

Comparison of Efficacy of Staining by Papinicalaou, Hematoxylin and Eosin and Giemsa Staining in Exfoliative Cytological Smears from Oral Lichen Planus Patients-A Case Control Study

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Abstract: Background: Oral lichen planus is a T-cell mediated chronic inflammatory oral mucosal disease of unknown origin. Oral cytology should be a part of every oral examination in which the dentist detects even the least suspicious disease. [1,2] The aim of this study was to Compare the efficacy of staining by Hematoxylin and eosin, Papinicalaou (PAP), and Giemsa in exfoliative cytological smears from oral Lichen Planus patients. Materials and method: The study consists of twenty lichen planus patients n=20 (study) and twenty normal patients (control). Smears were collected and stained by using three different staining methods, papinicalaou stain, H and E stain and Giemsa stain. The subjects were microscopically analysed and scored based on following Batch characteristics like Cytoplasmic stain, Nuclear stain, appreciation of nuclear characteristics, Degree of differentiation and Amount of debris present. Data collected were tabulated and statistically analysed. Results: Comparing the characteristics of staining, PAP staining (mean =6.50) was proved to be more efficient staining method for diagnosis of Lichen Planus patients than H and E (5.15) and Giemsa staining (4.50). Statistical analysis also showed significant results ($p < 0.005$). Conclusion: The study showed that PAP smear staining is the most appropriate staining for Lichen Planus and demonstrate cellular features in much detail when compared to the H and E and Giemsa stain. Hence Papinicalaou staining which is a cost effective and relatively simple method can be used effectively as a preliminary adjunct in diagnose the lichen planus patients in a given population.

Keywords: Lichen planus, PAP stain, H and E stain, Giemsa stain

1. Introduction

Lichen Planus is a immune mediated mucocutaneous disorder. OLP is considered a "potentially malignant disorder." Oral cytology helps the Clinician to confidently start treatment as it gives a good insight on the pathology especially in mucocutaneous disorders including Lichen Planus where biopsy is not done often unless and until required. Oral cytology helps the Clinician to confidently start treatment as it gives a good insight on the pathology especially in mucocutaneous disorders including Lichen Planus where biopsy is not done often unless and until required.

disease when it affects the oral mucosa is termed oral lichen planus (OLP). OLP usually affects 1-2% of the adult population [3,4]. OLP has been described as a disease of the middle aged, predominantly in adults over the age of 40 and more common in women than men [5]. Various etiologic factors have been described over the years for this disease including stress, foreign bodies such as restorations, trauma, bacterial and viral pathogens. The pathogenesis for this disease is considered to be a T cell mediated immune mechanism. [6] The buccal mucosa and the tongue are the most common sites where this disease manifests and usually is seen bilaterally. [7] Six clinical types of OLP lesions may be seen individually or combined: Papular, reticular, plaque type, atrophic, erosive and bullous. [8] It is a well-known fact that OLP is considered a "potentially malignant disorder." [9]

Exfoliative cytology and cytomorphometry have been considered to be an important adjuvant for the assessment of malignant changes in the oral cavity. Exfoliative cytology is

a simple and harmless procedure, which has been a controversial technique according to its real validity in oral pathology [10]. Lately it has reemerged due to its application in oral precancer and cancer as a diagnostic and predictive method as well as for monitoring patients. [11,12] Hematoxylin and Eosin staining is the most common staining method used in routine practice. Giemsa staining is used for vesiculobullous lesions and PAP staining is used most commonly to detect cervical cancer and also being for for diagnosis of oral malignant lesions. [13] The advantage of this method is that it differentiates between Mature and immature cells in the exfoliative smears. There is lack of scientific data in Comparison of these staining methods in identification and diagnosis of Oral Lichen planus. Rapid PAP staining is found to be less time consuming and more efficient than the conventional PAP staining. Thus this study aims at comparing the efficacy of PAP, Giemsa and H and E staining in diagnosis of oral lichen planus.

