

CORRELATION OF P21 EXPRESSION IN HEAD AND NECK SQUAMOUS CELL CARCINOMA WITH CLINICOPATHOLOGIC AND PROGNOSTIC PARAMETRES

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Abstract

Purpose: Head and neck tumours include tumours of nose, paranasal sinuses, oropharynx, larynx and mouth. Squamous cell carcinoma (SCC) is the most common head and neck malignant tumour which accounts for 90% of head and neck malignant tumours. p21 is an important immunohistochemical marker which has significant role in predicting prognosis of head and neck SCC (HNSCC). Increased p21 expression in HNSCC is associated with bad prognosis in terms of increased risk of nodal metastasis, increased rate of recurrence and decreased survival rate. The purpose of this study was to evaluate the expression of p21 in HNSCC with various clinicopathologic and prognostic parametres.

Materials and Methods: A total of 110 patients ($n = 110$) of HNSCC (oral cavity $n = 98$ and laryngeal tumours $n = 12$) were included in the study which were diagnosed and treated between January 2008 and January 2011 at a tertiary care centre in Pakistan. Mean age was 51 years (age range 15–78). 65 (59%) were male and 45 (41%) were female. Tumours were classified as p21 positive when $\geq 10\%$ tumour cells were immunoreactive for p21. p21 expression was noted and correlated with T-stage, nodal metastasis, perineural and depth of invasion, recurrence and 2-year survival rate.

Results: Of 110 cases, p21-positive cases were 88 (80%) compared to 22 (20%) negative. T1–T2 tumours with p21 expression were 59 (67.04%) while 17 cases (77.27%) had negative for p21 ($P = 0.44$). Amongst T3–T4, 29 cases showed p21 expression (32.96%) while 5 cases (22.73%) were negative ($P = 0.44$). Nodal metastasis was seen in 51 cases (57.95%) with p21 positivity as compared to 4 cases (18.8%) with no p21 expression ($P = 0.0015$). Perineural invasion was seen in 15 carcinomas (17.04%) having p21 positivity and 1 case (4.54%) with p21 negativity ($P = 0.18$). 48 cases (54.54%) had depth of invasion > 1 cm with positive p21 as compared to 4 cases (18.18%) without (P value = 0.0035). 39 cases (44.31%) showed recurrence along with p21 expression while 3 cases (13.63%) showed recurrence without ($P = 0.0076$). 2-year survival rate was 56.81% ($n = 88$) in p21-positive cases, whereas it was 90.90% ($n = 20$) those with negative p21 ($P = 0.0026$).

Conclusion: Positive p21 expression in HNSCC correlates with intermediate grade, late stage, increased nodal metastasis, tumour recurrence and decreased survival. p21 should be considered as an important prognostic and predictive marker in HNSCC to detect tumours at early stage and to improve therapy and prognosis.

Key words: Head and neck squamous cell carcinoma, lymph node metastasis, p21, recurrence, survival rate

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Introduction

Head and neck tumours include tumours of nose, paranasal sinuses, oropharynx, larynx and mouth.^[1] Squamous cell

carcinoma (SCC) is the most common head and neck malignant tumour which accounts for 90% of head and neck malignant tumours.^[2] Etiological factors include tobacco smoking, alcohol intake, betel nut chewing, ultraviolet light, certain occupational chemicals and few viruses like human papilloma virus.^[3]

In recent years, certain risk factors such as betel nut chewing and use of smokeless tobacco (snuff) have led to increased incidence of head and neck SCC (HNSCC).^[4] HNSCC diagnosed on biopsies is treated by surgery, radiation therapy, chemotherapy, targeted therapy or combination of treatments. Failure of treatment in HNSCC is most likely due to local and regional recurrence. Lymph node metastasis leads to poor prognosis and decreased survival rate.^[5] Head and neck cancers include many other types, but SCC is most common and most frequent malignant tumour in this region. Multiple steps are involved in carcinogenesis of HNSCC including activation and suppression of tumour suppressor genes. Cell cycle balance is disturbed and uncontrolled cell growth starts as compared to cell death.

Many cell cycle regulators such as cyclins, cyclin-dependent kinases (CDKs), oncogenes and tumour suppressor genes play an important role in maintaining cell cycle and regulating balance between cell growth and cell death.

p21 is a new-emerging immunohistochemical marker which has significant role in predicting prognosis of HNSCC. Increased p21 expression in HNSCC is associated with worse prognosis in terms of increased risk for nodal metastases, increased rate of recurrence and decreased survival rate. p21 is a CDKs inhibitor and plays a significant role in various steps of cell cycle and tumour cell death.^[6]

The purpose of this study was to determine the expression of p21 in HNSCC with various clinicopathologic and prognostic parameters.

