ORIGINAL RESEARCH

Assessment And Comparison of Keratin, Epithelial And Inflammatory Thickness In Oral Lichen Planus And Oral Lichenoid Reaction Using Picrosirius Red Stain

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ABSTRACT

Introduction: Lichen planus is a common, chronic autoimmune disorder of oral cavity. Other lesions often simulate oral lichen planus, most common being oral lichenoid reaction. In spite of having existent criteria defined by WHO, it often becomes difficult to sign out correct diagnosis. We hypothesized that Picrosirius red stain can aid to arrive at a correct diagnosis.

Aim: We aimed to validate criteria like keratin and epithelial thickness as diagnostic parameters, which may help to differentiate between oral lichen planus and oral lichenoid reaction.

Material and method: Fifteen cases of oral lichen planus and oral lichenoid reaction each, and ten cases of buccal mucosa as a comparison group were taken and stained with Picrosirius red stain. Epithelial, keratin and inflammatory thickness was measured used eye-piece reticule (10X magnification; Olympus CH20i, NA of 0.25). The thicknesses measured were then tabulated.

The obtained thicknesses were compared using one way ANOVA test. The comparison within groups was made with Tukey-HSD test.

Results: Significant differences were seen in thickness of keratin, epithelium and inflammation in lichen planus, lichenoid reaction and normal buccal mucosa (p value=0.002, <0.001 and <0.001 respectively). Keratin thickness and inflammatory component thickness were found to be maximum in lichenoid reaction, whereas epithelial thickness was measured highest in buccal mucosa.

Conclusion: Picrosirius red stain can be used as an adjunct to routine haematoxylin and eosin stain for arriving at correct diagnosis in lichenoid lesions. Keratin and epithelial thickness can also be considered while differentiating lichenoid lesions.

Keywords
Picrosirius red stain, keratin, epithelial and inflammatory thickness, oral lichen planus

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INTRODUCTION

Lichen planus is a chronic autoimmune, mucocutaneous disease, with a prevalence of 0.5-2.6% in the oral cavity.[1] Wilson, in 1869 described this disease as a cell-mediated immunopathological response of keratinocytes in the skin and mucosa to antigenic alterations. Clinically lichen planus exhibits six distinct forms which include reticular, papular, plaque-like, erosive and bullous type. Pain and burning sensation are often associated with erosive, atrophic and bullous type. Lichen planus shows characteristic clinical features of symmetrically bilateral or multiple distribution of lesion and presence of fine white crisscrossing lines called Wickham's striae.[2,3] Histopathologically, it shows the presence of hyperkeratinisation,

liquefactive necrosis of basal cell layer, disruption of basement membrane, saw tooth rete pegs, degenerating keratinocytes and juxtaepithelial dense band of lymphocytic inflammatory cell infiltrate.[1]

Oral lichenoid reaction can result as a contact hypersensitivity to varied dental materials such as amalgam, composite and dental acrylics or drugs like dapsone, beta blockers and sulfonylureas. It simulates lichen planus in its clinical presentation, however features like unilaterality of lesions, presence of erosion and occurrence on sites atypical for oral lichen planus like palate helps to differentiate between these two conditions. Histopathologically, the lichenoid reaction shows the presence of deep inflammatory cell infiltrate (chiefly plasma cells, neutrophils and eosinophils) and a focal perivascular infiltrate.[1] Though studies using Picrosirius red stain have been previously done to study collagen fibres in the connective tissue stroma of lichenoid lesions[4], none of them have assessed epithelial parameters by this stain.

Trichrome stains used for staining of collagen fibres, in addition to their poor and variable staining, tend to fade. Picrosirius red stain has the ability to detect thin fibres, contrary to stains employed by other investigators, which have poor specificity for thin fibres.[5] In the present study we measured and compared the thickness of keratin, epithelium and inflammation in lichen planus and lichenoid reaction. Picrosirius red stain was employed as it clearly demarcate the basement membrane even in the presence of dense inflammatory zone owing to its specificity for thin collagen fibers. Picrosirius red stained keratin, yellowish green and the basement membrane, dark red.

