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IMMUNOHISTOCHEMICAL LABELLING OF PHOSPHO-HISTONE H3 FOR HISTOPROGNOSTIC GRADING OF ORAL SQUAMOUS CELL CARCINOMAS- A RETROSPECTIVE STUDY

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ABSTRACT

Background: The hallmark of cancer is excessive proliferation of cells, that indeed is due to abnormally increased mitosis. Counting mitotic figures is one of the most essential factors for determining the histologic grade of OSCC. The identification of mitotic figures and the areas of highest mitotic activity in H&E stained slides is a tedious and subjective task. As histone H3 phosphorylation at serine 10 is closely linked to chromosomal condensation, a new antibody directed to phosphorylated histone H3 was recently proposed to detect mitotic cells. **Aim:** To qualitatively identify by light microscopy the presence of associated PHH3 antigens in sections of proven cases of formalin-fixed, paraffin embedded OSCC with immunohistochemistry. In addition, to distinguish mitotic figures from apoptotic bodies and karyorrhectic debris, faster identification and more accurate labelling of mitotic figures for grading. **Materials and Methods:** 45 tissues of histologically diagnosed cases of OSCC and 10 of normal oral mucosa (controls) were included in the study. They were sectioned and evaluated for the selectivity of H&E and PHH3 immunohistochemical marker for demonstration of mitotic figures. **Results and Conclusion:** In all the tissues tested with PHH3, mitotic figures were easily detected, allowing a rapid identification of the area of highest mitotic activity. The mean no. of mitotic figures detected was highest in PHH3 labelled sections in both the study and control group when compared to H&E. In different grades of OSCC the mean no.of mitotic figures using PHH3 was found to be higher in PDSCC as compared with MDSCC and WDSCC. Increased mitotic figures observed using PHH3 proves it to be an accurate marker and a step ahead of routine H&E staining in detecting MFs efficiently. Identification of prophase nuclei and rapid detection of mitotic figures even at low power magnification was possible by PHH3 staining making it a sensitive marker thus allowing easy and objective differentiation of mitotic figures from apoptotic nuclei.

KEYWORDS : Mitotic Figures, Oral Squamous Cell Carcinoma, PHH3 Marker, H&E.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) constitutes a large subgroup of head and neck squamous cell carcinoma and occupies more than 90% of malignancies in the oral cavity.[1] More than 300,000 new cases of OSCC are diagnosed annually worldwide,[2] and the incidence rate of OSCC is continuously increasing in many countries.[3]It is the most common cancer in the males and the third most common cancer in the females in India.[4]

One of the hallmarks of cancer is high rate of proliferation of cells owing to cell division by mitosis.[5] Identification of the high mitotic figures is one of the most important features for early diagnosis of Oral Cancer. This identification of mitotic figures can be effectively done by various markers which are classified as cell proliferation/cell cycle regulator markers, apoptosis control markers, angiogenesis markers and cellular adhesion/metastasis markers.[6]

Mitotic indexing is a common method for grading neoplasms of various types, such as meningiomas, melanomas, carcinomas and soft tissue tumors. Historically the mitotic figures would be counted using Hematoxylin and Eosin (H&E) stains or Ki-67 immunohistochemistry. These results varied in both accuracy and efficiency of interpretation. Ambiguous morphologies would not allow for distinction from apoptotic figures, and lack of specificity for mitotic figures would not allow for distinction from distorted, pyknotic, or karyorrhectic nuclei.[7] Histone H3, a chromatin protein that becomes phosphorylated during eukaryotic mitosis, is detected after phosphorylation step during the prophase, metaphase, anaphase, and telophase stages.[8] Hence, immunohistochemical detection of Phosphohistone H3(PHH3) may be the most accurate method of labeling mitotic figures and allows for the fastest counting of mitotic figures for mitotic indexing of any histological stain.[7]

Until now, no study has shown the correlation between PHH3 mitotic indexing (MI) and recurrence-free survival in a large cohort of oral squamous cell carcinomas. Hence the present study is aimed at evaluating the mitotic figures by PHH3 marker and comparing it with that of H&E in different grades of OSCC, in an attempt to assess the reliablitity of PHH3 as a histoprognostic marker.

