Applied Cancer Research

RESEARCH ARTICLE Open Access

Low $p27^{kip1}$ expression in head and neck squamous cell carcinoma: association with risk factors and adverse outcomes

Atif Ali hashmi[1*], Saher Aijaz[2], Muhammad Irfan[1], Zubaida Fida Hussain[1], Shumaila Kanwal Hashmi[3], Huda Asif[3] and Naveen Faridi[1]

Abstract

Background: Squamous cell carcinoma (SCC) of head and neck is highly prevalent in South-asian countries, owing to high consumption of areca nut/gutka and chewing tobacco. $p27^{kip1}$ is a tumor suppressor gene, thought to be downregulated in oral squamous cell carcinoma. Therefore, in the present study we used immunohistochemical analysis to investigate an association between low $p27^{kip1}$ expression in SCC of the head and neck and adverse outcomes/risk factors.

Methods: Total 105 cases of SCC of head and neck excision specimens were selected from records of pathology department archives that underwent surgeries at Liaquat National hospital, Karachi from January 2008 till December 2013. Clinical and pathologic characteristics of patients were evaluated and $p27^{kip1}$ immunohistochemistry was applied on tumor blocks.

Results: In our study, low expression of $p27^{kip1}$ in SCC of head and neck was seen in 39(37.1%) cases while 66(62.9%) of the cases showed high expression for $p27^{kip1}$. Significant association of $p27^{kip1}$ expression with pan/gutka usage ($p = 0.004$), and recurrence ($p = 0.001$) was noted; however, no significant association of $p27^{kip1}$ expression with other clinicopathologic features was seen. Multivariate binary logistic regression showed cases with history of pan/gutka usage were more likely to show low $p27^{kip1}$ expression. Similarly, we also found that recurrence was more likely to develop in patients with low expression of $p27^{kip1}$ in comparison to cases showing high $p27^{kip1}$ expression.

Conclusion: Loss of $p27^{kip1}$ expression is a significant event involved in the pathogenesis of SCC head and neck especially that of oral cavity. Significant association of gutka/areca nut with low $p27^{kip1}$ expression in our study suggests that loss $p27^{kip1}$ expression is a major event involved in areca nut induced SCC of head and neck in this part of the world; however, more large scale molecular based studies are required to validate this observation. Moreover, significant association of low $p27^{kip1}$ expression with tumor recurrence suggests its importance as a prognostic biomarker in SCC of head and neck.

Keywords: Head and neck squamous cell carcinoma, Gutka, $p27^{kip1}$, Pan, Oral squamous cell carcinoma

* Correspondence: doc_atif2005@yahoo.com
[1]Liaquat National Hospital and Medical College, Karachi, Pakistan
Full list of author information is available at the end of the article

Introduction

Squamous cell carcinoma (SCC) of the head and neck is highly prevalent in South-Asian region [1–4]. In developing countries like Pakistan, SCC of head and neck has been reported to be epidemiologically associated with smoking, tobacco consumption and areca nut (pan/gutka). The possible mechanism that leads to the development of malignant changes in the oral cavity is the disruption of cell proliferation control [5]. Deregulation of cyclin-dependent kinases and cyclin-dependent inhibitors has been largely associated with these malignant changes [6]. p27 is a kinase inhibitor protein that inhibits the function of two cyclins complexes E−cdk 2 and A−cdk 2 through binding mechanism [7]. It plays a significant role in negative-regulation of cell cycle, mainly during the stages G0 and G1 [8]; however, it is degraded during the G1 to S phase via ubiquitin-proteasome−dependent degradation mechanism [9]. The tumor suppressor gene CDKN1B encoding for cell cycle inhibitor $p27^{kip1}$ has been identified as a driver gene mutated in many human cancers like breast carcinoma, prostate cancer and endocrine tumors. It is noteworthy that, the mutations of CDKN1B in human cancers are non-sense or small deletions/insertions resulting in the formation of truncated protein. The resulting loss of inhibitory effect of $p27^{kip1}$ on cell cycle leads to un-controlled cell proliferation and ultimately carcinoma development [6].

