



ORIGINAL ARTICLE

# Increased cyclooxygenase 2 expression in association with oral lichen planus severity



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## KEYWORDS

cyclooxygenase 2;  
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visual analog pain scale

**Abstract** *Background/purpose:* Although some studies have shown induction of cyclooxygenase 2 (COX-2) in oral lichen planus (OLP), an association between COX-2 upregulation and OLP clinical severity has not been investigated. Therefore, we aimed to compare COX-2 expression in OLP with that in normal oral tissues, and to determine correlations between COX-2 expression and both clinical criteria and visual analog scale (VAS) scores.

*Materials and methods:* COX-2 expression was studied in 25 OLP and 13 normal oral tissues by immunohistochemistry. Both clinical criteria and VAS scores were used to evaluate the clinical severity of OLP. The differences in COX-2 expression between OLP and normal tissues, and the correlations between COX-2 expression and clinical severity were determined by the nonparametric statistical tests.

*Results:* COX-2 expression was significantly increased in OLP epithelium when compared with normal epithelium ( $P < 0.001$ ), and intense COX-2 staining in inflammatory infiltrates was observed in the OLP lamina propria. COX-2 expression in OLP epithelium and inflammatory infiltrates was significantly correlated with the clinical criteria score ( $r = 0.428$ ,  $P = 0.007$ , and  $r = 0.681$ ,  $P < 0.001$ , respectively), whereas a significant correlation with the VAS score was observed only in OLP inflammatory infiltrates ( $r = 0.605$ ,  $P < 0.001$ ).

*Conclusion:* Enhanced COX-2 expression in both OLP epithelium and inflammatory infiltrates correlates well with the clinical severity. An association between VAS score and

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COX-2 expression in OLP inflammatory infiltrates suggests an important role of additional COX-2 expression from inflammation in causing pain in OLP patients.

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## Introduction

Oral lichen planus (OLP), a chronic inflammatory lesion, is commonly found in the adult population and affects women more than men.<sup>1–3</sup> The ratio of female to male varies across different geographical areas, and this ratio can be as high as 4:1 in Thai patients.<sup>4</sup> Usually, OLP patients seek therapy because of burning sensation from atrophic or ulcerative epithelium, caused by epithelial apoptosis.<sup>5,6</sup> The definitive diagnosis of OLP is confirmed by the histopathologic features of epithelial hyperkeratosis, subepithelial band-like mononuclear infiltration, and liquefaction degeneration of the basal and parabasal cells.<sup>7</sup> OLP etiology is multifactorial and involves an aberration of T-cell-mediated immune responses to unknown foreign antigens,<sup>6</sup> such as dental materials, drugs, and bacterial and viral infections. Different intrinsic factors within individual patients are also implicated in the etiology.<sup>6–8</sup>

Cyclooxygenase 2 (COX-2) is a 72-kDa inducible enzyme essential for prostaglandin production. With respect to oral diseases, COX-2 expression is shown to be upregulated in oral squamous cell carcinoma (OSCC)<sup>9,10</sup> and chronic inflammatory disorders, including gingivitis, periodontitis, apical periodontitis, and OLP.<sup>11,12</sup> Enhanced COX-2 expression is shown in different cell types ranging from normal cells to cancer cells in these diseases. Moreover, COX-2 induction is proposed to be involved with malignant transformation.<sup>13</sup> Neppelberg and Johannesen<sup>12</sup> have, instead, suggested that COX-2 expression cannot be used as a reliable marker for malignant transformation in OLP.

Eight previous studies have demonstrated COX-2 expression in OLP,<sup>12,14–20</sup> but only four studies have shown a significant increase in COX-2 expression in OLP lesions compared with normal tissues.<sup>14–16,18</sup> Out of these four studies, only one<sup>16</sup> has reported differences in COX-2 expression between different types of OLP. Particularly, a significantly greater COX-2 expression was found in the ulcerative/erosive type than in the atrophic type. Therefore, it is possible that different classification systems and definitions used to categorize OLP types and severities are involved in the inconsistent findings. In 1992, Thongprasom and coworkers<sup>21</sup> proposed the clinical criterion to evaluate OLP severity. This criterion has been well accepted; we, therefore, used it as a tool to clinically evaluate OLP types and severities. In this study, we aimed to compare COX-2 expression in OLP lesions with that in normal oral tissues by immunohistochemistry, and to find correlations between COX-2 expression and OLP severity, assessed by the clinical criteria and visual analog scale (VAS)<sup>22</sup> scores.

