

Evaluation of protein activity in different grades of oral dysplasia using Phosphotungstic acid-haematoxylin stain (PTAH stain) –A Pilot Study

¹Dr Venkatesh Kulkarni, ²Shravani Parandekar, ³Riyaa Ranade

¹Professor, ²Intern, ³Intern

¹ Oral Pathology and Microbiology,

¹Bharati Vidyapeeth Dental College and Hospital, Pune, India.

Abstract: Staining of tissue specimens using gold standard haematoxylin and eosin stain enables the evaluation of the morphology of cell but not the protein activity. Phosphotungstic Acid-Haematoxylin Stain (PTAH Stain) demonstrates the protein activity within the cells as cells with higher protein activity take up more stain. Higher the protein activity, faster is the transformation of a normal cell into a malignant cell. Hence, this study has been taken up to evaluate the protein activity within the cell which helps in early diagnosis, treatment plan and prognosis of the lesion. In mild and moderate dysplasia cases, PTAH stain have found ability in exploring undermined high protein activity in initial dysplastic cases. So, PTAH stain can be used as a potential marker for protein activity and transformation of mild to moderate dysplasia, moderate to severe dysplasia and severe to malignant dysplastic transformation of cell.

IndexTerms – PTAH, protein activity, dysplasia.

I. INTRODUCTION

Staining of tissue specimens using gold standard haematoxylin and eosin stain enables the evaluation of the morphology of cell but not the protein activity. Phosphotungstic Acid- Haematoxylin Stain (PTAH stain) demonstrates the protein activity within the cells as cells with higher protein activity take up more stain. Higher the protein activity, faster is the transformation of a normal cell into a malignant cell. Hence, this study has been taken up to evaluate the protein activity within the cell which helps in early diagnosis, treatment plan and prognosis of the lesion.

15 tissue sections from archival blocks earlier diagnosed as dysplasia will be taken and stained with PTAH stain to evaluate the protein activity within the cell. The sections will be studied under Nikon Model YS100 Compound microscope.

5 samples -Mild dysplasia

5 samples- Moderate dysplasia

5 samples- Severe dysplasia

II. AIMS

To evaluate protein activity at the molecular level in mild, moderate and severe dysplasia using PTAH stain under light microscope.

III. OBJECTIVES

To evaluate protein activity in mild dysplasia.

To evaluate protein activity in moderate dysplasia.

To evaluate protein activity in severe dysplasia.

To compare the protein activity in mild, moderate and severe dysplasia using PTAH stain.

IV. MATERIALS AND METHODS

15 tissue sections from archival blocks earlier diagnosed as dysplasia will be taken and stained with PTAH stain to evaluate the protein activity within the cell. The sections will be studied under Nikon Model YS100 Compound microscope.

5 samples -Mild dysplasia

5 samples- Moderate dysplasia

5 samples- Severe dysplasia

Method of data analysis/Statistical test used: Statistical analysis will be performed using Statistical Product and service solution (SPSS) version 16 for Windows (SPSS Inc, Chicago, IL). Descriptive data will be expressed in proportions (percentages) Chi Square Test will be used to compare percentage /proportion of cases of mild, moderate, severe dysplasia having protein activity detected with PTAH stain. Confidence interval is set at 95% and probability of alpha error set at 5% Power of study set is 80%.

V. RESULTS

table 1: comparison of protein activity using phosphotungstic acid (ptah) in different grades of oral dysplasia (n=15)

	LOW PROTEIN ACTIVITY	MODERATE PROTEIN ACTIVITY	HIGH PROTEIN ACTIVITY	TOTAL
MILD DYSPLASIA	2/5 (40%)	2/5 (40%)	1/5 (20%)	5/5 (100%)
MODERATE DYSPLASIA	0/5 (0%)	2/5 (40%)	3/5 (60%)	5/5 (100%)
SEVERE DYSPLASIA	0/5 (0%)	0/5 (0%)	5/5 (100%)	5/5 (100%)

table 2: comparison of protein activity using phosphotungstic acid (ptah) in earlier diagnosed mild dysplasia cases (n=5)

	MILD DYSPLASIA	MODERATE DYSPLASIA	SEVERE DYSPLASIA	Chi-square test	p value
DIAGNOSIS	5/5 (100%)	0/0 (0%)	0/0 (0%)	Chi = 6.20	p = 0.027*
	MILD/LOW	MODERATE	SEVERE/HIGH		
PROTEIN ACTIVITY	2/5 (40%)	2/5 (40%)	1/5 (20%)		

p >0.05 – not significant * p <0.05 – significant difference **p<0.001 – highly significant difference

TABLE 2 shows the following result interpretation:

In earlier diagnosed mild dysplasia cases using PTAH stain, low protein activity is only observed in 40% cases. Moderate protein activity was observed in 40% in mild dysplasia cases. High protein activity was observed in only 20% of mild dysplasia cases. There was significant statistical difference (p<0.05) found in relation of diagnosis of dysplasia and level of protein activity using PTAH stain in mild dysplasia cases.

