Original Article

Sensitivity, Specificity and Profile of Direct Immunofluorescence in Oral Lichen Planus

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Abstract

Oral lichen planus (OLP) is a chronic immune-mediated mucocutaneous disease. The clinical features of OLP can sometimes resemble several autoimmune diseases. The histopathology and direct immunofluorescence (DIF) are useful methods to confirm the diagnosis. Our aim was to evaluate the sensitivity and specificity of DIF in OLP diagnosis. OLP DIF profiles were also investigated. Patients attending Oral Medicine Clinic, Faculty of Dentistry, Srinakharinwirot University with the clinical diagnosis of OLP were recruited. The demographic data, histopathology and DIF results, were collected from the patient records. Descriptive statistic was used to analyze the data. Fifty-seven patients were included. The mean age±SD was 52.25±12.93 years. Male to female ratio was 1:6. The final diagnosis based on clinical features, histopathology and DIF results was 46 cases of OLP and 11 of others. The sensitivity and specificity of histopathology in OLP diagnosis were 84.78 % and 90.91 % in that order. While those of DIF were 86.96 % and 100 % respectively. The shaggy fibrinogen deposition at the basement membrane zone (BMZ) was found the most in 84.78 % of the OLP cases. The percentage of OLP diagnosis was increased when histologic features and DIF profiles were included. To conclude, the sensitivity of histopathology is comparable to that of DIF in OLP diagnosis. Both techniques demonstrate high specificity. Most common immune deposition in OLP is fibrinogen. Therefore, clinical, histopathological and DIF features should be utilized for OLP diagnosis, especially in cases that lack clinical characteristics.

Keywords: Autoimmune diseases, Diagnosis, Direct immunofluorescence, Histopathology, Oral lichen planus

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Introduction

Oral lichen planus (OLP) is a chronic mucocutaneous inflammatory disease that affects 0.5-2.2 % of the population and is mainly found in women in their fifth or sixth decades of life.[1,2] The etiology remains unclear and the immunopathogenesis is complex. OLP can appear in the mouth in several different forms. The white, reticular pattern is commonly found and sometimes referred to as Wickham's striae. The reticular lines can be found together with atrophic and/or erosive lesions that usually lead to pain and burning sensation in the mouth[3,4] The diagnosis of OLP is usually based on clinical features and histopathological results.[1] OLP can sometimes clinically resemble other autoimmune diseases such as oral lupus erythematosus, chronic ulcerative stomatitis (CUS), pemphigus vulgaris and mucous membrane pemphigoid.[1] Although histopathology is considered to be the gold standard in the diagnostic protocol, it may be inconclusive. Direct immunofluorescence (DIF) provides additional information that helps to distinguish among various autoimmune diseases especially in cases without clinical and/or histopathological characteristics.[1] However, DIF is a more expensive method and the cost-benefit value should be considered. There are limited data on the sensitivity and specificity of histo-pathology and DIF in the diagnosis of OLP in Thailand. Therefore, the objective of this study was to study the sensitivity and specificity of DIF in the diagnosis of OLP. DIF profiles of OLP patients were also investigated.

Materials and Methods

The study was approved by the Committee on Human Rights Related to Human Experiment, Faculty of Dentistry, Srinakharinwirot University (DENT-SWU-IRB-14/2559). The retrospective study was performed on patients attending the Oral Medicine Clinic, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand with oral lesions clinically diagnosed with OLP from year 2007-2016. Patients who did not have complete oral medicine records or did not receive DIF studies were excluded.

Demographic and clinical data

Age and sex of the patients were retrospectively investigated from dental records. In addition, clinical features of oral lesions, location of lesions, biopsy methods and biopsy sites were documented. Diagnosis of OLP was based on the following criteria. The final diagnosis of OLP was made on patients with at least two criteria from clinical features, histopathologic features or DIF profiles.

