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Original Article

Dentistry Section

Immunohistochemical Expression of Cyclin B1 in Epithelial Hyperplasia, Dysplasia and Oral Squamous Cell Carcinomas - A Comparative Study

KOCHLI CHANNAPPA NIRANJAN[1], AMSAVARDANI TAYAAR[2], G.S. KUMAR[3], REKHA KRISHNAPILLAI[4], KAVERI HALLIKERI[5], SANTOSH HUNASGI[6]

ABSTRACT

Introduction: Cyclin B1 is important in the cell cycle progression from G2 to M phase. Cyclin B1 binds to CDC2, which then becomes dephosphorylated and gets relocated to the nucleus, ensuring the transition toward mitosis.

Aim: Over expression of Cyclin B1, has been reported more recently in breast, colon, prostate, oral and esophageal carcinomas. Thus, the aim of the present study was to examine the expression of Cyclin B1 in hyperplasia, dysplasia and Oral Squamous Cell Carcinomas (OSCC).

Materials and Methods: A total of 64 histopathologically diagnosed cases of epithelial hyperplasias, dysplastic oral epithelium and OSCC were included in the study. Immunohistochemical procedure was carried out using the monoclonal mouse Cyclin B1 antibody (Clone V-152). The Cyclin B1 positive tumor cells counted were expressed as percentage of positive tumor cells. Nuclear and cytoplasmic labeling index (n&cLI) were calculated. The results were tabulated and statistically analyzed by Kruskal Wallis test- One Way ANOVA and Mann Whitney U- test.

Results: Combined n&cLI was considered only in 28.57% of epithelial hyperplasias, 40.7% of oral epithelial dysplasias and 72% of OSCC showed over expression of Cyclin B1 with p value being 0.029. Cyclin B1 expression was not significantly different between the grades of dysplasia, between the grades of OSCC and between the marginal groups.

Conclusion: The present study demonstrates more than 50% of the study group showing less than 20% of nuclear staining. The importance of such variations within a type of lesion requires further investigation, since Cyclin B1 has proved useful in many studies from esophageal and laryngeal squamous cell carcinoma as a prognostic indicator, an indicator of recurrence and as an indicator for tumor sensitivity to radiotherapy. Further studies are to be extended towards evaluating the role of Cyclin B1 as a prognostic indicator.

Keywords: Carcinogenesis, Cell cycle regulators, Immunohistochemistry, Oral lesions

INTRODUCTION

Cell division is a serious event in tumor development and abundant molecules concerned in this progression has been the subject of powerful investigation in tumor biology [1].

Alteration of normal growth results in increased cell proliferation is a common feature of many human tumors. A number of genetic alterations have been identified in oral carcinomas that, in part, contribute to altered cellular homeostasis leading to increased cell proliferation [2].

Regular cell proliferation involves a particular series of actions that is prohibited by specific factors, namely Cyclins. Cyclins bind to consequent Cyclin-dependent kinases and start a compound flow that regulates the chain and timing of cell cycle phase transition [3,4].

Cyclins A, D, and E regulate the passage from G1 phase to S phase, where as Cyclins A and B direct the transition from G2 phase to M phase [2].

Cyclin B1 was identified as 45KDa sub unit; Cyclin B1-CDC2 is the principal mitotic Cyclin-dependent kinases (CDK) complex that regulates the G2/M transition (MPF). Cyclin B1 is important in the cell cycle progression from G2 to M phase. Cyclin B1 attaches to CDC2, which after that becomes dephosphorylated and relocated to the nucleus, making sure the conversion towards mitosis. While the CDC2 level is classically stable all through the cell cycle, Cyclin B1 expression is cyclic with a minimal expression in G1 phase, an increased level in S phase, and a peak at the G2/M transition [5-7].

Altered regulation of the cell cycle is a hallmark of human cancers. Cell cycle progression is governed by a series of Cyclins and CDKs. Individual Cyclins act at different phases of the cell cycle by binding and activating corresponding CDKs. Of the various Cyclin-CDK complexes involved in the cell cycle regulation, Cyclin D1/CDK4/6 and Cyclin B1/CDC2 are of meticulous concentration because the previous marker directs G1/S transition phase and the later controls G2/M phase check point surveillance, which are in turn essential for DNA synthesis and cell proliferation. Abnormal expression of these Cyclins, CDKs, or both may direct to uncontrolled cell proliferation and malignant transformation [8].