$p27^{kip1}$ has been reported to be actively expressed in non-proliferating cells [10]. Although genetic alterations leading to loss-of-function mutations are more commonly reported in p16 and p53 tumor suppressor proteins, deregulation of p27kip1 has been found in tumors of the breast, lungs, colorectal as well as SCC of the head and neck [3, 8]. The main mechanisms involved in $p27^{kip1}$ deregulation in human cancers include down-regulation of its expression and exclusion from the nuclear compartment. Research also highlighted that down-regulated $p27^{kip1}$ expression may result from altered proteasome-mediated degradation [11, 12]. Evidence also suggested that downregulation of $p27^{kip1}$ correlates with poor prognosis and more aggressive nature of the tumor [13, 14]. Down regulation of $p27^{kip1}$ is associated with loss of Immunohistochemical expression of p27 protein. Hence in the current study, we aimed to evaluate the frequency of low p27kip1 expression in cases of SCC head and neck in our population and its association with prognostic parameters.

Methods

Total 105 cases of head & neck SSC excision specimens were included in the study. All these patients were biopsy proven SCC of head and neck. The study was conducted in Liaquat National hospital, Karachi from January 2008 till December 2013 over a period of 6 years. The study was approved by institutional research and ethical review committee. Informed written consent was taken from the patients prior to surgery. Hematoxylin and eosin stained slides and paraffin blocks of 77 cases were retrieved and new sections were cut. Slides of all cases were re-evaluated by two senior histopathologists independently and pathologic characteristics were recorded. Clinical records of these patients were reviewed from institutional files to evaluate history of gutka/pan use history, and recurrence status. Moreover, representative tissue blocks of 105 cases were used for $p27^{kip1}$ immunohistochemistry (IHC). After retrieving tissue blocks of these 105 cases, IHC was performed manually in batches of 15 cases and positive and negative controls were run along with each batch. All the slides were similarly processed and the required IHC was completed in 5 days and then evaluated by 2 histopathologists independently and blinded with other histopathological findings.

Immunohistochemistry

$p27^{kip1}$ immunohistochemistry was performed using $p27^{Kip1}$(EP104) antibody purchased from Cell Marque and DAKO EnVision kit according to manufacturers protocol. Only nuclear staining for p27 was quantitatively and qualitatively evaluated. Intensity of staining was categorized into no staining (0), weak (1+), intermediate (2+), strong (3+) as follows,

0 (no staining): no nuclear staining
1+ (weak): Faint nuclear staining.
2+ (intermediate): Moderate nuclear staining.
3+ (strong): Strong nuclear staining.

Percentage of positively stained cells was measured as continuous variable from 0 to 100. Percentage of positively stained cancer cells was multiplied by intensity score to formulate an H-score. Cases with less than 200 H-score were labeled as low $p27^{kip1}$ expression, while tumors with more than 200 score were categorized as high $p27^{kip1}$ expression (Fig. 1).

No set guidelines are available for $p27^{kip1}$ IHC interpretation and different studies used variable cutoffs for low vs. high expression. In studies with semi-quantitative p27 interpretation, cutoffs ranging from 5 to 30% were used [8] and [15]. Only a few studies took into account the intensity of expression. We incorporated both intensity and percentage of p27 expression to construct an H-score with 200 cutoff, similar to that used in many biomarker analysis like ER/PR, androgens etc. to evaluate p27 expression in our study.

Follow-up and recurrence

Recurrence status and follow-up were evaluated by reviewing hospital medical records. Disease free survival