MATERIALS AND METHOD

The ethical clearance for the study was obtained from the Institutional ethics committee after which the archival formalin fixed paraffin embedded tissues were obtained from Department of Oral Pathology. Sample size of forty was estimated based on a pilot study keeping a power of 80% and error of 5%. This included fifteen cases each of lichen planus and lichenoid reaction and ten control cases of buccal mucosa as a comparison group.

Four micron thick sections (using Histocut Rotary microtome, Reichert Jung, Germany) of each case were taken and stained with Picrosirius red stain.

Staining protocol of Picrosirius red stain was used (adapted from protocol suggested by Puchtler et al)[6]:-

- De-wax and hydrate paraffin sections
- Stain nuclei with Weigert's Haematoxylin for 8 minutes, and then wash the slides for 10 minutes in running tap water.
- Stain in Picrosirius red stain for one hour.
- Wash in two changes of acidified water (Add 5 ml glacial acetic acid to 1 liter of water).
- Physically remove most of the water from the slides by vigorous shaking.
- Dehydrate in three changes of 100% ethanol.
- Clear in xylene and mount.

The thickness of keratin, epithelium and inflammation in lichen planus, lichenoid reaction and buccal mucosa were measured in ten non overlapping fields with an eye piece reticule under magnification of 10x (using Olympus CH20i, NA of 0.25, Figure 1 and 2).

STATISTICAL ANALYSIS

The keratin, epithelium and the inflammatory zone thickness were then compared using one way ANOVA test between the three groups. The comparison within groups was made with Tukey-HSD test.

RESULTS

Significant differences were seen in thickness of keratin, epithelium and inflammation in lichen planus, lichenoid reaction and buccal mucosa (p value = 0.002, <0.001, <0.001 respectively, One way ANOVA test, Table 1). The keratin thickness was found to be maximum in lichenoid reaction, with statistical difference seen between lichen planus and lichenoid reaction (p value = 0.027, Tukey-HSD test, Table 2) and buccal mucosa and lichenoid reaction (p value = 0.004, Tukey-HSD test, Table 2). The difference in epithelial thickness measured was statistically

significant between lichen planus and lichenoid reaction (p value = 0.006, Tukey-HSD test, Table 2), lichen planus and buccal mucosa (p value = <0.001, Tukey-HSD test, Table 2) and lichenoid reaction and buccal mucosa (p value = 0.018, Tukey-HSD test, Table 2). The inflammatory thickness measured was maximum in lichenoid reaction, with statistical difference noted between lichen planus and lichenoid reaction (p value = <0.001, Tukey-HSD test, Table 2), lichen planus and buccal mucosa (p value = 0.004, Tukey-HSD test, Table 2) and lichenoid reaction and buccal mucosa (p value = <0.001, Tukey-HSD test, Table 2).

DISCUSSION

Equilibrium between proliferation and apoptosis determines final epithelial morphology. Insults in the form of inflammation, mechanical trauma or exposure to toxic agents can dismantle this equilibrium leading to varying degrees of apoptosis, increased epithelial proliferation, expression of Major Histocompatibility complex class II antigens and decreased epithelial thickness. These alterations could be a mirror of disease activity taking place.[7] Lichen planus and lichenoid lesions are examples of such inflammatory lesion of the oral cavity.[1]