1. Clinical features

OLP was diagnosed clinically based on WHO criteria.[2] Characteristic clinical features include well-defined intersecting white lines or striae on minimal to significant erythema background. The desquamative gingivitis, which cannot be distinguishable clinically from other autoimmune diseases, was also included.

2. Histopathologic features

Hematoxylin & Eosin-stained sections of all the cases were reviewed by an oral and maxillofacial pathologist. The diagnosis of OLP was based on modified WHO criteria.[3] Briefly, the specimens should demonstrate well-defined, band-like zone of cellular infiltration consisting mainly of lymphocytes, confined to the superficial lamina propria. In addition, liquefaction degeneration of basal cell layer should be present and no epithelial dysplasia should be observed (Fig. 1A, B).

Cases that presented artefacts such as tangential sectioning, superficial sectioning or did not demonstrate characteristic features of OLP were diagnosed as non-specific chronic mucositis, or descriptively. In addition, cases that exhibited features of other specific diseases were diagnosed as those particular diseases.

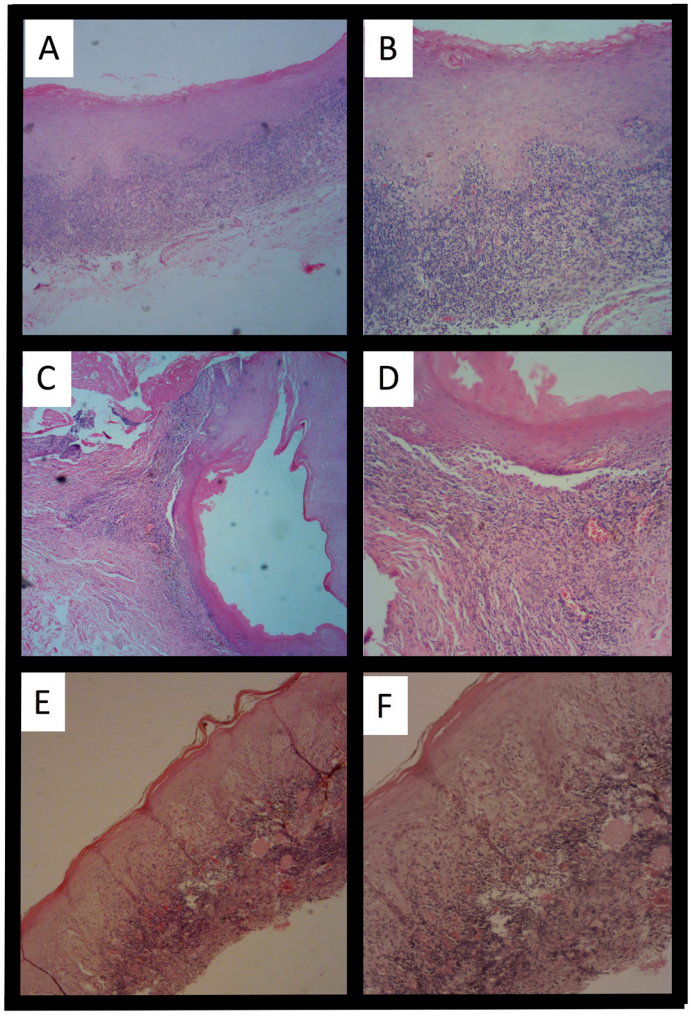

Figure 1 *Histopathological features of OLP (Hematoxylin & Eosin stain) at 100X (A, C, E) and 400X (B, D, F) magnification. A and B: OLP characteristic features. C and D: artefactual separation at the epithelium-connective tissue junction. E and F: Superficial biopsy.*

3. Direct immunofluorescence profiles

All specimens were stored in Michel's solution and submitted for analysis within 7 days to the Dermato-immunology laboratory, Department of Dermatology, Faculty of Medicine Siriraj Hospital (ISO 15189) for the presenceof IgG, IgM, IgA, C3 and fibrinogen. Study interpretation and the presence of each marker were retrieved from the reports. The diagnosis of OLP was based on previously reported studies.[1,4-6] Briefly, the specimen should demonstrate shaggy deposition of fibrinogen at the basement membrane zone with or without deposition of immunoglobulin and/or C3 as colloid bodies. In addition, weak deposition of C3 at the basement membrane zone maybe seen. Cases exhibited features of other specific diseases were diagnosed as those particular diseases, namely lupus erythematosus (course granular deposition of immunoglobulin and C3 along the basement membrane zone). Cases that did not demonstrate any particular pattern were considered non-specific or negative.