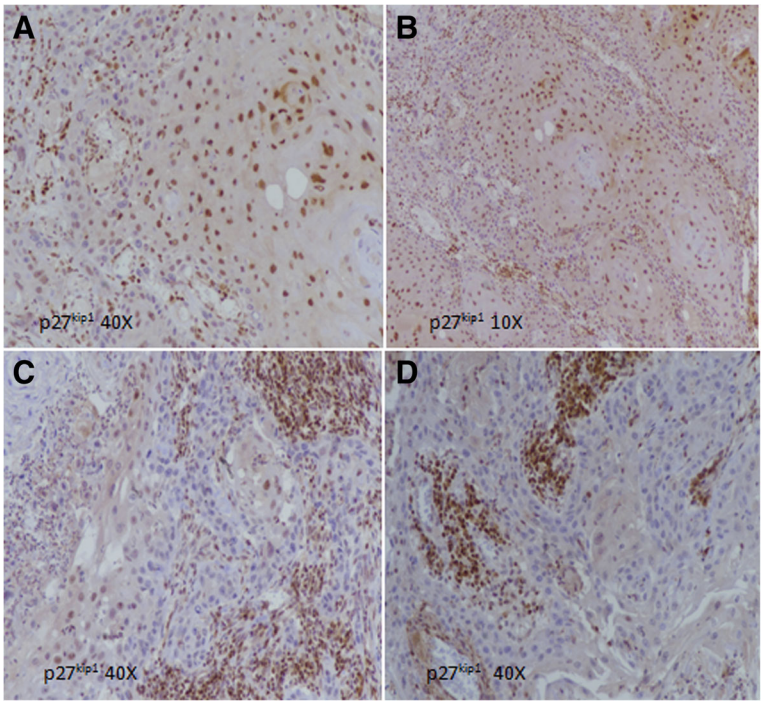

Fig. 1 p27^{kip1} expression in Squamous cell carcinoma. **a** & **b**. High expression of p27^{kip1}, showing diffuse nuclear positivity of p27^{kip1}; **c** & **d**. Low expression of p27^{kip1}, only a few tumor cells showing mild nuclear positivity for p27^{kip1}, majority of tumor cells revealing loss of expression . Lymphocytes serve as positive control

was taken as time between surgical excision and local recurrence or distant metastasis, death or last follow-up.

Statistical analysis

Statistical package for social sciences (SPSS 25) was used for data compilation and analysis. Mean and standard deviation were calculated for quantitative variables. Frequency and percentage were calculated for qualitative variables. Chi-square and fisher exact test was applied to determine association. Shapiro wilk test was used to check normality of data. Student t test and Mann-Whitney test were applied to compare difference in means among groups. Odds ratios were calculated by Multivariate binary logistic regression for variables with significant association. Survival curves were plotted using Kaplan- Meier method and the significance of difference between survival curves were determined using log-rank ratio. *P*-value ≤0.05 was taken as significant.

Results

Mean age of the patients involved in the study was 50.9 $\pm$ 12.3 years with male to female ratio of 3:1. Most of the cases (i.e 50.5%) fall in T2 category (2-4 cm) with mean depth of invasion of 1.1 $\pm$ 0.7 cm. Oral cavity is the most common site of the tumor (68.5%) and keratinizing squamous cell carcinoma being the most common histology. History of pan/gutka usage was revealed in 77% of cases. Detailed patient characteristics are presented in Table 1.

In our study, low expression of p27^{kip1} in SCC of head and neck was seen in 39(37.1%) cases while 66(62.9%) of the cases showed high expression for p27^{kip1}.

Significant association of p27^{kip1} expression with pan/gutka usage ($p = 0.004$) and recurrence ($p = 0.001$) was noted; however, no significant association of p27^{kip1} expression with other clinicopathologic features was seen as shown in Table 2. Patients with low p27kip1 expression were found to have poorer disease free survival compared to those with high p27^{kip1} expression as shown in Fig. 2.

Multivariate binary logistic regression shows cases with history of pan/gutka usage were more likely to show low p27^{kip1} expression. Similarly, we also found that recurrence was more likely to develop in patients with low expression of p27^{kip1} in comparison to cases showing high p27^{kip1} expression (Table 3).

Discussion

In the present study, we found low p27kip1 expression in 37.1% cases of SCC of head and neck. Moreover, significant association of loss of p27kip1 expression was noted with pan/gutka use and poorer disease free survival. To our knowledge, this is the first study conducted in Pakistan evaluating p27^{kip1} expression in SCC of head

Table 1 Clinicopathologic characteristics of patients with Squamous cell carcinoma included in the study