Overexpression of Cyclin B1, has been reported more recently in breast, colon, prostate, oral and esophageal carcinomas [2,9-12].

Most of the studies concentrated on the controlled G1 to S transition phase, which is commonly changed in tumorigenesis. Over expression of Cyclin B1 has been considered in various tumors and some of the reports accentuated the significance of Cyclin B1 expression as an indicator of the malignant potential of the tumors [3,9,11,13].

The localization and shift of the Cyclin B1 appearance in cancer cell cycles have been a focal point of concentration by many researchers. However, the association connecting the localization of Cyclin B1 protein expression either in the nuclei or in the cytoplasm and the biological activities of the carcinoma cells is still to be clarified [7,14].

AIM

The aim of the present study was to examine the expression of Cyclin B1 in normal, hyperplastic, dysplastic oral epithelium and Oral Squamous Cell Carcinoma (OSCC). Additionally, we referred to the difference in staining pattern of Cyclin B1, including nuclear and cytoplasmic leading expression.

MATERIALS AND METHODS

This laboratory based study involved the use of buffered formalin fixed, paraffin embedded tissues of previously diagnosed cases of normal, hyperplastic, dysplastic oral epithelium and OSCC obtained from Department of Oral and Maxillofacial Pathology, SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India.

A total of 64 cases were evaluated immunohistochemically for Cyclin B1 protein expression. These included 5 cases of Normal Tissue (NT), 7 cases of Hyperplastic Tissue (HT), 27 cases of Dysplastic oral epithelium (D) and 25 cases of OSCC. The 27 cases of dysplastic oral epithelium were subdivided in to Mild-Dysplasia (MD) (11 cases), Moderate-Dysplasia (MOD) (10 cases) and Severe-Dysplasia (SD) (6cases). The 25 cases of oral squamous cell carcinoma were subdivided by Broder's histopathological grading into Well-Differentiated (WDSCC) (10 cases), Moderately-Differentiated (MDSCC) (8 cases) & Poorly-Differentiated Carcinoma (PDSCC) (7 cases) by three independent observers, and were taken up for evaluation.

Immunohistochemical procedure was carried out using the monoclonal mouse Cyclin B1 antibody (Clone V-152) and the detection kit, LSAB+ visualization kit (DAKO Cytomation, USA). The stained sections were assessed for positivity of Cyclin B1. Positive tumor cells were counted by two individual examiners A&B to eliminate inter-observer bias.

The representative areas were cautiously chosen and at least 500 tumor cells were counted below high power magnification (X40). All the cases showed nuclear staining and variable intensities of cytoplasmic staining. The Cyclin B1 positive tumor cells counted were expressed as percentage of positive tumor cells. The results were tabulated and statistically analyzed by Kruskal Wallis test-One Way ANOVA and Mann Whitney U- test.

RESULTS

Various sub-cellular staining locations like nuclear, cytoplasmic (granular/homogenous) and peri-nuclear region were observed. Nuclear and faint cytoplasmic staining showed in the basal, parabasal layers of normal oral mucosa [Table/Fig-1].

In cases of epithelial hyperplasia's presence of nuclear and faint cytoplasmic staining in the basal, parabasal layers and relatively uniform staining of cytoplasm in the suprabasal layers were observed with the absence of staining in the keratinized layer, if present [Table/Fig-2]. There was no definitive change in the pattern of staining as observed between the grades of dysplasias [Table/Fig-3-5].

In squamous cell carcinoma sections, both cytoplasmic and nuclear staining was appreciated. In few of the cases, the nuclear staining was observed in the peripheral cells of the tumor islands of WDSCC [Table/Fig-6], while in MDSCC and PDSCC the nuclear stained cells were seen distributed throughout the tumor islands. In the cells of WDSCC, MDSCC and PDSCC varying number of peripheral and central cells showed cytoplasmic staining. Many of the central cells of the islands especially in WDSCC showed no immunostaining. Few of the cases in WDSCC, MDSCC and PDSCC showed predominant cytoplasmic staining [Table/Fig-7-10].

The values obtained from histopathological analysis were used in our study to deduce nuclear Labeling Index (nLI), cytolplasmic Labeling Index (cLI) and combined nuclear and cytoplasmic Labeling Index (n&cLI). The parameters thus calculated were submitted for statistical analysis so as to correlate with the selected study groups. Non parametric ANOVA was performed.