Statistical analysis

The data was analyzed by descriptive statistics.

Results

Demographic data of study population

A total of 57 patients presented with the clinical diagnosis of OLP were included in the study. There were 49 women and 8 men with the mean age±SD of 52.25± 12.93 years. The final diagnosis based on clinical features, histopathology and DIF results was 46 cases (80.70 %) of OLP, 8 cases (14.03 %) of non-specific chronic mucositis, 1 case (1.75 %) of lupus erythematosus (LE), 1 case (1.75 %) of erythema multiforme (EM) and 1 case (1.75 %) of mild to moderate epithelial dysplasia. The example of clinical features of the study population was shown in Figure 2.

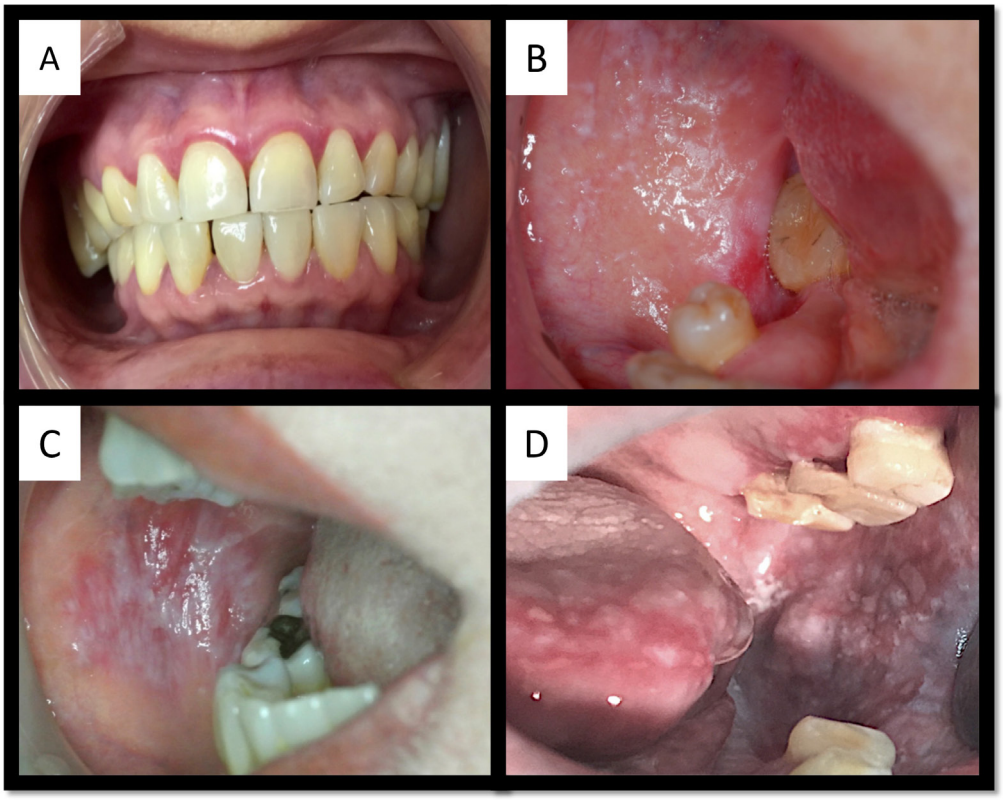

Figure 2 *Clinical features of the study population. A: Erythematous lesion at the gingiva. B: White reticular and papule with atrophic area. C: White plaque on the erythematous base. D: White plaque with erythematous and ulcerative areas.*