## Materials and methods

### Patient selection

Twenty-five new patients clinically and histopathologically confirmed to have OLP were recruited from the Oral Medicine Clinic, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand. The inclusion criteria were as follows: (1) patients clinically presented with a white and/or red oral lesion that was later confirmed histologically as OLP without evidence of dysplastic changes; (2) patients did not have a history of taking drugs that are reported to cause lichenoid drug reactions and the OLP lesion was not adjacent to dental restoration<sup>2,23</sup>; and (3) patients have neither other oral mucosal lesions nor a history of lichenoid-related systemic conditions. The exclusion criteria were as follows: patients who had received systemic or topical steroid treatment for oral lesions in the past 3 months and those who were pregnant or breast-feeding. The demographic data on age, gender, and site of the lesion for patients with OLP are summarized in Table 1. The research protocol was approved, and a certificate of ethical clearance (#16/2014) was issued by the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University. Informed consent was obtained prior to sample collection.

### Assessment of OLP severity and collection of tissue specimens

The clinical severity of OLP was evaluated by the VAS<sup>22</sup> and clinical criteria scores.<sup>21</sup> The VAS score was obtained from each patient at the first visit to subjectively describe his or her pain using a 0–10 scale, where 0 indicates no pain and 10 the worst imaginable pain. The clinical criteria score was given for the most severe site of OLP as follows: 0 = no lesion, normal mucosa; 1 = mild white striae, no erythematous area; 2 = white striae with an atrophic area smaller than 1 cm<sup>2</sup>; 3 = white striae with an atrophic area larger than 1 cm<sup>2</sup>; 4 = white striae with an ulcerative area smaller than 1 cm<sup>2</sup>; and 5 = white striae with an ulcerative area larger than 1 cm<sup>2</sup>. An incisional biopsy was conducted at the most severe site of OLP, as a representative area for each patient, fixed in 10% formalin buffer for 24 hours, and embedded in paraffin blocks. The OLP specimens were sectioned for a definitive histopathologic diagnosis by hematoxylin and eosin staining.

### Immunohistochemistry

Twenty-five OLP specimens as well as 13 normal oral specimens from both vestibular and buccal mucosae,

**Table 1** Demographic data and sites of lesions in patients with OLP.

Group	No. of participants	Gender (%)		Mean age, y (range)	Site of lesion (%)					
		Male	Female		Buccal mucosa	Gingiva	Mucobuccal fold	Tongue	Labial mucosa	Multiple sites
OLP	25	5 (20.0)	20 (80.0)	48.76 (19–69)	25 (100.0)	7 (28.0)	6 (24.0)	2 (8.0)	2 (8.0)	10 (40.0)

OLP = oral lichen planus.

retrieved from the archives of the Dental Hospital, Faculty of Dentistry, Chiang Mai University, were serially cut at 5  $\mu$ m thickness and processed by immunohistochemistry according to our previously published protocol.<sup>24</sup> The sections were deparaffinized, gradually rehydrated, and quenched for the tissue peroxidase activity by incubation in 3% H<sub>2</sub>O<sub>2</sub> for 10 minutes. Antigen retrieval was performed by heating the sections in 1mM EDTA for 15 minutes and left at room temperature for 20 minutes. The sections were incubated in 1.5% normal horse serum for 20 minutes and with the mouse monoclonal antibody specific for COX-2 at 1:50 dilution (sc-166475; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight. Thereafter, the sections were reacted with the biotinylated antimouse antibody (Ready-to-Use (R.T.U.) Vectastain elite ABC kit; Vector Laboratories, Burlingame, CA, USA) for 20 minutes, and incubated with avidin-biotinylated horseradish peroxidase (ImmunoCruz ABC Staining Systems; Santa Cruz Biotechnology) for 20 minutes at room temperature. The sections were stained with 3,3'-diaminobenzidine (Vector Laboratories) for 4 minutes and then counterstained with hematoxylin for 20 seconds. The OLP section, in which the primary antibody was omitted and replaced with normal horse serum, was used as a negative control. The section with a histopathologic diagnosis of OSCC, retrieved from the Dental Hospital, was used as a positive control for COX-2 staining.<sup>9</sup> Digitalized images were captured by a charge-coupled device camera (D70; Olympus, Tokyo, Japan), attached to a bright-field microscope (BX41; Olympus) and a computer desktop system.

