Original Article

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Direct Immunofluorescence in Oral Lichen Planus

Dentistry Section

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ABSTRACT

Introduction: Oral lichen planus (OLP) is a common immune-mediated oral mucosal disease. Diagnosis of OLP depends mainly on both clinical and histopathological features. Direct immunofluorescence (DIF) is a useful investigation method to distinguish between similar lesions and to confirm diagnosis in cases of uncharacterized features.

Aim: The purpose of this study was to evaluate the prevalence and pattern of DIF in a group of Thai patients with OLP.

Materials and Methods: Records of clinically and histologically diagnosed OLP patients attending the Oral Medicine Clinic, Faculty of Dentistry, Mahidol University, Bangkok, Thailand were consecutively reviewed for DIF results. The DIF patterns in these patients were analysed.

Results: There were 82 atrophic and/or erosive OLP patients with a mean age of 51.6 years. Male to female ratio was 1:5. Of these, 82.9% showed positive DIF. Buccal mucosa was superior to the gingiva and palate in terms of sensitivity for DIF. All specimens except one (98.5%) demonstrated deposition of fibrinogen at the basement membrane zone (BMZ) in a shaggy pattern. The most common DIF pattern was shaggy fibrinogen at BMZ with IgM deposition on the colloid bodies (CB) (35.3%) followed by shaggy fibrinogen along BMZ (27.9%).

Conclusion: The prevalence of positive DIF in Thai OLP patients was 82.9%. The most common finding was shaggy fibrinogen at BMZ. The typical pattern was shaggy fibrinogen along BMZ with or without positive IgM at CB. DIF pattern could be evaluated for the diagnosis of OLP lacking clinical and/or histopathological characteristic features.

Keywords: Diagnosis, DIF, Pattern, Prevalence

INTRODUCTION

Lichen planus is a chronic immune-mediated mucocutaneous disease [1,2]. It commonly affects oral mucosa with a prevalence rate of about 1-2% of the population [3]. It has been reported that only 15% of patients with oral lichen planus (OLP) have skin involvement [4]. OLP may appear as white reticular, papular or plaque-like forms which are usually asymptomatic. Atrophic (erythematous) and erosive (ulcerated) forms are painful [5-7]. Lesions are mostly found on the buccal mucosa, followed by the tongue, gingiva, and lower vermilion border. Definite diagnosis of OLP depends mainly on clinical and histopathological features [5]. Atrophic and erosive OLP may sometimes clinically resemble oral lupus erythematosus (LE) [8,9] as well as other vesiculobullous lesions including oral pemphigus and oral mucous membrane pemphigoid [10,11]. In addition, in some cases, the histopathological diagnosis of OLP is inconclusive [12] as essential features cannot always be found [13]. In these circumstances, direct immunofluorescence (DIF) in OLP is of importance for diagnosis [5]. The reported DIF patterns of OLP include shaggy staining with anti-fibrinogen in the basement membrane zone, positive anti-IgM staining of colloid bodies [14-17], and weak anti-C3 staining within the basement membrane zone [17,18]. The criteria of DIF patterns for diagnosis of OLP are inconsistent [16,19] as similar patterns of immune deposits have been found in oral LE [9,20].

DIF in OLP has mostly been studied in western countries [14-16,21] with only one study in a small number of Thai patients with both oral and skin lesions [17]. The purpose of this study was to evaluate the prevalence and pattern of DIF in a group of Thai patients with OLP. Based on our review of previous studies, this study was the first to report on DIF in a large number of OLP patients in Thailand. The results of this study might provide useful data to support the diagnosis of OLP.

MATERIALS AND METHODS

This retrospective study was conducted on Thai OLP patients attending the Oral Medicine Clinic, Faculty of Dentistry, Mahidol University, Bangkok, Thailand from 1995 to 2008. The study was approved by the Committee on Human Rights Related to Human Experimentation, Mahidol University (MU-IRB 2008/262.2512). Records of 356 OLP patients were reviewed for data regarding history, clinical features, and laboratory investigations. For this type of study, formal informed consent is not required since data are anonymised. In order to analyse DIF, OLP patients without DIF results were excluded. DIF results were collected from OLP patients diagnosed according to clinical and histopathological criteria (WHO, 1978) [22]. The prevalence and pattern of the DIF were analysed.