Kruskal-Wallis test was individually performed for all three parameters to see if there is significant difference between the study groups for the expression of Cyclin B1. It was found that both nLI and cLI did not statistically show any significant difference between the study samples when studied in groups (NT, HT, D, and SCC) and singly (NT, HT, MD, MOD, SD, WDSCC, MDSCC, and PDSCC).

When combined n&cLI was considered, there was significant statistical difference observed, only when the study samples were grouped together [Table/Fig-11] and not when they were considered separately [Table/Fig-12].

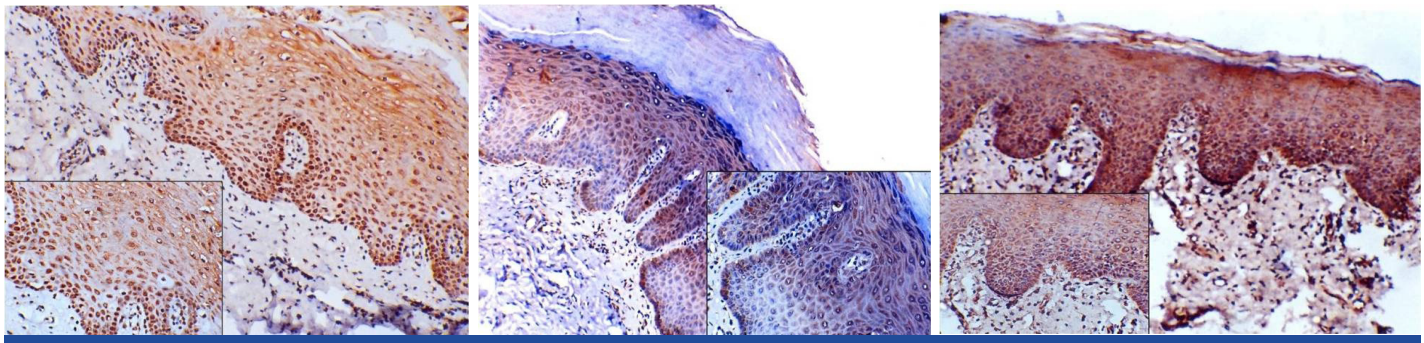
Mann-Whitney test was performed between the study samples in groups to see if any batch of two has significant difference in the expression of Cyclin B1. Significant difference was obtained only between dysplasia and SCC [Table/Fig-13].

Kruskal-Wallis test was performed between D, WDSCC, MDSCC, PDSCC [Table/Fig-14]; and between MD, MOD, SD and SCC [Table/Fig-15]. This revealed only significant difference between D and PDSCC; MD and SCC; MOD and SCC. No significant difference was appreciated between the marginal cases. So we believe that Cyclin B1 may not prove useful as a consequential indicator.

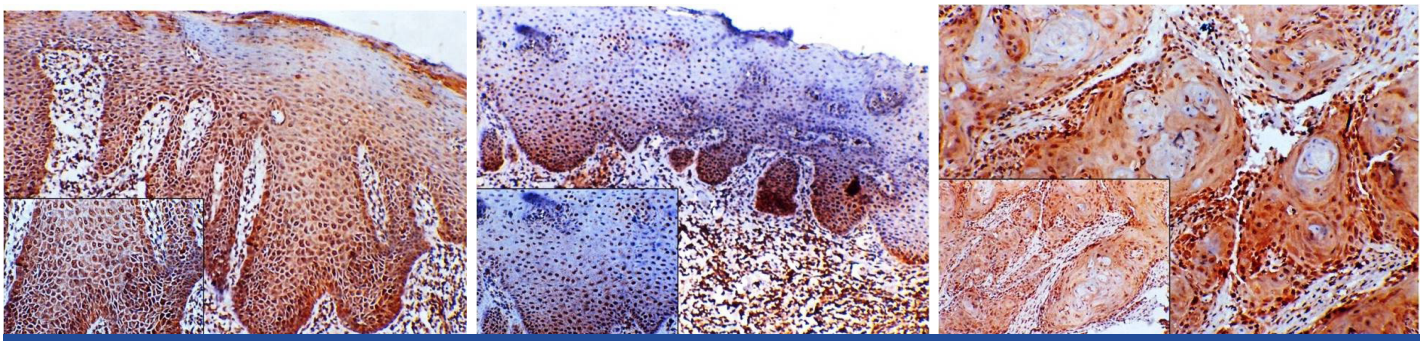
DISCUSSION

In the present study all the cases including the controls showed nuclear staining and variable intensities of cytoplasmic staining.