MATERIALS AND METHODS

This retrospective study was undertaken to compare the staining of MFs using H&E with PHH3 immunohistochemical marker in 45 cases with initial diagnosis of OSCC. It was carried out by retrieving the paraffin embedded tissue blocks of previously diagnosed cases of OSCC from the archives of Oral & Maxillofacial Pathology, Sri Sai College of Dental Surgery, Vikarabad and Mehdi Nawaj Jung Institute of Oncology, Hyderabad. 10 normal oral mucosa tissue samples served as controls.

Selection Criteria

45 cases of OSCC were included in the study out of which 15 were of WDSCC, 15 of MDSCC and 15 of PDSCC. 10 tissue biopsies of normal oral mucosa were obtained from control subjects.

Inclusion Criteria

1. Patients with clinically and histopathologically diagnosed OSCC.
2. Normal healthy subjects as controls.

Exclusion Criteria

1. Patients with secondary SCC metastasizing to oral cavity.
2. Patients undergoing chemotherapy and radiotherapy.

1. Ployclonal rabbit Phosphohisotne H3 Antibody (Biocare medical) and MACH4 detection system HRP/DAB (Biocare medical) were used for the study. The sections were then lightly counterstained by using Mayer's hematoxylin for 10 seconds, after which sections were gently washed in running tap water for 60 seconds.

Mitotic figure (MF) counting on PHH3 and H&E stained slides: The MI is defined as the sum of MFs per 10 consecutive high power fields (HPFs) in the area of highest mitotic activity.

An area of highest mitotic activity was selected and MI was obtained by summing the number of MFs in 10 consecutive HPFs.

For PHH3:

Assessment of PHH3 positive cells was performed using a binocular light microscope at 40X. The criteria used to define PHH3 positive cells was:
Positive brown staining of the nucleus by chromogen.

For H&E:

Quantitative analysis was performed on one section per biopsy. Criteria followed for identification of MFs was given by Van Diest PJ (1992):[9]

1. The nuclear membrane must be absent, so cells must have passed the prophase.
2. Clear, hairy extensions of the nuclear material (condensed chromosomes) must be present, either clotted (beginning metaphase), in a plane (metaphase/anaphase), or in separate clots (telophase). Regular extensions with an empty central zone favor non-mitosis.
3. Two parallel, clearly separate chromosome clots are to be counted as if they are separate mitoses, however obvious it is that only 1 MF is concerned.

MFs were counted manually in 10 HPFs in the area of highest mitotic activity.

For interobserver variability in counting the MFs, all the stained sections were evaluated independently by a second observer with sound knowledge of Van Diest PJ's (1992)[9] criteria for identifying MFs.

Statistical Analysis

All the analysis was done using SPSS version 16. A p-value of <0.05 was considered statistically significant. Comparison of mean mitotic figures between H&E staining and PHH3 was done with Paired t test. Comparison of mean mitotic figures with H&E and PHH3 between study and control groups was done using Independent sample t test. The inter-observer reliability was assessed using Pearson correlation and the intra class correlation was done using ANNOVA with Post-hoc tukey's test.

RESULTS AND OBSERVATIONS

In our study, the inter observer reliability was assessed using pearson correlation which was 0.994 for H&E and 0.998 for PHH3 and this was significant at 0.01 level, suggesting a strong significant positive correlation between observer 1 and observer 2. Hence, the results obtained by observer 1 were taken into consideration for further statistical analysis. (Table 4)

The mean age group of patients included in study and control groups was 44.91yrs and 28yrs and the male to female ratio was 2:1 and 3:2 respectively. Statistical analysis using Independent sample t test for comparision of age & chi square test for comparison of sex between study and control group yielded p values of 0.455 and 0.723 respectively, which were statistically not significant with respect to both age and sex.

In the control group, a mean MI of 17.66/10HPF (SD=4.12) was obtained in the H&E stained sections, while a mean MI of 19.66/HPF (SD=4.06) was obtained in sections stained with PHH3 immunohistochemical marker using student t test. A p value of <0.001 was obtained which was statistically significant.

