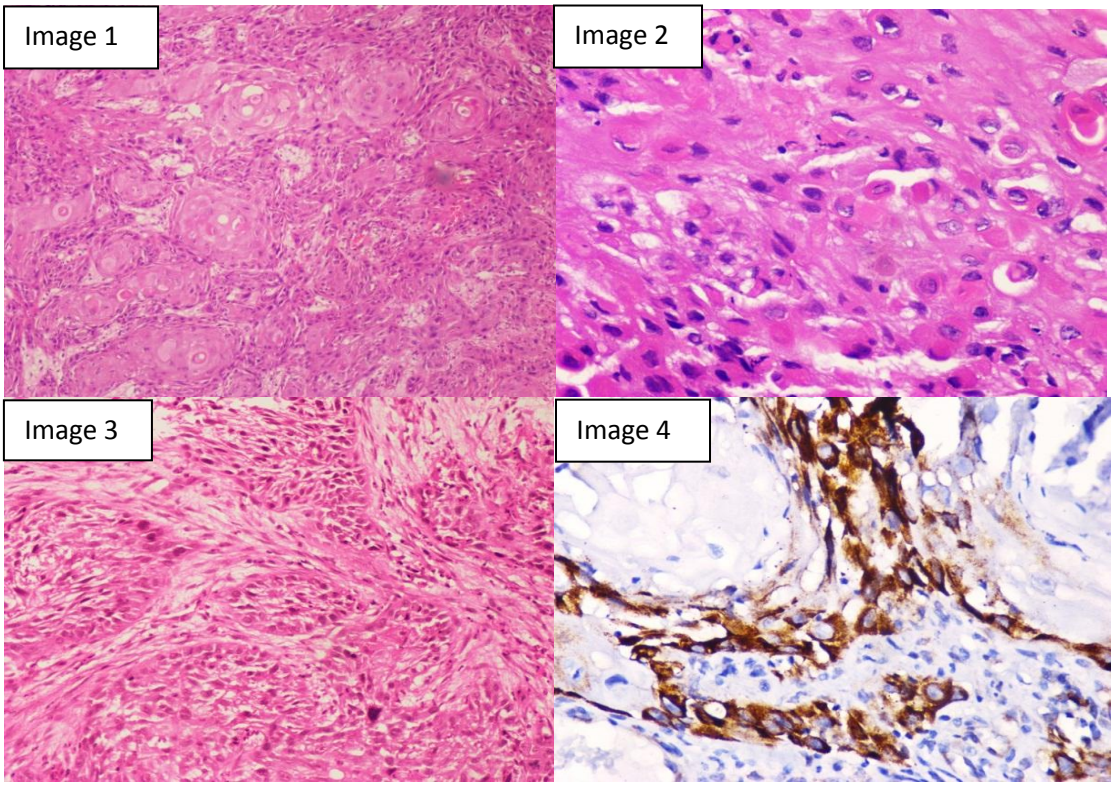
TABLE 2: CORRELATION OF COEXPRESSION OF p16 AND EGFR IN HNSCC

p16/EGFR STATUS	No. of cases (n=100)	Percentage (%)	Pearson's Chi Square Test Value (p Value)
p16+/EGFR+	**43**	**43**	
p16+/EGFR-	**17**	**17**	
p16-/EGFR+	**15**	**15**	
p16-/EGFR-	**25**	**25**	**11.50 (0.001)***
Total	**100**	**100**	

***Statistically significant**

TABLE 3: CORRELATION OF p16AND EGFR EXPRESSION WITH HISTOLOGICAL GRADING

Histological Grading	p16 (n=60)	EGFR(n=58)
I (WDSCC)	33	30
II (MDSCC)	24	25
III (PDSCC)	3	3
Pearson's Chi Square Test Value (p Value)	2.25(0.36)	4.92(0.09)