ONE WAY ANOVA		Descriptives			Welch statistics	df1	df2	Sig.
		N	Mean (microns)	Std. Deviation				
Final Keratin thickness	Lichen Planus	15	34.839	13.957	8.341	2	22.158	**0.002**
	Lichenoid reaction	15	51.157	22.518				
	Buccal mucosa	10	27.75	4.861				
	Total	40	39.186	18.797				
Final Epithelial thickness	Lichen Planus	15	270.45	92.779	16.916	2	20.025	**<0.001**
	Lichenoid reaction	15	424.071	136.16				
	Buccal mucosa	10	574.95	161.02				
	Total	40	404.183	173.88				
Final Inflammatory thickness	Lichen Planus	15	254.846	97.286	64.113	2	24.108	**<0.001**
	Lichenoid reaction	15	610.136	173.31				
	Buccal mucosa	10	79.166	57.581				
	Total	40	344.160	251.45				

Table 1 – Comparison of parameters between groups using One way ANOVA

Dependent Variable	(I) V3	(J) V3	Mean Difference (I-J)	Std. Error	Sig.
Final Keratin thickness	Lichen Planus	Lichenoid reaction	-16.317*	6.014771	**0.027**
		Buccal mucosa	7.089	6.724719	0.548
	Lichenoid reaction	Buccal mucosa	23.407*	6.724719	**0.004**
Final Epithelial thickness	Lichen Planus	Lichenoid reaction	-153.619*	47.01772	**0.006**
		Buccal mucosa	-304.497*	52.56741	**<0.001**
	Lichenoid reaction	Buccal mucosa	-150.878*	52.56741	**0.018**
Final Inflammatory thickness	Lichen Planus	Lichenoid reaction	-355.289*	45.83002	**<0.001**
		Buccal mucosa	175.680*	51.23952	**0.004**
	Lichenoid reaction	Buccal mucosa	530.970*	51.23952	**<0.001**

Table 2 – Comparison of parameters within groups using Tukey-HSD test

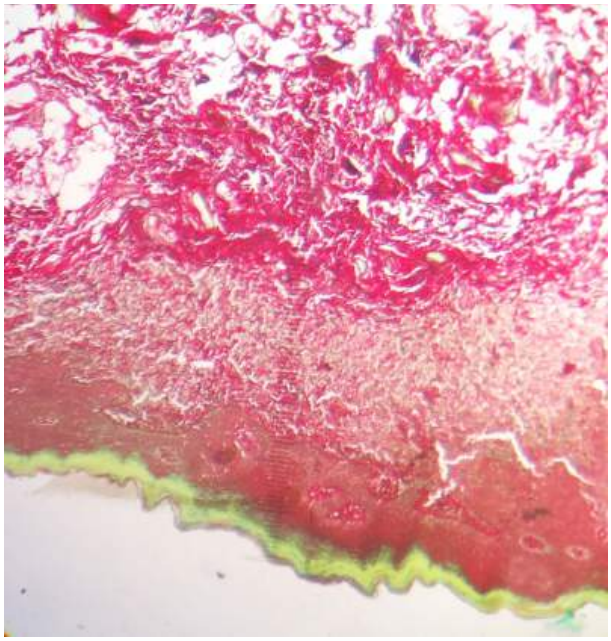

Figure 1 – Oral lichenoid reaction – Picrosirius red stain (10X): note the yellow green stratum corneum layer.

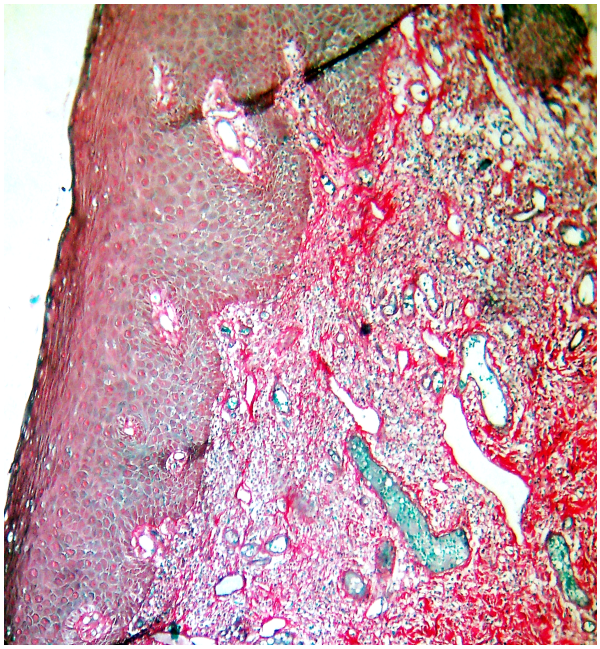

Figure 2 – Oral lichen planus – Picrosirius red stain (10X): Note the relatively thin layer of keratin on top as compared to Lichenoid reaction