table 3: comparison of protein activity using phosphotungstic acid (ptah) in earlier diagnosed moderate dysplasia cases(n=5)

	MILD DYSPLASIA	MODERATE DYSPLASIA	SEVERE DYSPLASIA	Chi-square test	p value
DIAGNOSIS	0/5 (0%)	5/5 (100%)	0/0 (0%)	Chi =4.286	p =0.038*
	MILD/LOW	MODERATE	SEVERE/HIGH		
PROTEIN ACTIVITY	0/5 (0%)	2/5 (40%)	3/5 (60%)		

p >0.05 – not significant * p <0.05 – significant difference **p<0.001 – highly significant difference

TABLE 3 shows the following result interpretation:

In earlier diagnosed moderate dysplasia cases using PTAH stain, low protein activity is not observed in any cases. Moderate protein activity was observed in 40% in moderate dysplasia cases. High protein activity was observed in only 60% of moderate dysplasia cases. There was significant statistical difference (p<0.05) found in relation of diagnosis of dysplasia and level of protein activity using PTAH stain in moderate dysplasia cases.

table 4: comparison of protein activity using phosphotungstic acid (ptah) in earlier diagnosed severe dysplasia cases (n=5)

	MILD DYSPLASIA	MODERATE DYSPLASIA	SEVERE DYSPLASIA	Chi-square test	p value
DIAGNOSIS	0/5 (0%)	0/5 (0%)	5/5 (100%)	Chi = 0.0	p =1.000
	MILD/LOW	MODERATE	SEVERE/HIGH		
PROTEIN ACTIVITY	0/5 (0%)	0/5 (0%)	5/5 (100%)		

p >0.05 – not significant * p <0.05 – significant difference **p<0.001 – highly significant difference

TABLE 4 shows the following interpretation:

In earlier diagnosed severe dysplasia cases using PTAH stain, low protein activity is not observed in any cases. Moderate protein activity was also not observed. High protein activity was observed in all of moderate dysplasia cases (100%). There was no significant statistical difference (p>0.05) in relation of diagnosis of dysplasia and level of protein activity using PTAH stain in moderate dysplasia cases.

VI. DISCUSSION

Sumeet Khandelwal et al (2010) showed in the study that the mean value for the cellular area and cellular diameter of keratinocytes obtained from the mucosa of tobacco abuser was greater than those of OSCC lesions. We observed that with the progression from normal to malignancy, the nuclear dimensions increased and cellular dimensions decreased. Similarly, we found that higher the protein activity, faster is the transformation of a normal cell into a malignant cell.^[1]

Urmila Udayashankar et al (2016) noted in their study that the superficial epithelial cells do contain nuclei, thus alterations in these cells can serve as reliable indicators of dysplastic or neoplastic changes. It is the nucleus that expresses the genotypic alterations caused in the process of malignancy. Thus, it elucidates that cytomorphology is a valuable parameter to assess the influence of tobacco on the oral mucosa and in establishing a link to premalignant and malignant transformations before a lesion is noted. Thus, our study also describes that more denser staining of nucleus by PTAH stain indicates greater protein activity, further showing potential malignant transformation of cell.^[4]

Jordan RC et al (1998) study has found that a reduction in the proportion of cells expressing p27Kip1 protein is frequently associated with oral dysplasia. Conversely, our study showed that in mild and moderate dysplasia cases, PTAH stain have found ability in exploring undermined high protein activity in initial dysplastic cases.^[1]

VII. CONCLUSION

Phosphotungstic acid- haematoxylin stain (PTAH stain) demonstrates the protein activity within the cells as cells with higher protein activity take up more stain. Higher the protein activity, faster is the transformation of a normal cell into a malignant cell. Hence, this study has been taken up to evaluate the protein activity within the cell which helps in early diagnosis, treatment plan and prognosis of the lesion. In mild and moderate dysplasia cases, PTAH stain have found ability in exploring undermined high protein activity in initial dysplastic cases. So, PTAH stain can be used as a potential marker for protein activity and transformation of mild to moderate dysplasia, moderate to severe dysplasia and severe to malignant dysplastic transformation of a cell.

VIII. REFERENCES

Richard C.K Jordan et al: Reduced levels of the cell cycle inhibitor p27 in epithelial dysplasia and carcinoma of oral cavity, American Journal of Pathology, vol.152, no:2 (1998)¹

Khandelwal et al: Cytomorphological Analysis of Keratinocytes in Oral Smears from Tobacco Users and Oral Squamous Cell Carcinoma Lesions: A Histochemical Approach.