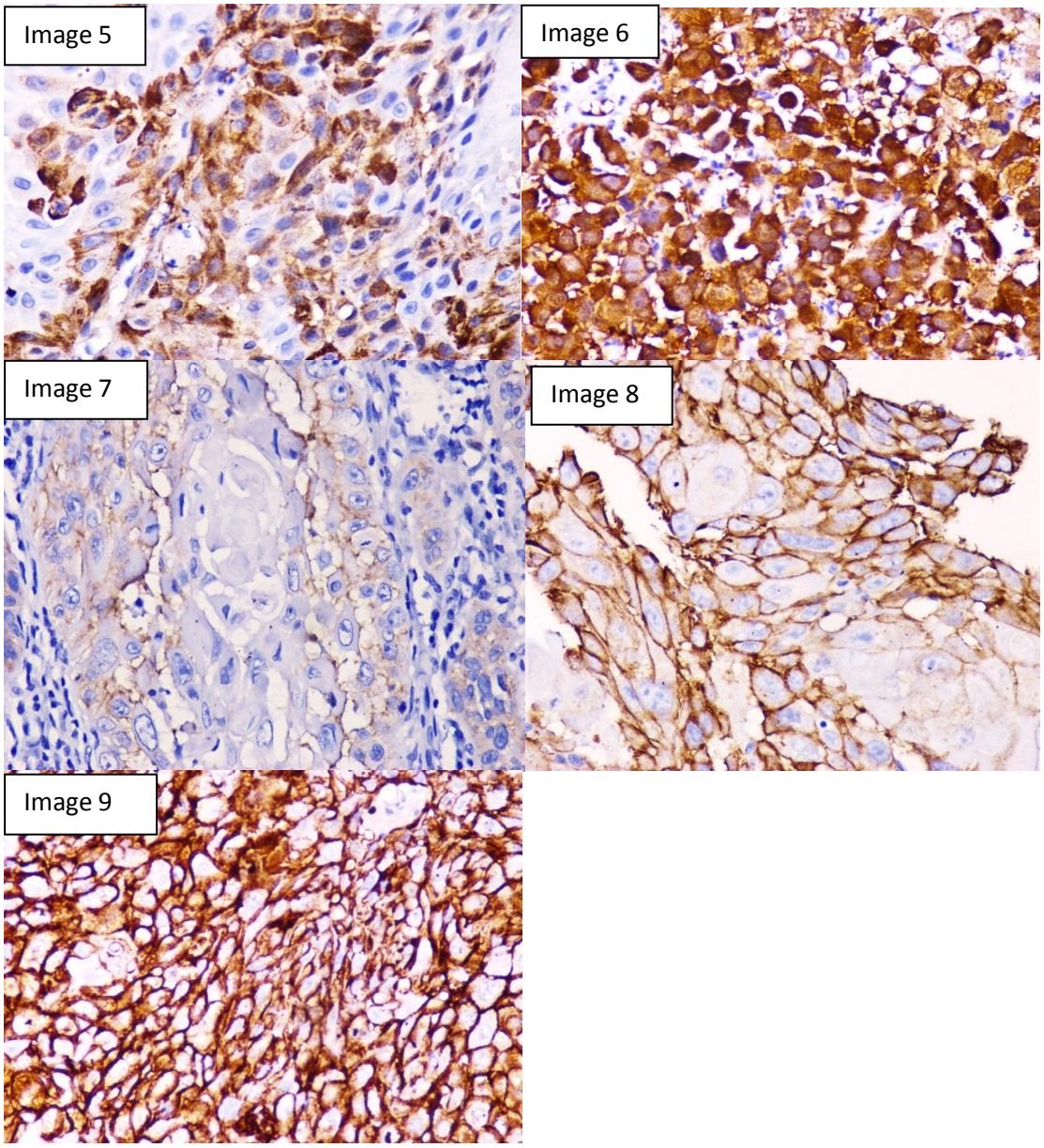


Image 1: Well Differentiated Squamous cell carcinoma showing keratin pearls and intracytoplasmic keratinization. (Hematoxylin and Eosin stain, X 100). Image 2: Moderately Differentiated Squamous cell carcinoma showing intracytoplasmic keratinization and nuclear pleomorphism. (Hematoxylin and eosin stain, X 400). Image 3: Poorly Differentiated Squamous cell carcinoma showing clusters of highly pleomorphic tumour cells with hyperchromatic nucleus and fair number of mitotic figures. (Hematoxylin and eosin stain, X 200). Image 4: IHC p16 - Score 1(IHC, X400). Image 5: IHC p16 – Score 2 (IHC, X400). Image 6: IHC p16 – Score 3, (IHC, X 400). Image 7: IHC EGFR – Score 1+, weak membranous staining (IHC, X400). Image 8: IHC EGFR – Score 2+, moderate membranous staining (IHC, X400). Image 9: IHC EGFR – Score 3+, strong membranous staining. (IHC,X400).

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