	Frequency	%
Age(years)		
Mean ± SD	50.95 ± 12.35	
Groups		
≤ 30 years	6	5.7
31–50 years	51	48.6
> 50 years	48	45.7
Tumor Size (cm)		
Mean ± SD	3.27 ± 1.80	
Groups		
T1(≤2 cm)	27	25.7
T2(2.1–4.0 cm)	53	50.5
T3(> 4 cm)	25	23.8
Tumor Depth (cm)		
Mean ± SD	1.14 ± 0.74	
Groups		
< 2 cm	89	84.8
≥ 2 cm	16	15.2
Disease Free Survival (months)	26.66 ± 25.31	
History of Pan/gutka		
Pan/gutka Duration (years)	15.45 ± 8.70	
History of pan		
Yes	81	77.1
No	24	22.9
Gender		
Male	79	75.2
Female	26	24.8
Site		
Oral cavity	72	68.6
Lip	4	3.8
Tongue	24	22.9
Soft Palate	5	4.8
Nodal Stage		
N0	54	51.4
N1	15	14.3
N2b	34	32.4
N2c	1	1
N3	1	1
Extranodal extention		
Present	29	27.6
Not Present	76	72.4
Histological Subtypes		
Non-keratinizing	17	16.2
Keratinizing	59	56.2
Keratinizing with maturation	29	27.6
Histological Grade		
Grade I	25	23.8
Grade II	70	66.7
Grade III	10	9.5
Lymhovascular Invasion		
Present	0	0
Not Present	105	100
Perineural Invasion		
Present	16	15.2
Not Present	89	84.8
Recurrence		
Yes	55	52.4
No	50	47.6
$p27^{kip1}$ expression		
Low Expression	39	37.1
High Expression	66	62.9

and neck where SCC prevalence is particularly high with different risk factors compared to the west (pan/gutka use vs. HPV/alcohol). In the western countries, HPV is considered as the most important risk factor in oral cancer pathogenesis with 70–90% oral cancers showing HPV infection [16, 17]. On the other hand, the data on HPV oral infections in Pakistan is limited, however, approximately 67% of cases of oral cancers were found to be infected with HPV in one study [18]. Tobacco use is considered as an important factor in oral carcinogenesis; however chewable tobacco in the form of betal nut/ pan is more prevalent than smoking tobacco in this part of the world.

p27 is a tumor suppressor gene. Low/loss of $p27^{kip1}$ expression is believed to be associated with rapid cell growth and proliferation, thus a significant contributor of tumor development. Also demonstrated by various studies, down-regulation of $p27^{kip1}$ reflects adverse disease outcomes and poor prognosis [6, 19] as seen in our study as well. Furthermore, in the present study, clinicopathologic features of the tumors revealed that low $p27^{kip1}$ expression was irrespective of age and gender of the patients. Only 37.1% tumors in our study showed low $p27^{kip1}$ expression in our study. These findings suggest a multi-step carcinogenesis of SCC of head and neck with involvement of other oncogenes and tumor suppressor genes. Our findings acknowledge the results of another study conducted by Jocelyn Yao, et al., in which no symbolic association was established between $p27^{kip1}$ expression in colorectal carcinomas and

Table 2 Association of $p27^{kip1}$ expression with various clinicopathologic characteristics and prognostic parameters in patients with head and neck Squamous cell carcinoma