The lesions were atrophic and/or erosive with white component. The most common site of OLP was buccal mucosa (73.91 %), gingiva (58.70 %) and mucobuccal fold (43.48 %) respectively. Most subjects have multiple sites of lesions (44 cases, 77.19 %) with 6 (10.53 %), 5 (8.77 %), 1 (1.75 %) and 1 (1.75 %) patients showing lesions confined to the gingiva, buccal mucosa, tongue and mucobuccal fold respectively. Four cases (7.02 %) had unilateral lesion including 3 patients with OLP and 1 patient with epithelial dysplasia. Demographic information of the study population classified according to the final diagnosis was shown in Table 1.

Table 1 *Demographic information of study population classified based on final diagnosis.*

General information	Clinical diagnosis: OLP (57 cases)				
	Final diagnosis				
	OLP (n=46)	Chronic mucositis (n=8)	SLE (n=1)	EM (n=1)	Dysplasia (n=1)
Gender					
Male	5	7	0	0	1
Female	41	1	1	1	0
Age (yrs) Mean ± SD	52.13±13.40	48.13±12.05	47	62	57
Location (%)					
- Buccal mucosa	73.91	87.50	100	100	0
- Gingiva	58.70	50	100	0	0
- Mucobuccal fold	43.48	25	0	0	0
- Tongue	23.91	37.5	0	0	100
- Lips	13.04	12.5	0	0	0
- Palate	6.52	0	0	0	0
Distribution (%)					
- Bilateral/Unilateral	93.50/6.50	100/0	100/0	100/0	0/100
- Multiple/single location	78.26/21.74	87.50/12.50	100/0	0/100	0/100
Biopsy technique (%)					
- Punch	69.57	87.50	100	100	100
- Scalpel	30.43	12.50	0	0	0

The sites of biopsy in this study were buccal mucosa (42 cases, 73.68 %), gingiva (10 cases, 17.54 %), vestibular area (1 case, 1.75 %) and tongue (4 cases, 7.01 %). Punch biopsy was used in 32 cases of OLP, 7 cases of chronic mucositis and 1 case each of LE, EM and dysplasia. Scalpel biopsy was used in 14 cases of OLP and 1 case of non-specific mucositis. All specimens were cut in half. One piece was fixed in 10 % formalin and submitted for H&E staining. The other piece was stored in Michel's solution and submitted for DIF study.

Histopathologic features of the study population

From a total of 46 cases with the final diagnosis of OLP, 39 cases (84.78 %) demonstrated histopathologic features characteristics of OLP, which allowed definitive diagnosis to be made. However, 7 cases (15.21 %) were called non-specific chronic mucositis due to the inability to evaluate some characteristic features of OLP in the submitted specimens. For example, band-like lymphocytic infiltrates could not be evaluated due to artefacts such as tangential sectioning or crush artefact. Some biopsied specimens were too superficial (Fig. 1E, F). Moreover, one case demonstrated artefactual separation at the epithelium-connective tissue junction, which made it difficult to differentiate that case from other autoimmune diseases such as mucous membrane pemphigoid or pemphigus vulgaris (Fig. 1C, D).

Two cases were diagnosed as other specific diseases based on histopathological features including mild to moderate epithelial dysplasia and erythema multiforme. The other 8 cases were called mucositis according to histopathological results and were diagnosed as non-specific chronic mucositis due to negative DIF results. Interestingly, one case was histopathologically diagnosed as lichen planus/ lichenoid mucositis, however, clinical and DIF studies supported the final diagnosis of lupus erythematosus. This could be because the specimen was too superficial precluding the evaluation of deep perivascular inflammation characteristics of lupus erythematosus. A summary of histopathologic results was shown in Table 2.