2. Materials and Method

Exfoliative cytological smears were obtained from twenty (n=20) clinically diagnosed cases of Lichen Planus and twenty normal control patients. After obtaining informed consent, buccal keratinocytes were collected from the lesional areas of patients with OLP and also from the normal appearing buccal mucosa of control group using a slightly moistened sterilized wooden spatula. The smears were then transferred and spread uniformly on a clean glass slide. Three smears one for each stain were prepared. The slides containing smears were fixed in 95% alcohol for 30 min. after fixing, the slides are subjected to staining using RAPID PAP kit, Giemsa stain, Hematoxylin and eosin

stain which was then mounted and examined microscopically. Scoring was done by two individual pathologists based on the five characteristics of staining like Cytoplasmic stain, Nuclear stain, appreciation of nuclear characteristics, Degree of differentiation and Amount of debris present. Scoring was done and data collected were tabulated and statistically analysed using One way ANOVA and between the groups using student T test and also kappa statistics test was done to eliminate interobserver bias.

3. Results

Comparing the overall five characteristics of staining, in $n=20$ subjects, PAP stain was found to have greater mean value (6.50) than that of H and E (5.15) and Giemsa stain (4.50). PAP stain showed greater degree of differentiation (40%) than that of Giemsa stain (20%) and H and E stain (15%). The amount of debris present was more in Giemsa stain (65%) than that of PAP stain (10%) and H and E stain (30%). Appreciation of nuclear characteristics was significantly greater in PAP (35%) and H and E stain (30%) but the nuclear characteristics were less appreciable in case of Giemsa stain (15%). Statistical analysis using One way ANOVA, demonstrated significant results ($p < 0.05$) and Kappa statistics comparing the scoring between the observers showed 78.8% (good agreement).

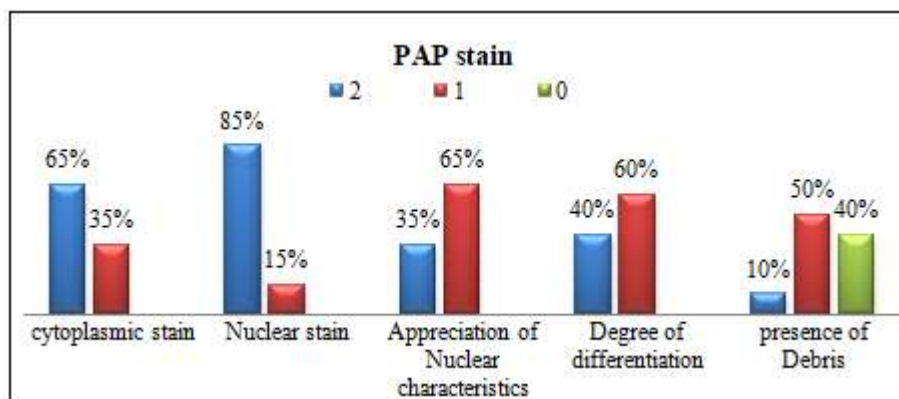


Figure 1

Figure 1 showing various characteristics of staining observed in the LP smears ($n=20$) by using PAP stain

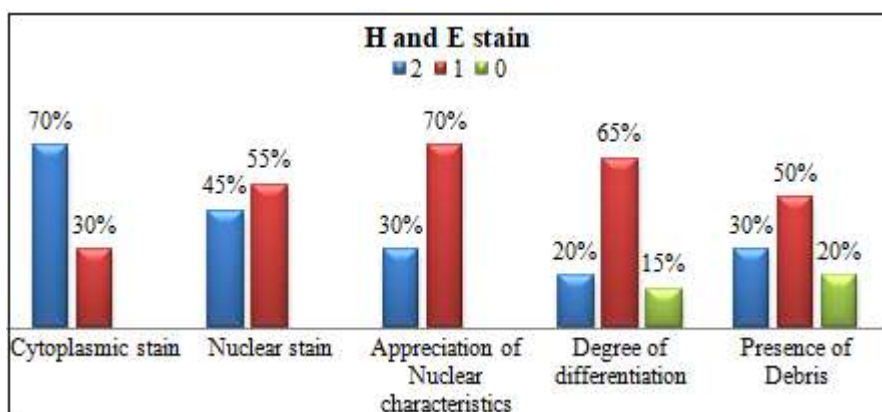


Figure 2

Figure 2 showing various characteristics of staining observed in the LP smears ($n=20$) by using H and E stain.