Lichen planus is a relatively common disorder of the stratified squamous epithelium.[3] It has been suggested that cell mediated immunity plays a principal role in its pathogenesis. At the cellular level, lichen planus probably occurs as a consequence of immunologically induced degeneration of basal layer. It is characterized by cytotoxic CD8+ cell response to altered keratinocytes surface antigen.[1] Histologically, lichen planus is characterized by dense subepithelial lympho-histiocytic infiltrate, increased number of intraepithelial lymphocytes

and degeneration of basal keratinocytes. Degenerating basal keratinocytes form colloid bodies, which are ultra-structurally shown to be apoptotic in nature.[8]

Oral lichenoid reaction, considered to be a variant of oral lichen planus by few, may be regarded as a disease by itself or an exacerbation of an existing oral lichen planus by the presence of medication or dental materials. The pathogenesis of this condition seems to involve different routes of antigen presentation. Though the exact mechanism is still unknown, positive patch test in patients with lichenoid reaction is highly suggestive of an allergic etiopathogenesis.

Histopathologically, the lichenoid reaction shows the presence of deep inflammatory infiltrate (chiefly eosinophils, plasma cells and neutrophils), and a focal perivascular infiltrate.[1] The same trend was observed in our study where we found the thickness of inflammation significantly increased in case of lichenoid reaction when compared to lichen planus.

Epithelial thickness was found to be significantly reduced in lichen planus than in lichenoid reaction. Initially in lichen planus keratinocyte antigen expression occurs on basal cells of epithelium, which could be induced by systemic drugs, contact allergen in dental restorative materials, mechanical trauma, bacterial or viral infection or any unclassified agent. As a consequence cytotoxic CD8+ cell response is initiated against altered keratinocytes surface antigen with release of pro-inflammatory and inflammatory cytokines. Factors like IL-6, IL-1β and GM-CSF further stimulates T-lymphocytes producing more TNF-α.[1] Reduced epithelial thickness can be attributed to the action of TNF- α, release of which can lead to cessation of growth and necrosis of epithelial cells.[9] Moreover, apoptosis might also contribute to altered thickness. TNF-α secreted by T cells could trigger apoptosis of altered keratinocytes. Other mechanisms for apoptosis of altered keratinocyte could include binding of Fas ligand on T cell surface to Fas on the keratinocytes surface, or entry of granzyme B into keratinocyte through perforin induced membrane pores.[1]

We also found significantly reduced keratin thickness in lichen planus compared to lichenoid reaction. Epidermal keratinocytes can undergo any of the two pathways, differentiation or activation.[10] Reduced keratin and epithelial thickness could be the result of premature or terminal differentiation of keratinocytes.[2] Due to the presence of cytokines like interleukin-1, TNF-α, transforming growth factor beta and interferon gamma keratinocyte activation process rather than differentiation gets exaggerated which could also lead to reduced keratin thickness in oral lichen planus.[10]

CONCLUSION

The parameters assessed in the present study are few most important histopathological modulators which alter the clinical presentation of these lesions. In addition, they signify the epithelial and connective tissue changes in the pathogenesis oral lichen planus and oral lichenoid reaction. The present study also highlights the effectiveness of Picrosirus red to delineate keratin and basement membrane. Apart from routine Haematoxylin and eosin stain, Picrosirius red could be used to classify lichenoid lesions based on keratin thickness and epithelial thickness.

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