Table 2 *Histopathology and DIF results of all lesions*

Lesion	Histopathology results		DIF results	
	OLP	Chronic mucositis or as specified	positive	negative/non-specific
Oral lichen planus	39	7	40 (OLP)	6
Chronic mucositis or others	0	8	0	8
Epithelial dysplasia	0	1 (dysplasia)	0	1
Erythema multiforme	0	1 (erythema multiforme)	0	1
Lupus erythematosus	1	0	1 (LE)	0

OLP DIF profile

The data from 46 patients with the final diagnosis as OLP was further investigated. Forty out of 46 patients (86.96 %) demonstrated characteristic or compatible DIF profiles of OLP. Nevertheless, all except 2 patients demonstrated the deposition of at least one immune component. Table 3 demonstrated DIF profiles of all 46 OLP patients.

Table 3 *DIF profiles of OLP patients.*

DIF profile	N (%)	Histopathologic diagnosis N (% within group)	
		Lichen planus	Chronic mucositis
DIF: LP / seen in LP			
Shaggy BMZ fibrinogen	3 (6.52)	2 (66.67)	1 (33.33)
Shaggy BMZ fibrinogen + granule C3	7 (15.22)	7 (100)	0
Linear BMZ fibrinogen + granule C3	1 (2.17)	1 (100)	0
Shaggy BMZ fibrinogen + granule C3 + CB IgM	5 (10.87)	5 (100)	0
Shaggy BMZ fibrinogen + CB C3, IgM, IgA	2 (4.35)	2 (100)	0
Linear BMZ fibrinogen + CB C3, IgM, IgA	2 (4.35)	1 (50)	1 (50)
Colloid BMZ fibrinogen + CB C3, IgM, IgA	1 (2.17)	1 (100)	0
Shaggy BMZ fibrinogen + granule C3 + CB IgM, IgA	3 (6.52)	2 (66.67)	1 (33.33)
Shaggy BMZ fibrinogen + CB IgM	3 (6.52)	2 (66.67)	1 (33.33)*
Shaggy BMZ fibrinogen + CB IgM, IgA	2 (4.35)	2 (100)	0
Shaggy BMZ fibrinogen + granule C3, IgM	2 (4.35)	2 (100)	0
Shaggy BMZ fibrinogen + granule C3 + CB IgA	1 (2.17)	0	1 (100)
Linear BMZ fibrinogen + granule C3, IgM, IgA	1 (2.17)	1 (100)	0
Shaggy BMZ fibrinogen + CB C3, IgM + Nuc IgG	1 (2.17)	0	1 (100)
Linear BMZ fibrinogen+granule C3+CB IgM+Nuc IgG	1 (2.17)	0	1 (100)
Shaggy BMZ fibrinogen+granule C3+ Nuc IgA, IgG	1 (2.17)	1 (100)	0
Shaggy BMZ fibrinogen+ granule IgM, IgA, IgG	1 (2.17)	1 (100)	0
Linear BMZ fibrinogen+Nuc IgG	1 (2.17)	1 (100)	0
Shaggy BMZ fibrinogen+CB IgA	1 (2.17)	1 (100)	0
CB IgM, IgA	1 (2.17)	1 (100)	0
TOTAL	**40**	**33**	**7**

Table 3 *DIF profiles of OLP patients. (cont.)*

DIF profile	N (%)	Histopathologic diagnosis N (% within group)	
		Lichen planus	Chronic mucositis
DIF: non-specific/negative			
CB IgA	1 (2.17)	1 (100)	0
C3, IgM superficial BV	1 (2.17)	1 (100)	0
CB C3	1 (2.17)	1 (100)	0
Granule C3 and CB IgM	1 (2.17)	1 (100)	0
All negative	2 (4.35)	2 (100)	0
TOTAL	**6**	**6**	**0**
TOTAL	**46 (100)**	**39 (84.78)**	**7 (15.21)**

**Differential diagnosis included chronic mucositis and mucous membrane pemphigoid due to artefactual separation between the epithelium and connective tissue.*

CB=colloid bodies, BMZ=basement membrane zone, C3=complement 3, NUC=nuclear, BV=blood vessel