	Low $p27^{kip1}$ Expression ($n = 39$)	High $p27^{kip1}$ Expression ($n = 66$)	*P*-Value	Relative Risk (95% CI)
Age(years)				
Mean ± SD[a]	49.46 ± 14.13	51.83 ± 11.20	0.175**	NA
Groups[d]				
≤ 30 years	4(10.3)	2(3)	0.323**	NA
31–50 years	19(48.7)	32(48.5)		
> 50 years	16(41)	32(48.5)		
Tumor Size (cm)				
Mean ± SD°	3.80 ± 1.44	2.96 ± 1.44	0.056**	NA
Groups[c]				
T1(≤2 cm)	9(23.1)	18(27.3)	0.079**	NA
T2(2.1–4.0 cm)	16(41)	37(56.1)		
T3(> 4 cm)	14(35.9)	11(16.7)		
Tumor Depth (cm)				
Mean ± SD°	1.04 ± 0.65	1.20 ± 0.79	0.534	NA
Groups®				
< 2 cm	35(89.7)	54(81.8)	0.275**	0.514 (0.154–1.723)
≥ 2 cm	4(10.3)	12(18.2)		
Disease Free Survival (months)°	16.08 ± 21.66	32.91 ± 25.37	0.000*	NA
History of Pan/gutka usage				
Duration (unit)[b]	15.72 ± 6.34	15.24 ± 10.28	0.532	NA
History of pan/gutka[c]				
Yes	36(92.3)	45(68.2)	0.004*	0.179 (0.049–0.647)
No	3(7.7)	21(31.8)		
Gender[c]				
Male	31(79.5)	48(72.7)	0.438**	0.688 (0.267–1.775)
Female	8(20.5)	18(27.3)		
Site[d]				
Oral cavity proper/buccal mucosa	27(69.2)	44(66.7)	0.165	NA
Lip	4(10.3)	1(1.5)		
Tongue	7(17.9)	17(25.8)		
Soft Palate	1(2.6)	4(6.1)		
Nodal Stage[d]				
N0	18(46.2)	36(54.5)	0.102**	NA
N1	3(7.7)	12(18.2)		
N2b	17(43.6)	17(25.8)		
N2c	1(2.6)	0(0)		
N3	0(0)	1(1.5)		
Extranodal extention[c]				
Present	11(28.2)	18(27.3)	0.918**	1.048 (0.433–2.534
Not Present	28(71.8)	48(72.7)		
Histological Subtypes[c]				
Non-keratinizing	7(17.9)	10(15.2)	0.738**	NA
Keratinizing	20(51.3)	39(59.1)		

Table 2 Association of $p27^{kip1}$ expression with various clinicopathologic characteristics and prognostic parameters in patients with head and neck Squamous cell carcinoma *(Continued)*

	Low $p27^{kip1}$ Expression ($n = 39$)	High $p27^{kip1}$ Expression ($n = 66$)	*P*-Value	Relative Risk (95% CI)
Keratinizing with maturation	12(30.8)	17(25.8)		
Histological Grade[d]				
Grade I	7(17.9)	18(27.3)	0.570**	NA
Grade II	28(71.8)	42(63.6)		
Grade III	4(10.3)	6(9.1)		
Lymphovascular Invasion				
Present	0(0)	0(0)	NA	NA
Not Present	66(100)	39(100)		
Perineural Invasion[c]				
Present	9(23.1)	7(10.6)	0.086**	2.529 (0.858–7.454)
Not Present	30(76.9)	59(89.4)		
Recurrence[c]				
Yes	29(74.4)	26(39.4)	0.001*	0.224 (0.094–0.536)
No	10(25.6)	40(60.6)		

[a]Independent t test was applied
[b]Mann-Whitney Test was applied
[c]Chi-Square Test was applied
[d]Fisher Exact Test was applied
P-Value≤0.05, considered as significant
*Significant at 0.05 level
**Significant at 0.05 level

clinicopathologic features of the tumor including age, gender, tumor stage, tumor size and histologic grade [20]. Similarly, our findings are consistent with another study conducted by Hiroyuki Mineta, et al., in which no significant association between tumor stage and loss of $p27^{kip1}$ expression was established [6].

It is noteworthy that low frequency of $p27^{kip1}$ expression was found in majority of tumors of the oral cavity, followed by tongue and we found a significant association of gutka/pan usage with loss of $p27^{kip1}$ expression. Gutka is a preparation of crushed areca nut mixed tobacco, paraffin wax, catechu and slaked lime. In our