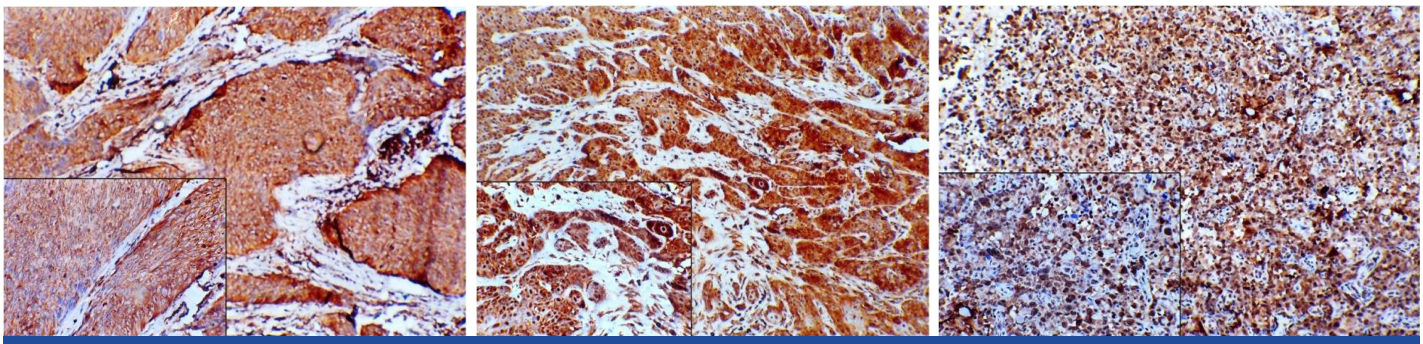
The normal oral mucosae stained with anti-Cyclin B1 demonstrated nuclear staining of few basal cells and parabasal cells with faint cytoplasmic staining. Few cells in the rest of the layers showed granular cytoplasmic staining and were seen distributed randomly.



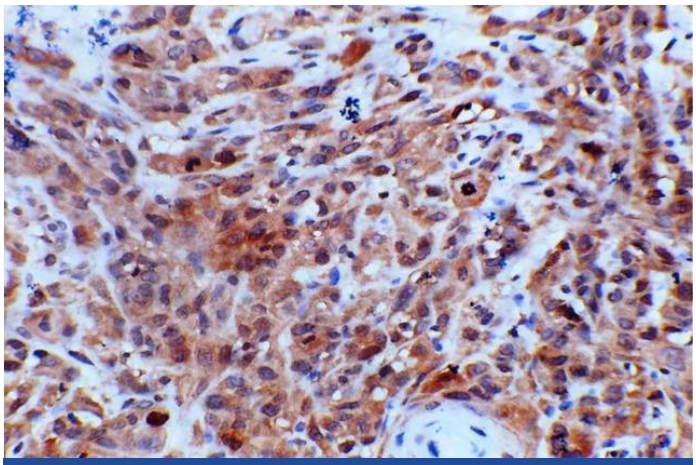
[Table/Fig-1]: Stratified squamous epithelium (normal oral epithelium) showing nuclear stain in basal and suprabasal layer with faint cytoplasmic stain; the above layers demonstrating few cells with cytoplasmic or perinuclear staining (X10 & X25, IHC-MoAb Cyclin B1).
[Table/Fig-2]: (Epithelial hyperplasia) Hyperorthokeratinized stratified squamous epithelium showing many cells with prominent cytoplasmic staining in almost all the layers except the orthokertinized layer. Nuclear staining is not a prominent feature in the basal and parabasal layer (X10 & X25, IHC- MoAb Cyclin B1).
[Table/Fig-3]: (Mild dysplasia) Hyperparakeratinized stratified squamous epithelium showing prominent cytoplasmic staining of almost all the cells from all the layers of the cells. Nuclear staining is less prominent feature in the basal and parabasal layer (X10 & X25, IHC-MoAb Cyclin B1).