The histopathological examination (H&E) and direct immunofluorescence testings (IgG, IgA, IgM, C3, and fibrinogen) of the OLP patients were performed as follows. The biopsy specimens from the OLP lesions were hemisected. One half was placed in 10% buffered formalin and sent for histopathological diagnosis by Oral Pathologists at the Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Mahidol University. In brief, histopathological procedures were as follows: The formalin fixed specimen was processed overnight in a tissue processor. They were then paraffin embedded and sectioned into 4μm thick. The section was stained with H&E, dried, and coverslipped. The other half of the biopsy specimen was placed in normal saline solution and immediately sent for DIF examination, as suggested by Vodegel et al., [23], at the Institute of Dermatology, Ministry of Public Health, Thailand, which was near our Faculty. The brief procedures for DIF were as follows. Biopsy specimens were frozen and sectioned into 4μm thick sections, were placed on microscope slides and dried at room temperature for 15 minutes. The slides were then fixed with acetone for two minutes and dried for about one hour. The fluorescein isothiocyanate (FITC)-labeled rabbit anti-human antibody (DAKO, Copenhagen, Denmark) against IgG, IgA, IgM, C3, and fibrinogen were used. The antibodies were diluted at 1:20 to 1:80 with PBS to produce working antibody concentration. Slides containing three tissue sections were then placed in a moist chamber, covered with working antibody dilutions and incubated for 30 minutes at room temperature in total darkness. After incubation, excess antibodies were removed. Slides were washed with PBS and dipped in PBS for five minutes twice, dried, and covered with mounting medium and coverslipped.

For specimen transport for DIF, we used normal saline solution as a transport medium instead of liquid nitrogen and Michel's fixative medium. The specimens were retrieved at the Institute of Dermatology within 1-2 hours after biopsy. With this method, the DIF results proved to be reliable [23].

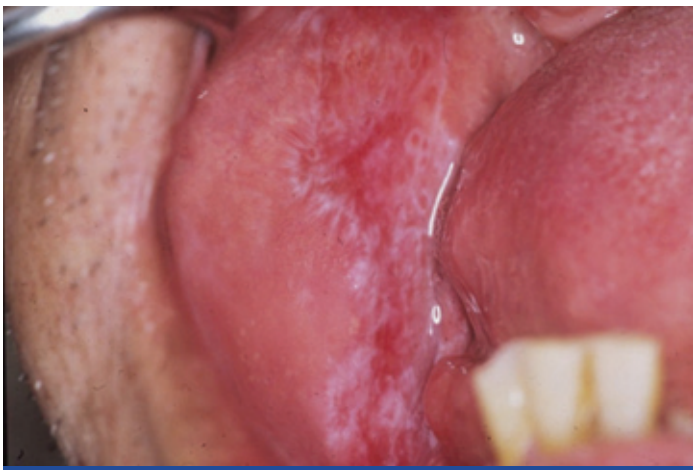
RESULTS

A total of 82 Thai OLP patients with DIF results were studied. They were diagnosed mainly by clinical and histopathological features with DIF as a supportive tool. The clinical characteristics of these OLP patients are shown in [Table/Fig-1]. The lesions were atrophic and/or erosive with or without white striae mostly found on the buccal mucosa, gingiva, and mucobuccal fold [Table/Fig-2,3]. The lesser frequent sites of lesions were on the tongue, palate, and vermilion border. Most patients had multiple sites of lesions with 11 patients (13.4%) showing lesions confined to the gingiva. Positive DIF patterns were found in 68 of 82 cases (82.9%). [Table/Fig-4] shows DIF findings according to the biopsy site and type of OLP specimens. Most of them were atrophic OLP with white striae from buccal mucosa followed by that from the gingiva. With respect to the final diagnosis, buccal mucosa was superior to the gingiva and palate in terms of sensitivity for DIF testing. The most common DIF pattern found in these patients was shaggy fibrinogen at the basement membrane zone (BMZ) with IgM deposition on the colloid bodies (CB) (35.3%) [Table/Fig-5]. The next common pattern was shaggy fibrinogen along BMZ (27.9%). It is noted that all these specimens except one (98.5%) demonstrated deposition of fibrinogen at the BMZ in a shaggy pattern [Table/Fig-6]. Granular IgM at the BMZ was found in three cases. C3 were also found at the BMZ with granular patterns (3 cases) and linear pattern (1 case).