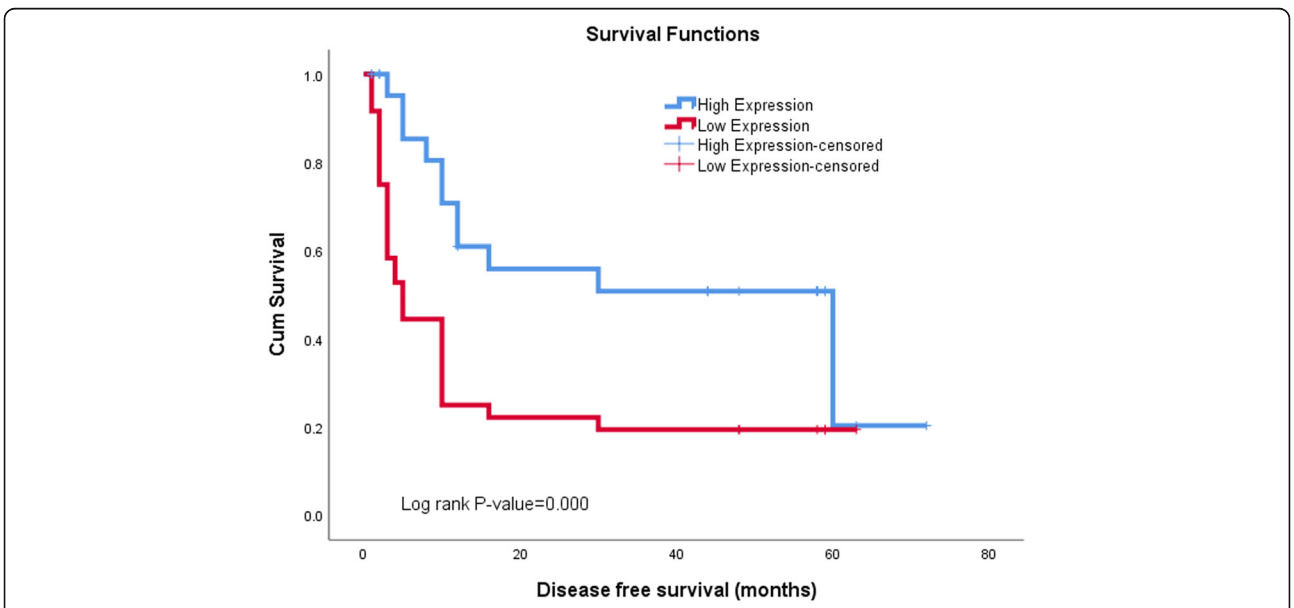

Fig. 2 Kaplan-Meier for $p27^{kip1}$ over expression (disease free survival), showing significant association of $p27^{kip1}$ expression with disease free survival. Cases with low $p27^{kip1}$ expression showing lower disease free survival compared to cases with high $p27^{kip1}$ expression

Table 3 Binary logistic regression for low expression of $p27^{kip1}$, association with history of pan/gutka usage and disease recurrence

	β	Odds Ratio	95% CI	*P*-value
History of pan				
Yes	1.155	3.176	0.801–12.588	0.100**
No[a]		1		
Recurrence				
Yes	1.166	3.209	1.262–8.156	0.014
No[a]		1		

Multivariate Binary Logistics Regression Applied
[a]Reference Category
P-Value≤0.05, considered as significant
**Not Significant at 0.05 level

setup, areca nut/gutka chewing has been asserted as a significant risk factor attributable to leukoplakia which subsequently develops into oral carcinoma [15]. Reduced expression of $p27^{kip1}$ was also demonstrated by a study of similar nature conducted by Jordan, et al. [21]. On account of these results and antecedent studies, we suggest further research to establish the role of carcinogens like tobacco smoking and areca nut chewing in altering the cell proliferation cycle and deregulation of biomarkers including $p27^{kip1}$.

We found a significant association of $p27^{kip1}$ loss with higher recurrence/poor disease free survival. Association of loss of $p27^{kip1}$ expression with recurrence and disease free survival has been evaluated by some authors. Mineta et al. and Venkatesan et al. in their studies involving 94 and 35 cases respectively found a significant association of $p27^{kip1}$ loss with disease free survival with low expression in 44 and 20% cases respectively [9, 22]. On the other hand, no such significant association was found by Fujieda et al. in a series of 60 cases with 58% low p27 expression [23]. On the contrary, we found $p27^{kip1}$ loss in 34% cases of SCC of head and neck. The difference in frequency of low expression and association with recurrence/ disease free survival may be due to variation in antibody dilution and cutoffs used to categorize low vs. high $p27^{kip1}$ expression. We used ready to use antibody and took a cut-off of 200 to divide low vs. high $p27^{kip1}$ expression. Therefore, we suggest that proper guidelines should be defined for low $p27^{kip1}$ expression. Zhang M et al. in a study involving 110 cases of SCC of head and neck evaluated expression of p21, p27 and survivin. They found that p27 immunoreactivity was negativity correlated with 5 year disease free survival [24]. Apart from the prognostic significance of p27 in SCC of head and neck, a few investigators also explored the role of p27 staining in differentiating ordinary/ reactive squamous mucosa from malignant (dysplastic/ invasive squamous cell carcinoma. In this regard, Queiroz AB investigated the same observation and found a significantly diffuse expression of p27 in normal/ reactive squamous mucosa compared to patchy/ focal p27 expression in squamous cell carcinoma [25].