The mean MI of the study group in total between H&E and PHH3 was analyzed using paired t test with a mean mitotic index of 23.23 (SD=7.31) and 28.58 (SD=10.72) respectively. A p value of 0.001 was obtained which was statistically significant. (Table 1 & Graph 1)

However in different grades of study group, the mean MI in H&E stained sections for WDSCC, MDSCC and PDSCC was 20.78/10HPF (SD=5.97), 23.47/10HPF (SD=9.61), 25.45(SD=5.32) and PHH3 stained sections was 24.03/10HPF(SD=10.12), 28.26/10HPF(SD=7.15), 33.45/10HPF(SD=12.64) respectively which were calculated using student t test. The p values obtained for WDSCC and MDSCC were 0.295 and 0.065 respectively which were statistically not significant and that for PDSCC was 0.004 which was statistically signifiant between H&E and PHH3. (Table 2 & Graph 2)

When cases of OSCC were compared with controls using independent sample t test, a p value of 0.024 and 0.013 was obtained for H&E and PHH3 stains between the two groups. This test showed a significantly higher mean MI in the study group as compared to the control group. (Graph 3)

Intra group correlation of the mean MFs between different grades of OSCC was done using ANNOVA with post hoc tuckey's test. The p value obtained between WDSCC vs MDSCC, MDSCC vs PDSCC and WDSCC vs PDSCC using H&E stain were 0.569, 0.734 and 0.192 respectively and using PHH3 stain were 0.499, 0.355 and 0.04 respectively. The p value of 0.04 between WDSCC vs PDSCC using PHH3 was found to be statistically significant. (Table 3 & Graph 4)

Table 1: H&E MI and PHH3 MI in OSCC

	H&E		PHH3		p-value	Paired t test
	Mean	SD	Mean	SD		
Study group	23.23	7.31	28.58	10.72	<0.001	p< 0.01(Significant)

Table 2: H&E MI and PHH3 MI in Various Grades of OSCC

Grade	H&E MI		PHH3 MI		p-value	independent Sample t test
	Mean	SD	Mean	SD		
WDSCC	20.78	5.97	24.03	10.12	0.295	P>0.05 (not significant)
MDSCC	23.47	9.61	28.26	7.15	0.065	P>0.05 (not significant)
PDSCC	25.45	5.32	33.45	12.64	0.004	P<0.05 (significant)

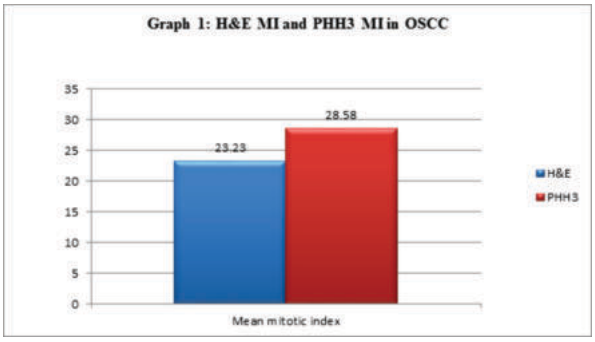
Table 3: Intra Group Correlation of the Mean MFs in Different Grades of OSCC

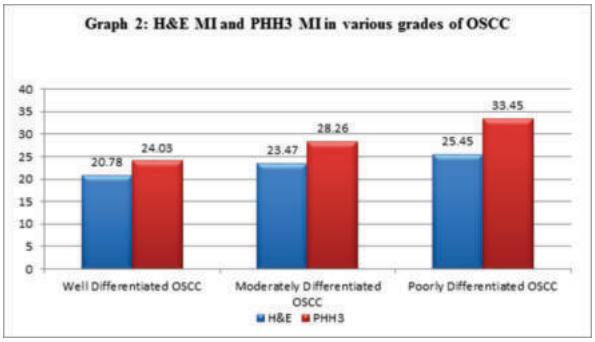
Stain	Grades	Mean Difference	p- value	Post-Hoc test
	WDSCC Vs MDSCC	-2.69	0.569	p>0.05 (not significant)
H&E	WDSCC Vs PDSCC	-4.67	0.192	p>0.05 (not significant)
	MDSCC Vs PDSCC	-1.98	0.734	p>0.05 (not significant)

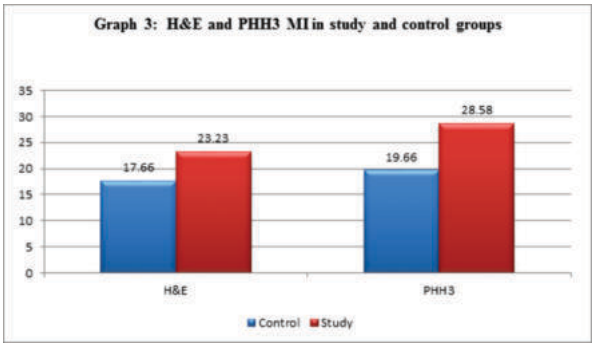
	WDSCC Vs MDSCC	-4.23	0.499	p>0.05(not significant)
PHH3	WDSCC Vs PDSCC	-9.42	0.040	p<0.05(significant)
	MDSCC Vs PDSCC	-5.19	0.355	p>0.05(not significant)