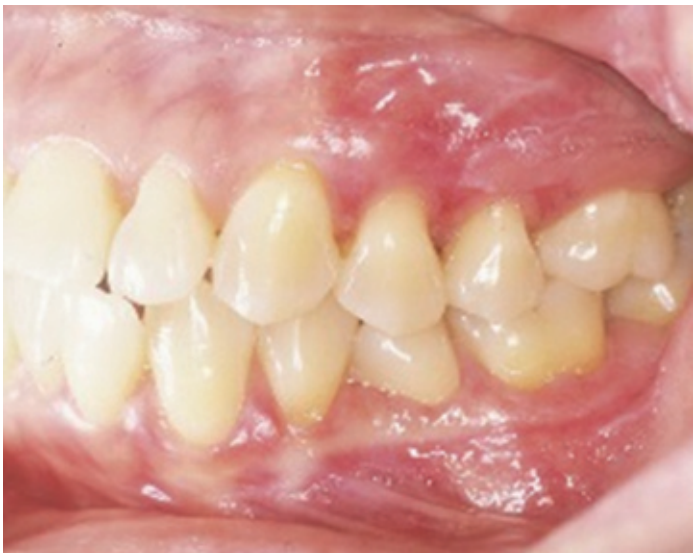
Gender (No.)		Mean age (yr.)	Mean Duration (mths.)	Location (%)*					
Male	Female			BM	G	MB	T	P	V
13	69	51.6	14.5	93.9	59.7	26.8	13.4	7.3	7.3

[Table/Fig-1]: Clinical characteristics of the study group

BM = buccal mucosa; G = gingiva; MB = mucobuccal fold;
T = tongue; P = palate; V = vermilion border
*Only common locations were presented; one patient might have lesions in more than one location



[Table/Fig-2]: Reticular and atrophic types of oral lichen planus affecting right buccal mucosa



[Table/Fig-3]: Oral lichen planus affecting buccal gingiva and alveolar mucosa of upper left posterior teeth

Biopsy site	OLP type	No. of patient	No. of positive DIF (%)	% Sensitivity
Buccal mucosa	Atrophic with white striae	62	54 (87.1)	94
	Atrophic without white striae	4	4 (100)	
Gingiva	Atrophic with white striae	9	6 (66.7)	64
	Atrophic without white striae	5	3 (60)	
Palate	Atrophic with white striae	1	1 (100)	50
	Atrophic without white striae	1	0 (0)	

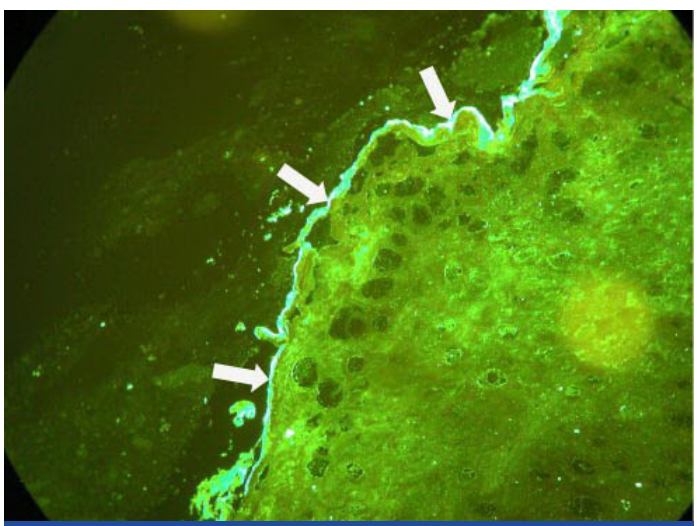
[Table/Fig-4]: Direct immunofluorescence findings in 82 patients with oral lichen planus according to the type of oral lichen planus and biopsy site