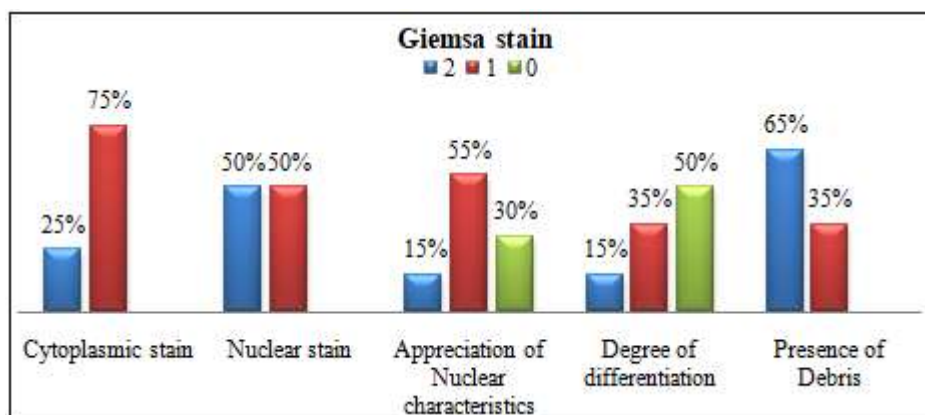


Figure 3

Figure 3: Showing various characteristics of staining observed in the LP smears ($n=20$) by using Giemsa stain.

4. Discussion

OLP is a chronic autoimmune disease mediated by T lymphocytes that involves the stratified squamous epithelial tissue. It presents as white striations, white plaque, erythema, erosions or blisters affecting predominantly the buccal mucosa, Tongue, gingiva. Multiple studies with different results have been carried out analysing the application of the cytology in the detection of dysplastic cells. The oral cytologic analysis is proposed as a useful early diagnostic method for epithelial atypia and therefore also for malignant oral lesions [14,15]. Lichen planus is considered to be one of the pre-malignant conditions. There are studies for demonstrating the role of exfoliative cytological smears in diagnosis of oral lichen planus by comparison of standard staining method and DNA cytomorphometry results. [16-19] But there is lack of scientific data correlating the different staining methods for diagnosis of Lichen planus. Hence this study aims at identifying the most appropriate staining method which can be used as chair side diagnostic technique in case of screening larger population. Earlier diagnosis of a oral lichen planus may help in treatment of the condition and prevent it from further progression into oral carcinoma. [18]

Analysis of the present study shows The cytoplasmic staining was more appreciable in Hand E staining (70%) than PAP stain (65%) and Giemsa stain (25%) comparing with the standard control group. Then comparing the Nuclear staining, it was found to be greater in using PAP stain (85%) followed by Giemsa stain (30%) and H and E staining (45%). The cytoplasmic and nuclear staining was observed more in PAP and H and E stain than Giemsa stain as both the cytoplasmic stain used in PAP and Eosin stain used in H and E staining had greater affinity towards the cytoplasmic component of the cell. The nuclear characteristics were increasingly appreciated in PAP stain (35%) and Hand E (30%) than that of Giemsa stain (15%) on comparison with the control group. Degree of differentiation was observed to be more in smears stained using Papanicolaou stain (40%) than H and E (30%) and Giemsa (10%). Microscopic observation revealed PAP stained smears (30%) showed lesser degree of debris compared to H and E and Giemsa stained smears. More debris was appreciated in Giemsa staining (65%). The reason for Giemsa stained smears showing increased presence of debris is that the staining method of Giemsa doesn't allow washing of the smear, which in turn increases the presence of debris. [figure: 1,2,3]

Comparing the overall characteristics of staining it was found that Papanicolaou staining (6.50) was more acceptable staining method for exfoliative cytological analysis of Lichen Planus patient compared to Giemsa (4.50) and H and E staining (5.15).

In the present study, despite the small number of cases, cytological features consistently observed in all the cases were indicative of a Lichen planus [19] and emphasized the fact that that exfoliative cytology and cytomorphometry can be used as an adjuvant rather than as a diagnostic tool. [20] However, further studies conducted on a larger study group

is required to establish the role of oral exfoliative cytology in predicting the Lichen planus at an earliest.

5. Conclusion

The study shows that PAP smear staining is the most reliable staining for Lichen Planus which can show the presence of matured superficial cells in the smear confirming the presence of hyperkeratosis in lichen Planus. Despite the small number of cases in the study, oral cytology can improve the accuracy of histology, and may be a useful adjuvant for the diagnosis of oral lichen planus. In the era where there is increasing incidence of medico legal issues, using the RAPID PAP kit will serve as faster and reliable adjuvant in diagnosis and treatment of lichen Planus.