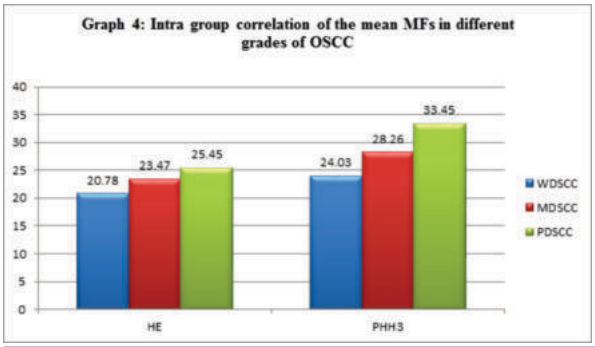
Table 4: Inter Observer Variability in Estimating the Mean no. of MFs in OSCC and Control Group Using H&E and PHH3

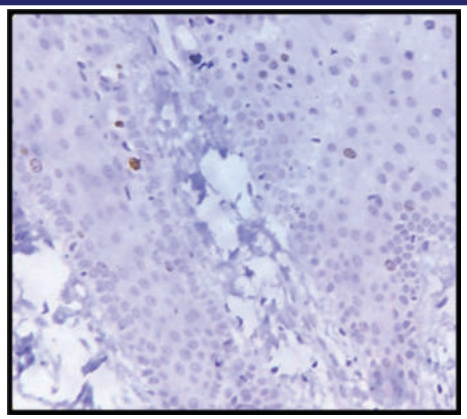
Stain	Observer	N	p-value	Pearson correlation (2- tailed)
H&E	Observer 1 Observer 2	55	0.994**	p<0.001 significant
PHH3	Observer 1 Observer 2	55	0.998**	p<0.001 significant



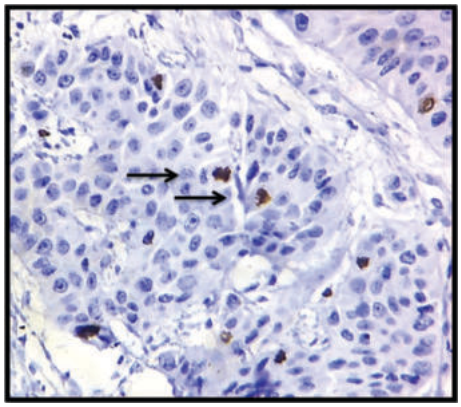




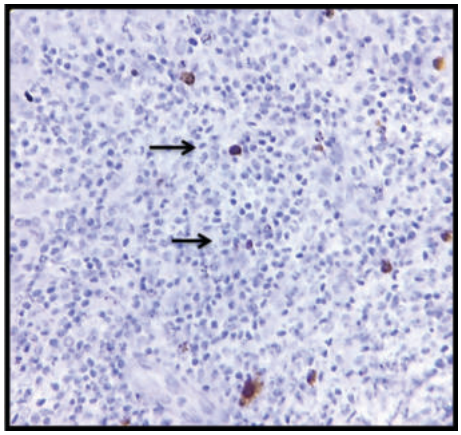




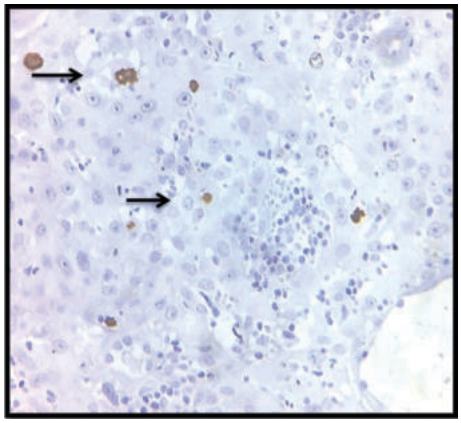
Photomicrograph 1: Normal Oral Mucosa Showing MF in the Basal Cell Layer of Epithelium (PHH3 40X)



Photomicrograph 2: WDSCC Showing MFs (PHH3 40X)



Photomicrograph 3: MDSCC Showing MFs (PHH3 40X)



Photomicrograph 4: PDSCC Showing MFs (PHH3 40X)

DISCUSSION

Tumors of head and neck comprise an important group of neoplasia. Carcinoma of the oral cavity is one of the most frequent malignant tumors worldwide with major predominance in South-East Asia and India. More than 95% of cancers of oral cavity are of SCC type and their high incidence is due to widespread habits of tobacco chewing, smoking and alcohol consumption.