We had 3 cases in which tumor location was lip. Although, alcohol and tobacco remains the most significant risk factors in the pathogenesis, it is noteworthy that solar radiations may also play a role in lip cancer pathogenesis.

One of the limitations of our study was that we evaluated only one biomarker in the molecular cascade of squamous cell carcinoma pathogenesis; moreover, molecular studies were not performed to confirm underlying gene mutations. However, this study can serve as a platform for future studies as SCC of head and neck is a major health problem in this resource limited region of the world.

Conclusion

A significant proportion of cases in our study revealed low $p27^{kip1}$ expression which reveals that down-regulation of this protein is a major event involved in the causation of SCC of head and neck in this part of the world. Moreover, association of low $p27^{kip1}$ expression with gutka/areca nut chewing and poor prognostic parameters like tumor recurrence suggests its importance as a prognostic biomarker in SCC of head and neck.

Abbreviations

IHC: Immunohistochemistry; SCC: Squamous cell carcinoma

Acknowledgments

We gratefully acknowledge all staff members of Pathology, Liaquat National Hospital, Karachi, Pakistan for their help and cooperation.

Ethical approval and consent to participate

Ethics committee of Liaquat National Hospital, Karachi, Pakistan approved the study. Written informed consent was obtained from the patients for the participation.

Funding

There was no funding available for this manuscript.

Availability of data and materials

Please contact author, Atif Ali Hashmi (doc_atif2005@yahoo.com) for data requests.

Consent to publish

Not applicable.

Authors' contributions

AAH and SA: main author of manuscript, have made substantial contributions to conception and design of study. MI, ZFH, SKH, HA and NF: have been involved in requisition of data. MI, ZFH, SKH, HA and NF have been involved in analysis of the data and revision of the manuscript. All authors read, revise and gave approval of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]Liaquat National Hospital and Medical College, Karachi, Pakistan. [2]Aga Khan University, Karachi, Pakistan. [3]CMH institute of medical sciences, Multan, Pakistan.