Int.J.oral.Sci, 2(1):45-52, (2010)¹Deepa .V. Babji et al: Histomorphometric Study to Compare Histological Changes Between Oral Squamous Cell Carcinoma And Apparently Normal Adjacent Oral Mucosa. Indian.J. Otolaryngol Head Neck Surg, 67(Suppl 1) :S21-S28: (2015) ¹

Urmila Udayashankar et al: Evaluation of cytomorphometric changes in tobacco users and diagnosed oral squamous cell carcinoma individuals, J. Cytol Jul-Sep 33(3), 125-129 (2016)¹

Yasusei et al: High Expression of S-Phase Kinase –Interacting Protein 2, Human F-Box Protein, Correlates with Poor Prognosis in Oral Squamous Cell Carcinomas, J. American Association For Cancer Research (2018)¹

IX. FIGURES AND CHARTS

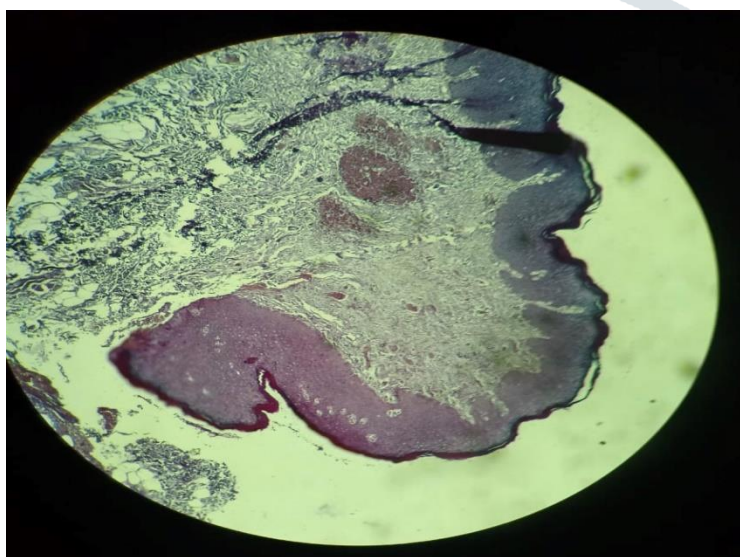


Fig.1 shows mild dysplasia showing protein activity in PTAH stain.
PHOTOMICROGRAPHY-1(Magnification:10x)

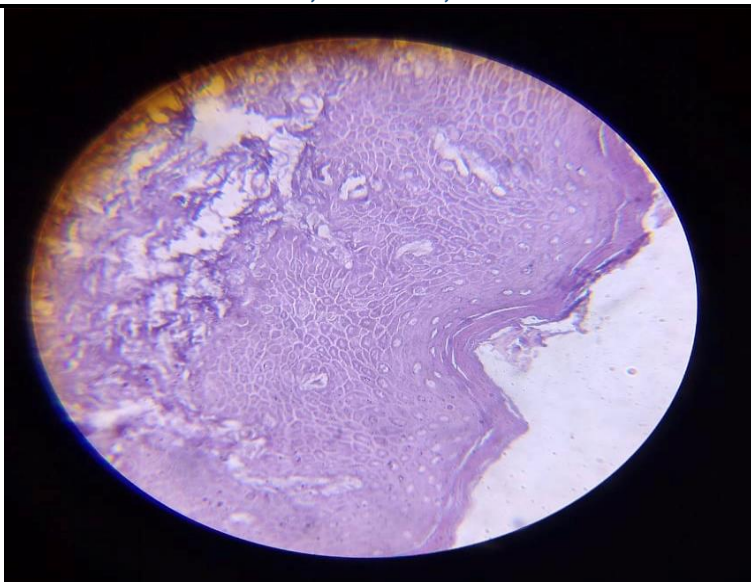


Fig.2 shows moderate dysplasia showing protein activity in PTAH stain.
PHOTOMICROGRAPHY-1(Magnification:10x)

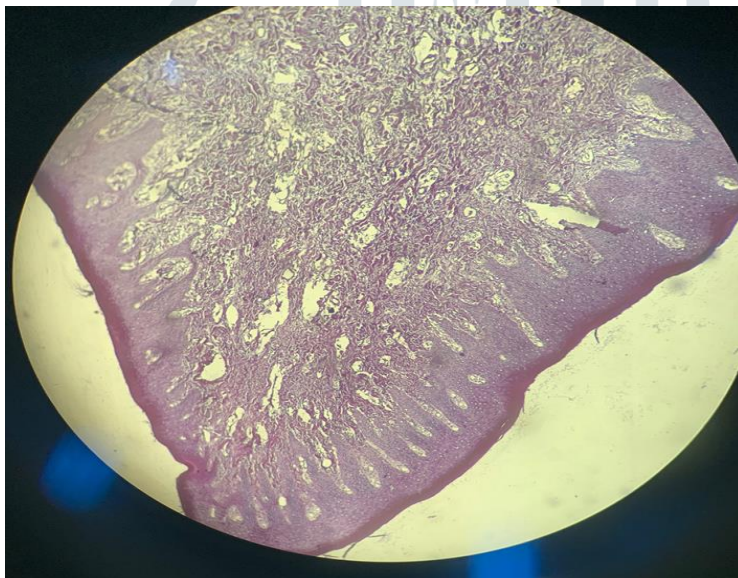


Fig.3 shows severe dysplasia showing protein activity in PTAH stain.
PHOTOMICROGRAPHY-1(Magnification:10x)