OSCC represents a worldwide problem, with a poor prognosis. Despite advances in the OSCC therapy, it remains as a disease of later diagnosis and high level of recurrence. Therefore, it is necessary to define molecular markers to allow early diagnosis, and to identify new therapeutic targets.

While many characteristics of oral cancer have endured over time, new research is revealing trends that are changing the way we approach its screening, diagnosis and treatment. Though the diagnosis of OSCC seldom presents difficulty, routine histopathology, exfoliative cytology and immuno-histochemistry remain the most efficient diagnostic tools.

One of the hallmarks of cancer is excessive proliferation of cells that is mostly attributed to increased mitosis. This increase in mitosis is helpful in understanding and grading their potential. The quantification of MFs has been on the backseat over the decades, owing to lack of precise histological techniques. However, even newer prognosticators are less feasible, when technique, cost and time factors are considered.

PHH3 antibody has gained much interest as a more distinct proliferation marker as it easily displays mitotic figures in tumor tissue. Immunohistochemical studies performed with anti-PHH3 antibody have shown that the antibody detects specifically the core protein histone H3 only when phosphorylated at serine 10 or serine 28. The phosphorylation of histone H3 is a rare event in interphase cells but is a process almost exclusively occurring during mitosis. Studies have also revealed no phosphorylation on the histone H3 during apoptosis. Therefore, PHH3 can serve as an effective mitotic marker.

Literature revealed many studies conducted in the past using PHH3 as a mitosis specific marker to assess mitosis in tumors like pulmonary neuroendocrine carcinomas, upper tract uothelial carcinomas, thin melanomas, aggressive ovarian carcinomas, uterine smooth muscle tumors, meningiomas, breast adenocarcinomas, pituitary adenomas and high risk endometrial cancer.

To the best of our knowledge this is the first study to be conducted using PHH3 as a mitosis specific marker to assess mitosis in OSCC. In view of the above mentioned probabilities, the present study was conducted wherein MFs stained with H&E were compared with PHH3 immuno-histochemical marker in OSCC and control groups.

In the control group, the mean no. of MFs obtained were higher using PHH3 (19.66/10HPF) when compared to H&E (17.66/HPF), and the difference between them was statistically significant.

The initial objective of the study was unambiguous identification of MFs in proven cases of paraffin embedded OSCC sections in the areas of highest mitotic activity. Yoo-Jin Kim (2007)[10] in his study defined MI as the sum of the MFs per 10 consecutive high power fields in the area of highest mitotic activity. Thus the MI in our study was obtained by counting the PHH3 positive nuclei with morphological features of prophase, metaphase, anaphase and telophase, characterized by strong and dense staining of chromatin clumps.

In the OSCC group, our study revealed that by using PHH3 significantly higher no. of MFs were detected than with H&E. The mean mitotic count in the study group was 23.23/10HPF using H&E and 28.58/10HPF using PHH3 which was significanty higher. This was in accordance with the study conducted by Koji Tsuta (2011)[11] in Pulmonary Neuroendocrine Carcinomas where a statistically significant strong correlation was found between HEMFs and PHMFs and the mPHMFs were 3.25 times higher than the mHEMFs.This was also in accordance with the studies conducted by Duregon E (2014)[12], Charalambos CS (2012)[13], Tobias T et al (2012)[14], Agni M (2011)[15], Guro Auno (2011)[16], Koji Tsuta et al (2011)[59] in various tumors.

In our study the mean no. of mitotic figures in different grades of OSCC using PHH3 were found to be higher in PDSCC as compared with MDSCC and WDSCC which were 33.45/10 HPF, 28.26/10HPF, 24.03/10HPF and 25.45/10HPF, 23.47/10HPF, 20.78/10HPF in PHH3 and H&E respectively . This was in accordance with the study conducted by Yoo-Jin Kim (2007)[10] who stated that the PHH3 counting method was more sensitive in the detection of MFs as compared with traditional H&E, with mean MF counts of 3.2 vs 1.6 in H&E stained sections in all 3 grades of meningiomas. This can be reasoned by the grading system proposed by Jakobsson PA et al (1973)[17] and Willen R et al (1975)[17], which states that less differentiated tumors showed increased mitotic rate as compared to highly differentiated tumors.