[Table/Fig-4]: (Moderate dysplasia) Dysplastic stratified squamous epithelium showing prominent cytoplasmic staining of cells from various layers with many cells in the basal, parabasal layer and the cells from suprabasal layer. Many cells also show a prominent perinuclear ring (X10 & X25, IHC-MoAb Cyclin B1).
[Table/Fig-5]: (Severe dyspalsia) Dysplasia stratified squamous epithelium showing prominent nuclear staining of basal, parabasal and several other cells from the suprabasal layer. Cytoplasmic staining was also observed in many cells (X10 & X25, IHC-MoAb Cyclin B1).
[Table/Fig-6]: (Well differentiated squamous cell carcinoma) Tumor epithelial islands showing nuclear staining in the cells at the advancing front; cytoplasmic staining of all the cells except the cells (Mature) forming the pearl. (Inset) Tumor islands showing only cytoplasmic staining and the central cells are devoid of staining (X10 & X25, IHC-MoAb Cyclin B1).



[Table/Fig-7]: (Moderately differentiated squamous cell carcinoma) Tumor epithelium islands showing cytoplasmic staining (X10 & X25, IHC- MoAb Cyclin B1).
[Table/Fig-8]: (Moderately differentiated squamous cell carcinoma) Tumor epithelial islands showing prominent nuclear and cytoplasmic staining (X10 & X25, IHC- MoAb Cyclin B1).
[Table/Fig-9]: (Poorly differentiated squamous cell carcinoma) Sheets of pleomorphic tumor epithelial cells with prominent nuclear staining and faint cytoplasmic staining (X10 & X25, IHC- MoAb Cyclin B1).



[Table/Fig-10]: (Poorly differentiated squamous cell carcinoma) Areas of tumor cells showing no nuclear staining but show only cytoplasmic staining (X25, IHC- MoAb Cyclin B1).

	Group 2	N	Mean Rank	Chi-Square	df	Significance
% +ve n&cLI	NT	5	32.00	9.040	3	0.029 (NS)
	HT	7	26.29			
	D	27	26.30			
	SCC	25	41.04			
	Total	64				

[Table/Fig-11]: Statistical analysis of combined n & cLI in a grouped study samples by a Kruskal-Wallis Test.
Test Statistics (a,b) a Kruskal-Wallis Test; b Grouping Variable: Group 2.
p<0.05 is statistically significant, p>0.05 is not statistically significant.
Normal Tissue (NT), Hyperplastic Tissue (HT), Dysplastic oral epithelium (D), Oral Squamous Cell Carcinoma (SCC).

	Group	N	Mean Rank	Chi-Square	df	Significance
% +ve n&cLI	NT	5	32.00	13.857	7	0.054 (S)
	HT	7	26.29			
	MD	11	20.27			
	MOD	10	25.50			
	SD	6	38.67			
	WDSCC	10	38.90			
	MDSCC	8	38.50			
	PDSCC	7	47.00			
	Total	64				

[Table/Fig-12]: Combined n&cLI in a study samples studied seperately by a Kruskal-Wallis Test.
Test Statistics (a,b) a Kruskal-Wallis Test; b Grouping Variable: Group
p<0.05 is statistically significant, p>0.05 is not statistically significant.
Normal Tissue (NT), Hyperplastic Tissue (HT), Mild-Dysplasia (MD), Moderate-Dysplasia (MOD), Severe-Dysplasia (SD), Well-Differentiated (WDSCC), Moderately-Differentiated (MDSCC), Poorly-Differentiated Carcinoma (PDSCC).

The cytoplasmic staining was faint compared to the nuclear staining. The oral mucosa used by Kushner et al., & Hassan et al., in their study showed similar pattern of nuclear staining [2,3]. There was no mention about the cytoplasmic staining of these cells.

It is a well known fact that the sub-cellular localization of various cell cycle related proteins decides the progression of cell division. Cyclin B1 is associated with the mitotic asters during the prophase and thereby it is located in the nucleus [10,14,15]. The authors in their article also have stated that the cytoplasmic localization of Cyclin B1 was observed during interphase (S-G2 phase) and this positioning was found to be due to its association with microtubule. This association prepares the establishment of mitotic spindle [16]. This could explain the reason why basal layer and parabasal layer exhibit such a staining pattern.