Received: 16 December 2018 Accepted: 30 April 2019
Published online: 10 May 2019

References

1. Gupta N, Gupta R, Acharya AK, Patthi B, Goud V, Reddy S, et al. Changing trends in oral cancer–a global scenario. Nepal Journal of Epidemiology. 2017 May 1;6(4):613–9.
2. Hashmi AA, Hussain ZF, Hashmi SK, Irfan M, Khan EY, Faridi N, et al. Immunohistochemical over expression of p53 in head and neck Squamous cell carcinoma: clinical and prognostic significance. BMC Res Notes. 2018 Jul 3;11(1):433.
3. Hashmi AA, Hussain ZF, Aijaz S, Irfan M, Khan EY, Naz S, et al. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in South Asian head and neck squamous cell carcinoma: association with various risk factors and clinico-pathologic and prognostic parameters. World J Surg Oncol. 2018 Jun 28;16(1):118.
4. Naveed H, Abid M, Hashmi AA, Edhi MM, Sheikh AK, Mudassir G, et al. Diagnostic accuracy of touch imprint cytology for head and neck malignancies: a useful intra-operative tool in resource limited countries. BMC Clin Pathol. 2017 Nov 25;17:25.
5. Li W, Thompson CH, Cossart YE, O'Brien CJ, McNeil EB, Scolyer RA, et al. The expression of key cell cycle markers and presence of human papillomavirus in squamous cell carcinoma of the tonsil. Head & neck. 2004 Jan 1;26(1):1–9.
6. Tannapfel A, Grund D, Katalinic A, Uhlmann D, Köckerling F, Haugwitz U, et al. Decreased expression of p27 protein is associated with advanced tumor stage in hepatocellular carcinoma. Int J Cancer. 2000 Jul 20;89(4):350–5.
7. Ladha MH, Lee KY, Upton TM, Reed MF, Ewen ME. Regulation of exit from quiescence by p27 and cyclin D1-CDK4. Mol Cell Biol. 1998 Nov 1;18(11): 6605–15.
8. Kudo Y, Kitajima S, Ogawa I, Miyauchi M, Takata T. Down-regulation of Cdk inhibitor p27 in oral squamous cell carcinoma. Oral Oncol. 2005 Feb 28; 41(2):105–16.
9. Mineta H, Miura K, Suzuki I, Takebayashi S, Amano H, Araki K, et al. Low p27 expression correlates with poor prognosis for patients with oral tongue squamous cell carcinoma. Cancer. 1999 Mar 1;85(5):1011–7.
10. Studzinski GP, Harrison LE. Differentiation-related changes in the cell cycle traverse. Int Rev Cytol. 1999 Dec 31;189:1–58.
11. le Sage C, Nagel R, Egan DA, Schrier M, Mesman E, Mangiola A, et al. Regulation of the p27 Kip1 tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J. 2007 Aug 8;26(15):3699–708.
12. Chu IM, Hengst L, Slingerland JM. The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. Nat Rev Cancer. 2008 Apr 1;8(4):253.
13. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer. 2009 Mar 1;9(3):153.
14. Porter PL, Malone KE, Heagerty PJ, Alexander GM, Gatti LA, Firpo EJ, et al. Expression of cell-cycle regulators p27Kip1 and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. Nat Med. 1997 Feb 1;3(2):222–5.
15. Patil TT, Kowtal PK, Nikam A, Barkume MS, Patil A, Kane SV, et al. Establishment of a tongue squamous cell carcinoma cell line from Indian Gutka chewer. J of Oral Oncol. 2014;2014:9.
16. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinoma worldwide: a systematic review. Cancer Epidermiol Biomarkers Prev. 2005;14:467–75.
17. Nasman A, Attner P, Hammarstedt L, Du J, Eriksson M, Giraud G, et al. Incidence of human papillomavirus (HPV) positive tonsillar carcinoma in Stockholm, Sweden: an epidemic of viral-induced carcinoma? Int J Cancer. 2009;125:362–6.
18. Ali A, Awan S. Prevalence of human papillomavirus infection in Pakistani patients with oral cancer. European J of Cancer. 2016;60(1):14.
19. Kuo MY, Hsu HY, Kok SH, Kuo RC, Yang H, Hahn LJ, Chiang CP. Prognostic role of p27 Kip1 expression in oral squamous cell carcinoma in Taiwan. Oral Oncol. 2002 Feb 28;38(2):172–8.
20. Yao J, Eu KW, Seow-Choen F, Cheah PY. Down-regulation of p27 is a significant predictor of poor overall survival and may facilitate metastasis in colorectal carcinomas. Int J Cancer. 2000 May 20;89(3):213–6.
21. Jordan RC, Bradley G, Slingerland J. Reduced levels of the cell-cycle inhibitor p27Kip1 in epithelial dysplasia and carcinoma of the oral cavity. Am J Pathol. 1998 Feb;152(2):585.
22. Venkatesan TK, Kuropkat C, Caldarelli DD, Panje WR, Hutchinson JC Jr, Chen S, et al. Prognostic significance of p27 expression in carcinoma of the oral cavity and oropharynx. Laryngoscope. 1999;109:1329–33.
23. Fujieda S, Inuzuka M, Tanaka N, Sunaga H, Fan GK, Ito T, et al. Expression of p27 is associated with Bax expression and spontaneous apoptosis in oral and oropharyngeal carcinoma. Int J Cancer. 1999;84:315–20.
24. Zhang M, Li J, Wang L, Tian Z, Zhang P, Xu Q, et al. Prognostic significance of p21, p27 and survivin protein expression in patients with oral squamous cell carcinoma. Oncol Lett. 2013;6:381–6.
25. Queiroz AB, Focchi G, Dobo C, Gomes TS, Ribeiro DA, Oshima CT. Expression of p27, p21 (WAF/Cip1) and p16 (INK4a) in normal oral epithelium, oral squamous papilloma and oral squamous cell carcinoma. Anticancer Res. 2010;30:2799–803.