References

- [1] Khandelwal S, Solomon MC. Cytomorphological analysis of keratinocytes in oral smears from tobacco users and oral squamous cell carcinoma lesions - A histochemical approach. *Int J Oral Sci.* 2010;2:45-52.
- [2] Ikeguchi M, Oka S, Saito H, Kondo A, Tsujitani S, Maeta M, et al. Computerized nuclear morphometry: A new morphologic assessment for advanced gastric adenocarcinoma. *Ann Surg.* 1999;229:55-61.
- [3] Sugerman PB, Savage NW, Williams SL, Joynson OB, Daley TJ, Cowpe JG. A quantitative cytological study of lesional and non-lesional mucosa in oral lichen planus. *Arch Oral Biol.* 1996;41:117-20.
- [4] Wilson E. On lichen planus. *J Cutan Med Dis Skin.* 1869;3:117-32.
- [5] Farhi D, Dupin N. Pathophysiology, etiologic factors, and clinical management of oral lichen planus, part I: Facts and controversies. *Clin Dermatol.* 2010;28:100-8.
- [6] Elenitas R, Murphy GF, Xu G. Autoimmune diseases of the skin. In: Elder DE, editor. *Lever's Histopathology of the Skin.* 10th ed. Philadelphia: Lippincott Williams and Wilkins; 2010. p. 228.
- [7] Mollaoglu N. Oral lichen planus: A review. *Br J Oral Maxillofac Surg.* 2000;38:370-7.
- [8] Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman PB, Thongprasom K. Current controversies in oral lichen planus: Report of an international consensus meeting. Part 1. Viral infections and etiopathogenesis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005;100:40-51.
- [9] Roopashree MR, Gondhalekar RV, Shashikanth MC, George J, Thippeswamy SH, Shukla A. Pathogenesis of oral lichen planus - A review. *J Oral Pathol Med.* 2010;39:729-34.
- [10] George A, Sreenivasan BS, Sunil S, Varghese SS, Thomas J, Gopakumar D, et al. Potentially malignant disorders of oral cavity. *Oral Maxillofac Pathol J.* 2011;2:95-100.
- [11] Holmstrup P. The controversy of a premalignant potential of oral lichen planus is over. *Oral Surg Oral Med Oral Pathol.* 1992;73:704-6.
- [12] van der Meij EH, Schepman KP, Smeele LE, van der Wal JE, Bezemer PD, van der Waal I. A review of the recent literature regarding malignant transformation of oral lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1999;88:307-10.

- [13] Mattsson U, Jontell M, Holmstrup P. Oral lichen planus and malignant transformation: Is a recall of patients justified? *Crit Rev Oral Biol Med.* 2002;13:390–6.
- [14] Eisen D. The clinical features, malignant potential, and systemic associations of oral lichen planus: A study of 723 patients. *J Am AcadDermatol.* 2002;46:207–14.
- [15] Gandolfo S, Richiardi L, Carrozzo M, Brocchetto R, Carbone M, Pagano M, et al. Risk of oral squamous cell carcinoma in 402 patients with oral lichen planus: A follow-up study in an Italian population. *Oral Oncol.* 2004;40:77–83.
- [16] Khandelwal S, Solomon MC. Cytomorphological analysis of keratinocytes in oral smears from tobacco users and oral squamous cell carcinoma lesions - A histochemical approach. *Int J Oral Sci.* 2010;2:45–52.
- [17] Ikeguchi M, Oka S, Saito H, Kondo A, Tsujitani S, Maeta M, et al. Computerized nuclear morphometry: A new morphologic assessment for advanced gastric adenocarcinoma. *Ann Surg.* 1999;229:55–61.
- [18] Ahmed HG, Idris AM, Ibrahim SO. Study of oral epithelial atypia among Sudanese tobacco users by exfoliative cytology. *Anticancer Res* 2003;23:1943-9.
- [19] Remmerbach TW, Weidenbach H, Pomjanski N, Knops K, Mathes S, Hemprich A, et al. Cytologic and DNA-cytometric early diagnosis of oral cancer. *anal Cell Pathol* 2001;22:211-21.
- [20] Jonathan G Cowpe, Quantitative exfoliative cytology of normal and abnormal oral mucosal squames: preliminary communication *Journal of the Royal Society of Medicine* Volume 77 November 1984