In a study conducted, by Prokocimer and Rotler, 1994 [17] on B-cell differentiation, they observed upregulation of p53 transcription and such an increase advances the cell to its next stage in differentiation. Krause et al., [18] has shown that such an action of p53 (as stated earlier) influences the G2 transition; p53 does this through Cyclin B1 transcriptional repression. In our study, only few cells from the suprabasal layer showed cytoplasmic

	Group 2	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Wilcoxon W	Z	Significance (2-tailed)
% +ve n&cLI	D	27	20.85	563.00	185.000	563.000	-2.79	0.005 (S)
	SCC	25	32.60	815.00				
	Total	52						

[Table/Fig-13]: Comparison of cyclin B1 expression between D & SCC by - Mann-Whitney Test.
Test Statistics (a) a Grouping variable: Group 2
p<0.05 is statistically significant, p>0.05 is not statistically significant.
Dysplastic oral epithelium (D), Oral Squamous Cell Carcinoma (SCC)

	Group 3	N	Mean Rank	Chi-Square	df	Significance
% +ve n&cLI	D	27	20.85	8.788	3	0.032 (S)
	WDSCC	10	30.70			
	MDSCC	8	30.75			
	PDSCC	7	37.43			
	Total	52				

[Table/Fig-14]: Comparison of cyclin B1 expression between D, WDSCC, MDSCC, PDSCC by Kruskal-Wallis Test.
a Kruskal-Wallis Test; b Grouping Variable: Group 3
p<0.05 is statistically significant, p>0.05 is not statistically significant
Dysplastic oral epithelium (D), Well-Differentiated (WDSCC), Moderately-Differentiated (MDSCC), Poorly-Differentiated Carcinoma (PDSCC).

	Group 4	N	Mean Rank	Chi-Square	df	Significance
% +ve n&cLI	MD	11	16.18	11.121	3	0.011 (S)
	MOD	10	20.40			
	SD	6	30.17			
	SCC	25	32.60			
	Total	52				

[Table/Fig-15]: Comparison of cyclin B1 expression between MD, MOD, SD & SCC by Kruskal-Wallis Test.
a Kruskal-Wallis Test; b Grouping Variable: Group 4
p<0.05 is statistically significant, p>0.05 is not statistically significant.
Mild-Dysplasia (MD), Moderate-Dysplasia (MOD), Severe-Dysplasia (SD), Oral squamous cell carcinoma (SCC).

staining which was faint. We believe this pattern could be due to the stage of differentiation of epithelial cells (mature cells) under the influence of p53.

In the cases of epithelial hyperplasia studied, there were focal areas of nuclear staining observed in the basal and parabasal layer as in the normal oral mucosa. Uniformly spread cytoplasmic staining was evident in the basal and parabasal layers. Keratinized layer, if present, did not show any staining due to Cyclin B1. The nuclear localization is often not recognizable as much as cytoplasmic presence in these cases, in spite of the basilar hyperplasia observed. This may be because the phase of mitosis is transient (is of short duration). Hassan et al., [3] in their study, the cytoplasmic staining in the cells that are preparing to undergo mitosis and the faint cytoplasmic staining observed in the cells of the differentiated compartment have been discussed earlier. This does not sufficiently explain the reason for such a homogenous accumulation in the cytoplasm of cells in the differentiated compartment of epithelial hyperplasia. It has been documented widely in the literature that G2 arrest is accounted in cells after DNA damage. Such an arrested cell seem to possess near maximal Cyclin B1 concentration and high levels of Cyclin B1-CDC2 association [19].

DNA damage in human cells also seems to arrest cells in G2 by stabilization of inhibitory phosphorylation sites of CDC2. This is done by inhibiting the phosphatase, CDC25c. The author also expresses the possibility that DNA damage might stabilize the cytoplasmic localization of Cyclin B1. Assessing the levels of DNA damage and the type of damage that could promote the cells to proliferate in a hyperplastic or malignant manner or to simply die off, is not within the scope of our study. Furthermore, since no studies are available in the literature, no co-relation could be achieved [20].

In the cases of dysplasias that were studied, there was no definitive pattern of staining appreciated. The basal and parabasal layers of all the grades of dysplasia showed areas of nuclear positivity and intense cytoplasmic positivity. The suprabasal cells in varying numbers showed nuclear and cytoplasmic staining.

In SCC sections, both cytoplasmic and nuclear staining was appreciated. In the cells of WDSCC, MDSCC and PDSCC varying number of peripheral and central cells showed cytoplasmic staining. Many of the central cells of the islands especially in WDSCC showed no immunostaining. Similar pattern was observed by Kushner et al., in his study on OSCC [2].