When evaluated the immune deposits in all cases, we found that fibrinogen deposition at the basement membrane zone (BMZ) was presented in the majority of cases (39 out of 46 cases, 84.78 %), followed by C3 (67.39 %), IgM (58.70 %) IgA (36.96 %), and IgG (10.8 %), respectively. Patterns of fibrinogen deposition included shaggy (32 out of 39 cases, 82.05 %), linear (6 out of 39 cases, 15.38 %) and colloid bodies (1 out of 39 cases, 2.56 %). When evaluated the immune deposition pattern, we discovered that the three most common DIF profiles in OLP patients were fibrinogen deposition in combination with granular C3 deposits (8 cases, 17.39 %), followed by fibrinogen deposition in combination with granular C3 deposits and IgM deposition at the colloid bodies (5 cases, 10.87 %) and fibrinogen in combination with C3, IgM and IgA deposition at the colloid bodies (5 cases, 10.87 %). Fibrinogen deposition alone was only observed in 3 cases (6.52 %). Interestingly, nuclear IgG deposition was observed in 4 OLP cases.

Sensitivity and specificity of histopathology and DIF

The sensitivity and specificity of histopathology for the diagnosis of OLP were 84.78 % and 90.91 % in that order. The sensitivity and specificity of DIF were 86.96 % and 100 % respectively. Table 2 demonstrated the diagnosis based on histopathology and DIF for each lesion. Six and 7 cases out of 46 OLP cases were unable to be diagnosed with DIF and histopathology, respectively. The data suggested that DIF and histopathology complemented each other in making OLP diagnosis. Although the sensitivity of histopathology and DIF in OLP diagnosis was similar, the percentage of OLP diagnosis increased approximately 15 % when both histopathology and DIF were performed. We then investigated whether or not biopsy sites affect the sensitivity and specificity of DIF results. Number of LP cases with corresponded DIF results and non-LP cases with negative DIF results according to the biopsy sites was shown in Table 4.

DIF was shown to give negative results for all of non-LP cases (11 out of 11 cases, 100 %) regardless of the biopsy site, while 29 out of 34 cases (85.29 %) and 9 out of 10 cases (90 %) of DIF positivity for LP were reported when the biopsy was performed at the buccal mucosa and gingiva, respectively. Tongue and vestibular area demonstrated 100 % of DIF positivity for LP. However only one case from each location were included in the study.

Table 4 *Number of LP cases with positive DIF and non-LP cases with negative DIF results according to biopsy site.*

Biopsy site	No. of LP cases with positive DIF* (%)	No. of non-LP cases with negative DIF** (%)
Buccal mucosa	29/34 (85.29)	8/8 (100)
Gingiva	9/10 (90)	0
Tongue	1/1 (100)	3/3 (100)
Vestibular area	1/1 (100)	0
TOTAL	**40/46**	**11/11**

**LP cases with positive DIF results represented cases with DIF results characteristic of LP*

***Non-LP cases with negative DIF results represented cases with non-LP DIF results*

Discussion

The diagnosis of OLP is usually based on clinical features and histopathology results. However, oral lesions of patients with other autoimmune diseases can sometimes be difficult to distinguish from OLP. For example, desquamative gingivitis can commonly found in OLP, but also in other autoimmune diseases such as pemphigus and pemphigoid.[7,8] Previous studies reported the usefulness of DIF in the diagnosis of these oral mucocutaneous lesions.[9,10] Inflammatory infiltrate of gingival biopsy may not be characteristics of OLP due to the coincided gingival disease. It may present as mixed lymphocytic and plasma cell infiltrates, precluding the definitive diagnosis of lichen planus histopathologically.[1] Therefore, DIF could provide additional information that aids in OLP diagnosis in these cases. In our study, 6 patients with desquamative gingivitis showed 100 % DIF positive results for LP when gingival biopsies were performed, supporting the benefits of DIF in the diagnosis of OLP cases that lack clinical and/or histopathological characteristic features.