This efficiency of PHH3 compared to H&E in selectively staining MFs can be attributed to the fact that histone H3, a chromatin protein that becomes phosphorylated during mitosis, is detected after this phosphorylation step during prophase, metapase, anaphase and telophase stages. Also MFs stain intensely and stand out distinctly against a light blue background of resting cells making it easier in their identification even at a low magnification. It also eliminates the erroneous inclusion of pyknosis, apoptosis and karyorrhexis as MFs, thereby eliminating false positive results, hence providing an easy discrimination.

In our study it was also noted that among various phases of mitosis, the no. of MFs in metaphase were more as compared to anaphase and telophase and it was attributed to the various biochemical studies which have shown that, histone H3 is phosphorylated at ser10 during prophase, reaches a maximum during metaphase, diminishes during anaphase and telophase and is lost during post-mitotic cytokinetic state. This finding was similar to the results obtained in a study conducted by Goodzari M et al (2009)[18].

Our study also showed that in normal mucosa, all MFs assessed by H&E staining present in the basal and parabasal cell layers were also strongly stained with the PHH3 antibody. This expression may be reasonably attributed to the presence of mitotic activity in the basal and parabasal layers (stratum germinatum), as it is the main site of proliferation in oral epithelium.

Apart from the above findings, the possible reason for strong positive correlation between the two observers using PHH3 could be attributed to the fact that it allows a clear identification of mitotic figures, especially the prophase nuclei; distinction of mitotic figures from the nuclei of cells undergoing apoptosis or necrosis. It also allows a rapid detection of the area of highest mitotic activity even at low power view. Similar views were expressed in the studies conducted by Yoo-Jin Kim (2007)[10], Guro Auno (2011)[16], Koji Tsuta (2011)[11], Tobias T (2012)[14], Brunner A (2012)[19].

CONCLUSION

In this study it was observed that in both OSCC and normal oral mucosa, significantly higher no. of MFs was identified

using PHH3 when compared to conventional H&E staining. Thus, PHH3 can be considered as a sensitive marker of mitosis when compared to H&E.

It was also observed that using PHH3 marker, maximum count of MFs was obtained in all grades of OSCC as compared with that of H&E and these exaggerated values obtained were attributed to the ability of PHH3 to stain cells in early prophase apart from the other phases of mitosis and easy identification of the MFs even at a low power view. Thus it proved to be the promising stain when compared to H&E in identifying MFs whatever may be the grade.

An increased value for mean no. of MFs was noticed in PDSCC as compared to MDSCC and WDSCC using PHH3. Thus, PHH3 immunolabelling was directly correlated with less-differentiated tumors, suggesting that this marker may contribute to understand the biological behavior of OSCC, and helps to distinguish risk groups of OSCC.

Thus it may be concluded that increased counts of mean MFs observed using PHH3 marker proves it to be a sensitive stain for MFs and a step ahead of routine H&E staining. Comparing the reliability and feasibility of PHH3 over H&E, identification of MFs using PHH3 is easy, rapid, accurate and more promising.

However, further studies on a greater sample size should be conducted on a broader aspect to standardize the selectivity of this stain for MFs and reproducibility becomes easy only when standardized staining protocol and identification criteria are strictly followed.

To the best of our knowlegde, this stands to be the first study to be conducted using PHH3 immunohistochemical marker to determine the histologic grade in prognostication of OSCC.