It is declared that spontaneous or constant appearance of Cyclin B1 throughout the cell cycle would direct the activation of already existing CDC2 which forces the cell in to mitosis. This variety of over expression might be the result of amplified synthesis or impaired degradation, or because of its inappropriate localization owing to failure in nuclear/cytoplasmic homeostasis [3].

Under the control of p53, in normal conditions, the cell can arrest in G2 phase by decreasing the $t^{1/2}$ of Cyclin B1 mRNA and also due to the decrease in Cyclin B1 promoter activity. It is also documented that 14-3-3σ through its p53 responsive elements arrests the cell at G2 by accumulating Cyclin B1 in cytoplasm [21,22].

Such a G2 arrest through the decrease in the $t^{1/2}$ of Cyclin B1 mRNA and retention of Cyclin B1 in cytoplasm can result if only functional p53 or wild type p53 exists. Since it has been shown from various studies that 15%-60% of oral carcinomas show mutant p53 over expression Garcia et al., a cytoplasmic accumulation/retention like what is observed in dysplasia and carcinoma cases could not be substantiated [23]. A study with colorectal cells showing p53 mutant was induced to express p53 wild type or mutant type selectively was performed by Krause et al., to understand the role of p53 on Cyclin B1 [18]. In their work, they observed wild type p53 expression caused an effective decrease in Cyclin B1 mRNA but not during the expression of mutant type p53. Also stated that, such cytoplasmic accumulation of Cyclin B1 rather than nucleus as an aberrant expression due to p53 inactivation or non-functional p53.

Kao H et al., mentions in his paper about 30% of mistranslated/misfolded proteins synthesized resulting in defective ribosomal products which are directed for proteosomal degradation [15]. This process might be exaggerated in the transformed cells according to the author. Thus, the cytoplasmic accumulation observed in dysplastic and malignant epithelial cells might be an exaggerated normal process as seen in our study.

Studies have used varying types of parameters to co-relate with the tumors arising from different parent tissues. In studies conducted LSCC-nuclear percentage [24]; Breast cancer-cytoplasmic percentage [16]; Esophageal SCC, NSCLC-not clearly stated [25,26]; Astrocytomas-nuclear and cytoplasmic LI counted separately [27]; Breast adenocarcinomas, HNSCCs, NSCLC, Lymphomas-combined nuclear and cytoplasmic percentage [8,10,28,29] were used.

In our study all these parameters were individually calculated and were co-related with the various study samples selected.

The study of normal oral mucosa, helped us to arrive at a cut-off value for Cyclin B1 expression. This percentage was obtained from the mean of the percentage of Cyclin B1 expression of the five cases studied. Thus, obtained value decides the negative and over expression cases in the rest of the study sample. A study conducted by Hassan et al., used 15% as the cut-off value [3]. He has used this from his earlier study on Esophageal SCC. Later in his study he has mentioned about three cases being positive for Cyclin B1 expression because they showed > 5% of positive cells. In a study conducted by Kushner et al., there was no note on cut-off value being used [2]. But the scoring was performed

by counting nuclear staining. In the work conducted by Hassan et al., it was not possible to comprehend as to whether he has considered cytoplasmic Cyclin B1 staining in calculating the cut-off percentage [3]. Since many authors have studied and often have mentioned cytoplasmic expression to be an aberrant expression in tumors [1,15], we found it to be appropriate to use it with great caution wherever possible.

On an average around 71.84% of cells in normal mucosa from Indian population has showed cytoplasmic positivity. The average nuclear positivity in these cases was found to be 10.64%. Both sum up to 82.49%.

Many studies have used cut-off values ranging from 15-20% [24,25]. Only when nuclear positivity 10.49% was considered for cut-off value, that it fitted in this range obtained from literature (15%-20%). When the average of nuclear positivity was considered for LI, the percentage of cases that showed over expression of Cyclin B1 was evaluated. It was found that 37.14% of Hyperplasias; 48% of Dysplasias (Mild-54.55%; Moderate-40%; severe 50%) and 52% of SCCs (WDSCC-50%; MDSCC-37.5%; PDSCC-71.43%) have over expressed Cyclin B1. In a study on tongue carcinoma conducted by Hassan et al., the author found that 37% of the SCC cases showed Cyclin B1 over expression which is slightly lower to the value 52% obtained by us [3].