OLP = oral lichen planus
DIF = direct immunofluorescence

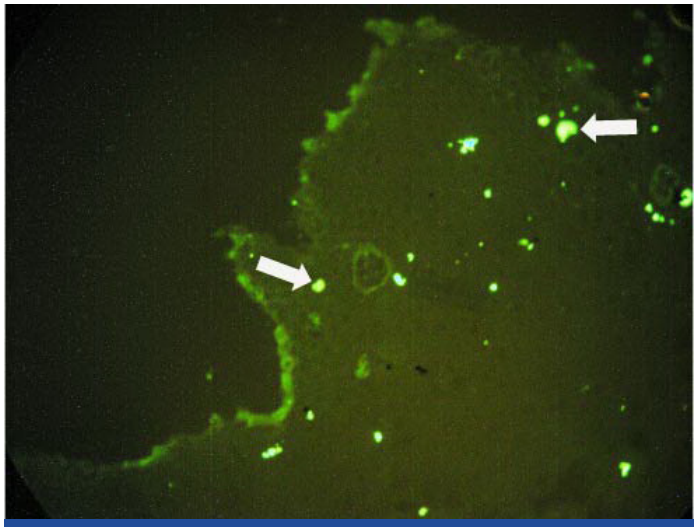
DIF pattern	No. of cases	%
Shaggy F along BMZ & IgM at CB	24	35.3
Shaggy F along BMZ	19	27.9
Shaggy F along BMZ & IgM, IgA at CB	8	11.8
Shaggy F along BMZ & IgM, IgA, C3 at CB	7	10.3
Shaggy F along BMZ & IgM, C3 at CB	3	4.4
Shaggy F, granular IgM along BMZ	1	1.5
Shaggy F, granular IgM along BMZ & IgM, IgA at CB	1	1.5
Shaggy F, granular IgM & C3 along BMZ & IgM at CB	1	1.5
Shaggy F, granular C3 along BMZ & IgM at CB	1	1.5
Shaggy F & linear C3 along BMZ	1	1.5
Shaggy F along BMZ & F, IgM, IgA at CB	1	1.5
Granular C3 along BMZ	1	1.5
Total	68	100

[Table/Fig-5]: The frequency of each direct immunofluorescence pattern in 68 patients with oral lichen planus

DIF = direct immunofluorescence
F = fibrinogen
BMZ = basement membrane zone
CB = colloid bodies



[Table/Fig-6]: Direct immunofluorescence stained section showing deposition of fibrinogen at the basement membrane zone in a shaggy pattern (arrows)



[Table/Fig-7]: Direct immunofluorescence stained section showing deposition of IgMon the colloid bodies in the upper connective tissue (arrows)

Authors	No. of Patients	No. of positive DIF	%
Laskaris et al., [14]	35	35	100
Firth et al., [15]	165	125	75.8
Helander and Rogers, [16]	178	110	61.8
Kulthananet al., [17]	27	21	77.8
Present study	82	68	82.9

[Table/Fig-8]: Studies on direct immunofluorescence in oral lichen planus patients
DIF = direct immunofluorescence

IgM deposition on the CB [Table/Fig-7] was detected in more than half of the positive DIF cases followed in decreasing frequency by IgA, C3, and fibrinogen [Table/Fig-5].

DISCUSSION

Clinical characteristics of OLP, bilateral white striae with or without atrophic/erosive areas, are essential for diagnosis. Histopathological examination is usually used to confirm the clinical diagnosis and also to exclude lesions with dysplastic or malignant changes [5]. In our study group, OLP patients were highly selective as they had to have DIF investigations. This implied that their clinical features could not be readily diagnosed as they were atrophic and erosive types. In addition, it seemed that final diagnosis in such cases required not only histopathological but also DIF results. These cases were, for example, OLP affecting only the gingivae causing desquamative gingivitis [24] or OLP appearing as ulceration with secondary infection. Therefore, differential diagnoses from vesiculobullous diseases were needed.