Materials and Methods

A total of 110 patients ($n = 110$) of NSCC were included in the study which were diagnosed and treated between January 2008 and January 2011 at a tertiary care centre

in Pakistan. Of 110 cases, 63 were from tongue, 16 from buccal mucosa, 4 from lip, 15 from alveolar mucosa and 12 from larynx. Mean age was 51 years (age range 15–78). 65 (59%) were male and 45 (41%) were female. Tumours were classified as p21 positive when $\geq 10\%$ tumour cells were immunoreactive for p21. Cutoff scores for p21

were determined by receiver operating characteristic curve analysis that is a cut point determination method.^[5] Nuclear positivity of p21 in HNSCC was noted, intensity of staining was not taken into consideration. p21 expression was noted and correlated with T-stage, nodal metastasis, perineural and depth of invasion, recurrence and 2-year survival rate.

Results

Of 110 cases, p21-positive cases were 88 (80%) compared to 22 (20%) negative. T1–T2 tumours with p21 expression were 59 (67.04%) while 17 cases (77.27%) had negative for p21 ($P = 0.44$). Amongst T3–T4, 29 cases showed p21 expression (32.96%) while 5 cases (22.73%) were negative ($P = 0.44$). Nodal metastasis was seen in 51 cases (57.95%) with p21 positivity as compared to 4 cases (18.8%) with no p21 expression ($P = 0.0015$). Perineural invasion was seen in 15 carcinomas (17.04%) having p21 positivity and 1 case (4.54%) with p21 negativity ($P = 0.18$). 48 cases (54.54%) had depth of invasion > 1 cm with positive p21 as compared to 4 cases (18.18%) without p21 ($P = 0.0035$). 39 cases (44.31%) showed recurrence along with p21 expression while 3 cases (13.63%) showed recurrence without p21 ($P = 0.0076$). 2-year survival rate was 56.81% ($n = 88$) in p21-positive cases, whereas it was 90.90% ($n = 20$) those with negative p21 ($P = 0.0026$).

Discussion

As part of cell cycle, p21 is an important cell cycle regulator such as p53 and p16. p21 binds to CDKs. Since cyclins inhibit apoptosis, it is thus a key factor in regulating the cell cycle at G1 phase.^[7]

Cyclin D/Cdk4 complex activated by p21, plays a major role in cell cycle.^[8] Cdk4-cyclin D interaction is controlled by p21 which forms complexes.^[9] p21 along with other regulators, such as p27 and survivin, controls apoptosis.^[10,11] p21 is an antiapoptotic factor and by inhibiting apoptosis interacts with normal cell cycle

progression.^[12] p21 also plays a role in cell cycle along with p53 which is also an important part of cell cycle.^[13]

Results: Association of p21 expression with clinicopathologic features:

Feature	p21 positive, n (%)	p21 negative, n (%)	P value
T classification			
T1–T2	59 (67.04)	17 (77.27)	0.44
T3–T4	29 (32.96)	5 (22.73)	
Nodal metastasis	51 (57.95)	4 (18.8)	0.0015
Tumour grade			
G1–G2	79 (89.77)	19 (86.36)	0.70
G3	9 (10.23)	3 (13.64)	
Depth of invasion			
<1 cm	40 (45.46)	18 (81.82)	0.0035
>1 cm	48 (54.54)	4 (18.18)	
Perineural invasion	15 (17.04)	1 (4.54)	0.18
Recurrence	39 (44.31)	3 (13.63)	0.0076
2-year survival rate	56.81 (n=88)	90.90 (n=22)	0.0026

Kaplan–Meier survival curve demonstrates 2-year survival rate of patients with p21-positive and negative expression.

Our study aimed at depicting antiapoptotic effect of p21 in HNSCC, to predict prognosis. Possible mechanism for the role of p21 role in predicting prognosis includes its proliferative effect in tumour and surrounding cells. Our study correlated with worse prognosis with positive p21 expression in HNSCC when compared with negative expression.

In our study, p21 clearly led to a higher incidence of nodal metastasis 57.95% compared with 18.8% in p21-negative carcinomas. Locoregional relapse was also high with p21-positive HNSCC as compared to p21-negative tumours (44.31% vs. 13.63%). 2-year survival rate was low with p21-positive carcinomas (56.81%) as compared to p21 negative (90.90%). A study showed that expression of this marker leads to increased lymph node metastases, recurrence and decreased survival rate, hence, predicting poor prognosis [Figures 1-5].