As mentioned earlier if cytoplasmic value was also taken into deciding the LI then cut-off percentage obtained was 82.49% which is quite a high value as compared to other studies documented in the literature. When 82.49% was taken for cut off percentage, it was found that 28.57% of Hyperplasias; 40.7% of Dysplasias (Mild-18.18%; Moderate-40%; Severe-83%) and 72% of SCCs (WDSCC-70%; MDSCC-62.5%; PDSCC-85.7%) have over expressed Cyclin B1. Thus the percentage of SCC cases over expressing Cyclin B1 with baseline as 82.49% were more.

In a study by Nozoe et al., on Cyclin B1 expression in esophageal SCC, the author found two different types of immunostaining [14]. One is nuclear dominant expression and the other is cytoplasmic dominant expression. Specimens with nuclear Cyclin B1 expression >20% were considered to show nuclear dominant pattern and those which show <20% of nuclear expression were regarded to have cytoplasmic dominant expression. The authors showed that the cases which show nuclear dominant expression were significantly unfavorable than that of tumors with cytoplasmic dominant expression. In our study when we divided our cases based on this, it was found that only 2/7 cases of epithelial hyperplasias; 4/11 cases of mild dysplasias; 3/10 cases of moderate dysplasias, 2/6 cases of severe dysplasias; 2/10 cases of well-differentiated squamous cell carcinomas; 3/8 cases of moderate-differentiated squamous cell carcinomas and 2/7 cases of poorly-differentiated squamous cell carcinomas showed nuclear dominant expression. That is, >50% of each study sample considered, showed cytoplasmic dominant expression. Such subset identification may prove useful to recognize the prognostically favorable subset of cases within a group.

Similarly Watanabe et al., and De Spindula et al., reported that Cyclin B1 is exported early to the nucleus by altering the nuclear import and export balance in cases of OSCC which correlated with tumour differentiation [30,31]. Recently, Patil et al., also observed that the Cyclin B1 be inclined to shift from the cytoplasm to the nucleus with grades of OSCC thus rising the mitotic index in higher grades [32]. In addition there was associated increase in Cyclin B1 overexpression from verrucous carcinoma to grades of SCC.

Literature search for last 15 years did not reveal any study on Cyclin B1 in oral epithelial hyperplasias and oral dysplasias to the best of our knowledge. Immunoexpression Cyclin B1 proved significant in differentiating oral epithelial dysplasia and oral squamous cell carcinoma.

LIMITATION

The limitation of the study is that Cyclin B1 expression cannot appreciably differentiate between the grades of dysplasia, between the grades of SCC and between the marginal groups (severe dysplasia-SCC; normal epithelium-dysplasia; normal epithelium-SCC and dysplasia-well-differentiated SCC). So it is clear that it is not a consequential indicator and a tumor marker.

Many of the findings observed in this study between the different lesions and its relation with squamous cell carcinoma needs to be further investigated with other proliferative and prognostic markers in a larger sample size.

CONCLUSION

The present study demonstrates over expression of Cyclin B1 only in a subset of epithelial hyperplasia, dysplasia and oral squamous cell carcinoma cases. Our study also showed more than 50% of the study group showing less than 20% of nuclear staining. The importance of such variations within a type of lesion requires further investigation, since Cyclin B1 has proved useful in many studies from esophageal and laryngeal squamous cell carcinoma as a prognostic indicator, an indicator of recurrence and as an indicator for tumor sensitivity to radiotherapy. Combined nuclear and cytoplasmic labeling index was comparatively more useful than nuclear labeling index and cytoplasmic labeling index for assessing the type of lesion.

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PARTICULARS OF CONTRIBUTORS:
1. Reader, Department of Oral and Maxillofacial Pathology, SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India.
2. Professor, Department of Oral and Maxillofacial Pathology, SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India.
3. Principal, Professor and Head of Department, Department of Oral and Maxillofacial Pathology, K.S.R. Institute of Dental Science and Research, Tiruchengode, Tamil Nadu, India.
4. Professor, Department of Oral and Maxillofacial Pathology, Anoor Dental College, Ernakulam, Kerala, India.
5. Professor, Department of Oral and Maxillofacial Pathology, SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India.
6. Professor, Department of Oral and Maxillofacial Pathology, Navodaya Dental College, Raichur, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Kochli Channappa Niranjan,
Reader, Department of Oral and Maxillofacial Pathology, SDM College of Dental Sciences and Hospital,
Sattur, Dharwad-580009, Karnataka, India.
E-mail: niranjankc29@gmail.com

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