REFERENCES

1. M. Perez-Sayans, J. M. Somoza-Martin, F. Barros-Angueira, M. D. Reboiras-López, J. M. Gándara Rey, and A. García-García, "Genetic and molecular alterations associated with oral squamous cell cancer (review)," Oncology Reports, vol. 22, no. 6, pp. 1277–1282, 2009.
2. L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet-Tieulent, and A. Jemal, "Global cancer statistics, 2012," CA: A Cancer Journal for Clinicians, vol. 65, no. 2, pp. 87–108, 2015.
3. E. P. Simard, L. A. Torre, and A. Jemal, "International trends in head and neck cancer incidence rates: differences by country, sex and anatomic site," Oral Oncology, vol. 50, no. 5, pp. 387–403, 2014.
4. Srivastava R, Sharma R, Singh RB, Wilson DW, Mishra S. Computational Studies and Molecular Dynamics of the Potent Biochemical and Molecular Markers in Relevance to Oral Cancer. Open Nutraceuticals J. 2012; 5: 213-218
5. Gutschner T, Diederichs S. The hallmarks of cancer: A long non-coding RNA point of view. RNA Biology. 2012;9(6):703-719. doi:10.4161/rna.20481.
6. Daniel FI, Fava M, Hoffmann R, Campos MM, Yurgel LS. Main Molecular Markers of Oral Squamous Cell Carcinoma. Appl Cancer Res. 2010; 30(3): 279-288
7. http://www.cellmarque.com/cmc/P_spotlighton_PHH3.php
8. Paula C. M. P., Techio V. H., Sobrinho F. S. and Freitas A. S. 2013 Distribution pattern of histone H3 phosphorylation at serine 10 during mitosis and meiosis in Brachiaria species. J. Genet. 92, 259–266
9. Van Diest PJ, Baak JP, Matze-Cok P, Wisse-Brekelmans EC, Van Galen CM, Kurver PH, Bellot SM, Fijnheer J, Van Gorp LH, Kwee WS. Reproducibility of mitosis counting in 2,469 breast cancer specimens: results from the Multicenter Morphometric Mammary Carcinoma Project. Hum Pathol. 1992 Jun; 23(6): 603-607
10. Kim YJ, Ketter R, Steudel WI, Feiden W. Prognostic Significance of the Mitotic Index Using theMitosis Marker Anti-Phosphohistone H3 in Meningiomas. Am J Clin Pathol 2007; 128: 118-125
11. Tsuta K, Liu DC, Kalhor N, Wistuba II, Moran CA. Using the Mitosis-Specific Marker Anti–PhosphohistoneH3 to Assess Mitosis in Pulmonary Neuroendocrine Carcinomas. Am J Clin Pathol 2011; 136: 252-259
12. Duregon E, Molinaro L, Volante M, Ventura L, Righi L, Bolla S, Terzolo M, Sapino A, Papotti MG. Comparative diagnostic and prognostic performances ofthe hematoxylin-eosin and phospho-histone H3 mitotic count and Ki-67 index in adrenocortical carcinoma. Mod Pathol. 2014.
13. Solomides CC, Bribe RC, Nicolaou N, Bagley D, Bibbo M. Does mitosis-specific Marker Phosphohistone H3 Help the Grading of Upper Tract Urothelial Carcinomas in Cell Blocks? Acta Cytol 2012; 56: 285-288
14. Tobias ST, Florian G, Mathias R, Sonali P, Antje S, Nicola B, Iris M, Dirk S, Uwe H. pHH3 Immunostaining Improves Interobserver Agreement of Mitotic Index in Thin Melanomas. Am J Dermatopathol.2012; 34(3): 266-269
15. Agni M, Damato B, Kalirai H, Dodson A, Taktak A, Coupland SE. Immunohistochemical assessment of mitotic count in uveal melanoma. Acta Ophthalmol. 2011 Mar; 89(2): 155-160
16. Aune G, Stunes AK, Tingulstad S, Salvesen O, Syversen U, Torp SH. The proliferation markers Ki-67/MIB-1, phosphohistone H3, and survivin may contribute in the identification of aggressive ovarian carcinomas. Int J Clin Exp Pathol 2011; 4(5): 444-453
17. Bhargava A, Saigal S, Chalishazar M. Histopathological Grading Systems in Oral Squamous Cell Carcinoma: A Review. J. Int Oral Health. 2010; 2 (4): 1-10
18. Goodarzi M, Correa http://www.nature.com/modpathol/journal/v22/n12/full/modpathol2009133a.html
19. AM, Ajani JA, Swisher http://www.nature.com/modpathol/journal/v22/n12/full/modpathol2009133a.html
20. SG, Hofstetter http://www.nature.com/modpathol/journal/v22/n12/full/modpathol2009133a.html
21. WL, Guha S, Deavers MT, Rashid http://www.nature.com/modpathol/journal/v22/n12/full/modpathol2009133a.html
22. A, Maru DM. Anti-phosphorylated histone H3 expression in Barrett's esophagus, low-grade dysplasia, high-grade dysplasia, and adenocarcinoma. Mod Pathol 2009; 22: 1612–1621
23. Brunner A, Riss P, Heinze G, Brustmann H. pHH3 and survivin are co-expressed in high-risk endometrial cancer and are prognostic relevant. Br J Cancer 2012; 107: 84-90