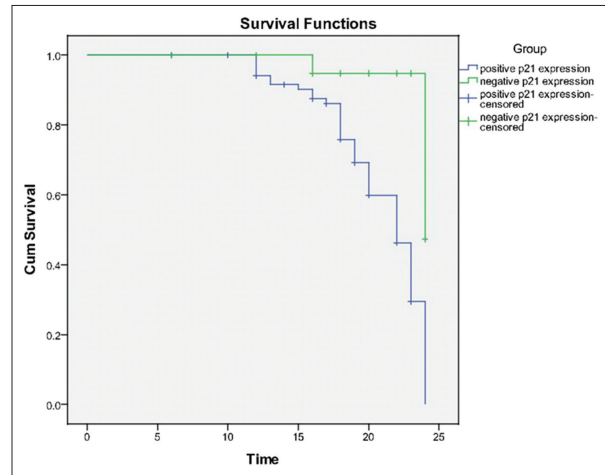


Figure 1: Survival functions

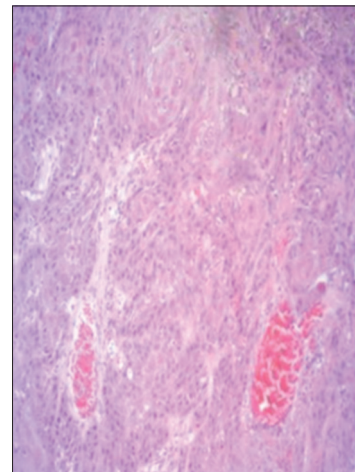


Figure 2: Squamous cell carcinoma, ×20, H and E stain

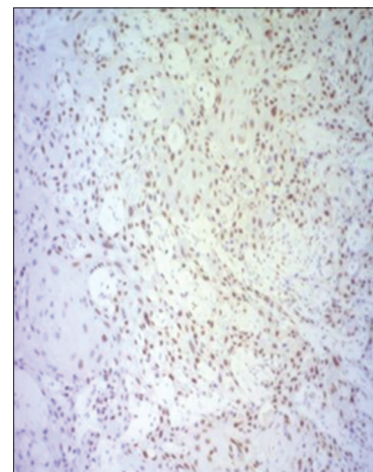


Figure 3: p21 staining (positive), ×20

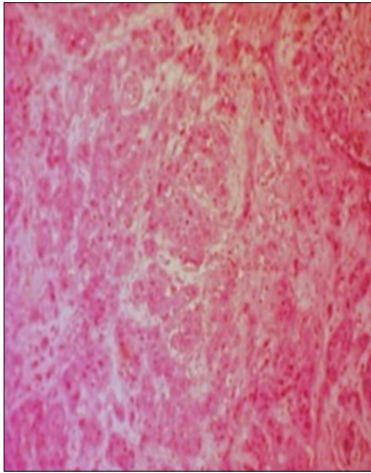


Figure 4: Squamous cell carcinoma $\times 20$, H and E stain

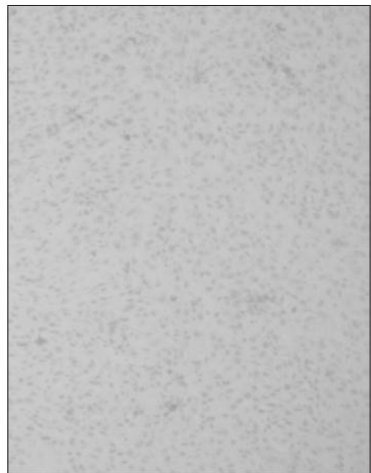


Figure 5: p21 staining, (negative), $\times 20$

In a study by Fischer *et al.* in which p21 expression was evaluated in 117 HNSCC and Ki67 expression in 116 cases, increased p21 expression led to increased nodal metastases, tumour recurrence and decreased survival rate. In their retrospective study with 117 patients of HNSCC, lymph node metastases were seen in 93.7% with p21-positive carcinomas and 66.7% with p21-negative tumours. In the same study, 5-year survival rate was 35.2% with positive p21 expression and 54.7% with negative expression, thus they confirmed decreased overall survival rate with positive p21 expression. They also showed 24.3% 5-year survival rate with Ki67 expression and 50.2% in that negative Ki67 expression, (Ki67 was considered positive when $\geq 60\%$ tumour cells were immunoreactive for Ki67), thus having similar result

like that for p21. In addition, coexpression of both p21 and ki67 showed worse prognosis with increased nodal metastases, recurrence and decreased survival rate.^[5]

In another study by Fizazi *et al.*^[16] related to p21 and Ki67 expression in prostatic cancer, it was concluded that p21 is an important prognostic and predictive marker of tumour recurrence and survival along with increased Ki67 expression.

Similar results were observed in the study by Lavertu *et al.*, they proposed regular follow-up of patients with increased expression of these markers to decrease recurrence, detect second primary at earlier stage and improve survival.^[17]

In our study, p21 positivity was observed in 80% of cases. This frequency of p21-positive HNSCC positively correlates with many studies, which showed frequency ranging from 58% to 82%.^[18-21]

The possible major mechanism of action for p21 in HNSCC includes its antiapoptotic effect,^[14] uncontrolled cellular proliferation and decreased cell death, hence, p21 reducing survival rate by developing resistance to therapy.^[15,22]

Conclusion

Positive p21 expression in HNSCC correlates with increased nodal metastases, tumour recurrence and decreased survival. p21 is an important prognostic and predictive marker in HNSCC. Its expression should be evaluated to detect tumours at early stage, to improve therapy and prognosis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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