In this study, the sensitivity of histopathology in the diagnosis of OLP was 84.78 %. Although, histopathology is considered a gold standard in OLP diagnosis, it has limitations such as depth of biopsy, orientation artefact and others. Histopathology results are also affected by inter and intraobserver variability.[3,11] Therefore, the DIF can be used as additional diagnostic tool in OLP.

Previous studies from other countries reported that the sensitivity of DIF in OLP diagnosis ranged from 61.8-100 %.[5,12-14] In this study, we found that the sensitivity of DIF was 86.96 %, which was comparable to the study in Thailand (82.9 and 75 %).[4,15] Factors affecting DIF sensitivity and specificity were studied by several groups including biopsy techniques and biopsy site.[4,5] Punch biopsy was shown to provide better DIF sensitivity than scalpel biopsy.[5] In contrast to previous report, our results showed that only 1 out of 14 cases (7.1 %) receiving scalpel biopsy demonstrated false negative DIF results, as compared to 7 out of 32 (21.9 %) of punch biopsy (data not shown).

Regarding biopsy site, Sano SM *et al.,* reported that buccal mucosa gave better DIF sensitivity (68.6 %) than dorsal tongue (62.5 %) and gingiva (58.82 %), respectively.[5] Consistently, Buajeeb W *et al.,* reported that buccal mucosa provided the best DIF sensitivity (94 %), followed by gingiva (64 %) and palate (50 %).[4] The majority of our OLP cases (73.91 %) were biopsied from buccal mucosa which provided 85.29 % DIF sensitivity. The sensitivity was lower than that of Buajeeb W *et al.* but higher than that of Sano SM *et al.* Notably, our study demonstrated that gingival biopsies provided better DIF sensitivity than other sites. Although tongue and vestibular mucosa provided 100 % specificity, only one case from each location were included. Further investigation including more cases may be beneficial.

Effects of transport media on DIF sensitivity were controversial. Some studies suggested that tissue storage in normal saline for less than 24 hours provided higher

DIF sensitivity than in Michel's solution, while others supported the use of Michel's solution for comparable results [16,17]. DIF sensitivity for OLP cases in our study was comparable to the other Thai study that used normal saline for tissue preservation, suggesting that Michel's solution is acceptable to be used as transport medium for OLP cases.[4]

When compared the DIF profiles of OLP in our study with previous studies[1,4,5,15,18,19], we found similar results. Shaggy fibrinogen deposition was reported to be the most frequent immune deposits in OLP ranging from 56.7-99 %, followed by C3 and IgM.[4,15,18] The most common findings in our study were shaggy fibrinogen deposition at the BMZ, deposition of C3 and IgM as granular pattern and at colloid bodies. This was in accordance with previous reports in skin LP that a combination of shaggy fibrinogen deposition at DEJ and immunoreactant deposits at colloid bodies is more typical of LP characteristics.[15,20,21]

Interestingly, IgG deposition was observed in four cases (8.69 %). The percentage was consistent with previous study that reported 3-30 % IgG deposits in non-lupus cases.[19] A specific pattern of nuclear IgG deposition was also reported in chronic ulcerative stomatitis (CUS), a rare mucocutaneous disease primarily involving mucosal surface and sometimes the skin. Clinically, CUS exhibits ulcerative and erosive lesions that resemble oral lichen planus. Routine histopathology may show feature of lichenoid mucositis. However, it can only be separated from OLP by immunofluorescence studies [22-24]. The separation between these two diseases is important as CUS is well-known for its resistance to conventional steroid treatment, but well respond to hydroxychloroquine [24].

Conclusion

The most common DIF profile of OLP in this study was shaggy fibrinogen deposition at the BMZ (84.78 %). Although the sensitivity of histopathology and DIF is comparable, both DIF and histopathological analysis have limitations. Therefore, clinical examination, histopathology and DIF should be performed in order to achieve the definitive diagnosis of OLP, especially in controversial cases.

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