Studies on the prevalence of positive DIF patterns of OLP revealed various findings ranging from about 62-100% [Table/Fig-8]. Our prevalence (82.9%) was comparable with most studies [14-17] although it was slightly higher than that in Thai patients with oral and/or skin lesions [17]. Most common DIF pattern (98.5%) was shaggy fibrinogen along BMZ with or without positive IgM at CB which was in agreement with other studies [14-16,21]. Therefore, fibrinogen along BMZ, alone or in combination with other deposits at CB, tends to represent OLP [14,17,21,25]. In this study, IgM was the most common deposit on the CB, comprising about half of the cases. This finding had a rather higher percentage than that of previous reports [14,21]. However, Kulthanan et al., showed positive IgM at the CB in 93% of Thai patients with oral and/or cutaneous lichen planus [17]. The differences could be related to lesion sites as more than half of their specimens were obtained from the skin. Kolde et al., demonstrated only IgM positive CB without fibrinogen deposit at BMZ in one of their 17 OLP specimens [21]. This pattern was not found in our 68 positive DIF cases.

In order to differentiate OLP from other diseases presenting similar clinical features, clinical, histological, immunostaining, and serological results are of importance [26]. A recent study has revealed that OLP is the most common cause of desquamative gingivitis followed by mucous membrane pemphigoid and pemphigus vulgaris [26]. In our study, 13.4% (11/82) of the patients had lesions confined to the gingiva. It has been reported that about 10% of OLP patients have only gingival lesions [27]. Therefore, DIF is an additional diagnostic tool in OLP appearing as desquamative gingivitis for its definitive diagnosis and exclusion from other vesiculobullous lesions [15,21,26,28,29]. Furthermore, DIF patterns might be useful to differentiate OLP from similar red and white oral lesions such as lupus erythematosus (LE) although other investigations such as histopathological study and autoantibodies profiles may also be essential [30,31]. Oral LE may show positive linear and/or granular IgG, IgA, IgM and complement at BMZ [32-35]. Laskaris et al., reported granular IgG and C3 deposit at BMZ [14] in discoid lupus erythematosus (DLE) in addition to other identical deposits found in OLP which were positive IgM and C3 at BMZ and colloid bodies [32-35]. In our OLP specimens, positive IgM on colloid bodies were common. We found neither IgG nor IgA deposit along BMZ. This result was different from a previous report on Thai patients by Kulthanan et al., [17]. There were only six cases with positive IgM and/or C3 plus fibrinogen at BMZ and one case with positive granular C3 at BMZ in our study. Yih et al., also demonstrated granular C3 at BMZ in a few cases of OLP [18]. Considering overlapped DIF findings in OLP and LE, the presence of granular IgG at the BMZ can be used to differentiate oral LE from OLP [30,36,37]. In addition, any immunoreactants at CB together with fibrinogen deposit at BMZ indicate OLP than oral LE. This is more site significant when DIF is determined in conjunction with histopathological assessment [36]. The histopathological criteria of OLP include presence of a lymphocytic band at superficial lamina propria and liquefaction degeneration in the basal cell layer without epithelial dysplasia [12]. The critical histopathological differences between OLP and oral LE include the following: more pronounced epithelial atrophy in OLP, thicker basement membrane in LE (H&E and PAS), more edema in the lamina propria in LE, deeper perivascular infiltrates, and PAS-positive thickening of vascular walls in LE [38].

Although gingiva may present nonspecific inflammatory change [39], Helander and Rogers [16] indicated that gingiva should be selected for biopsy site. They found that the gingiva has significantly higher sensitivity than buccal mucosa for DIF and histological findings. This was not supported by our findings in clinically and histologically diagnosed OLP. Our study indicated that buccal mucosa should be considered for the biopsy site in terms of sensitivity for positive DIF. Nevertheless, in cases with only gingival lesions, atrophic gingiva could represent histological features and typical DIF patterns.

CONCLUSION

In this study, the prevalence and pattern of DIF in Thai OLP patients were determined to help in the diagnosis of OLP lacking clinical and/or histopathological characteristic features. The prevalence of positive DIF OLP was 82.9%. Mostly presenting shaggy fibrinogen at BMZwith or without positive IgM at CB, DIF could be an additional investigation to differentiate from other oral lesions and to support the diagnosis of OLP.

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