### Determination of immunohistochemical staining scores

The scoring for each section was performed under Image J program twice by two observers who were blinded to its clinicopathological data. The intra- and interexaminer calibrations were performed with Cohen's kappa values equal to 0.90 and 0.85, respectively. Before scoring, each section was first observed to determine the orientation of epithelium and connective tissue, and then screened for a selection of five fields of vision in the epithelial and connective tissue layers, representing the characteristics of each section. Then, the scoring was performed in the epithelial and connective tissue layers under 200 $\times$  magnification power. The staining intensities were scored as follows: 0 = no staining, 1 = weak, 2 = moderate, and 3 = strong. Brown staining in the cytoplasm was considered to be positive regardless of the staining intensity. The number of positive cells in each field was counted, and the percentage of positive cells was calculated by dividing its

numbers with the total number of cells in each field. Then, the average percentage of positive cells was calculated from five fields of vision. The immunohistochemical staining score (0–3),<sup>25</sup> measured as (staining intensity score)  $\times$  (percentage of positive cells), was determined for COX-2 expression in both epithelial layer and inflammatory infiltrates.

### Statistical analyses

The Mann–Whitney *U* test was used to compare COX-2 expression between OLP and normal oral tissues. The Spearman or Pearson correlation coefficient was used to determine correlations between COX-2 expression and the clinical criteria or the VAS score, respectively. SPSS software version 17.0 for Windows (IBM, Chicago, IL, USA) was used for all statistical analyses.

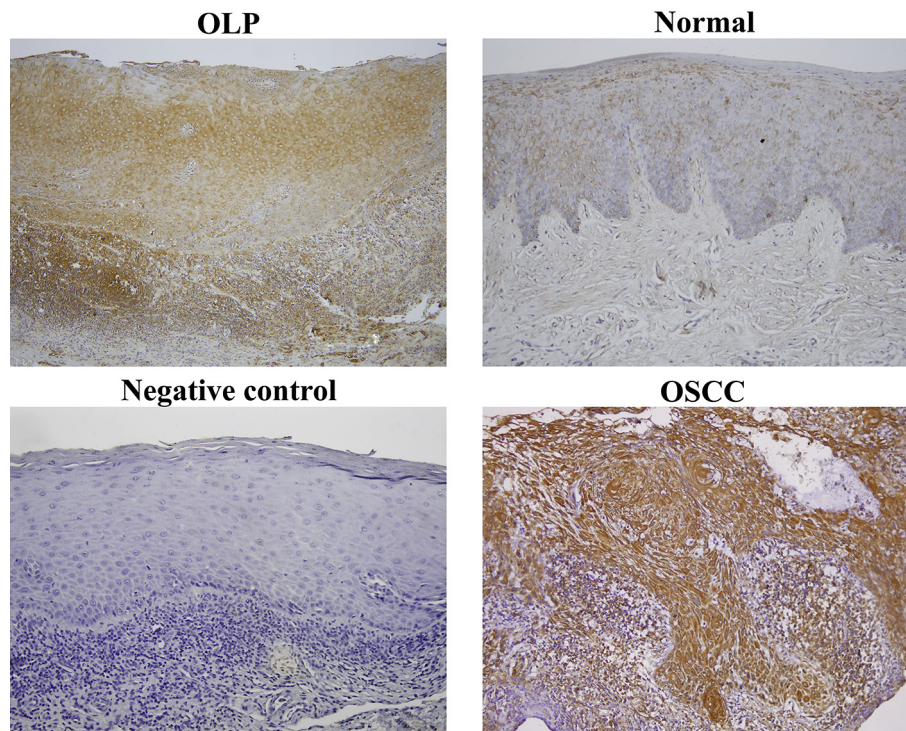
## Results

### Clinical findings

The chief complaints of OLP patients included burning sensation (76%) and pain (12%), and they were without any symptoms for the reticular type of OLP (12%). The VAS score ranged from 0 to 9.6, with a mean VAS score  $\pm$  standard deviation being equal to 2.87  $\pm$  3.17. When OLP and normal oral specimens were grouped by the clinical criteria score, there were 13 normal oral tissues with score 0 for no lesion; four OLP tissues with score 1 for reticular OLP; seven and six OLP tissues with scores 2 and 3, respectively, for atrophic OLP; and seven OLP tissues and one OLP tissue with scores 4 and 5, respectively, for ulcerative OLP.

### Increased COX-2 expression in OLP

By immunohistochemistry, COX-2 expression was found in the cytoplasm of the epithelial cells (brown staining) in all sections from both OLP and normal tissues (Figure 1), but COX-2 expression, assessed by the immunohistochemical staining score, in OLP epithelium was significantly greater than that in normal epithelium ( $P < 0.001$ ; Figure 2A). Intense staining of COX-2 expression was also detected in a band-like inflammatory infiltrate in the OLP lamina propria (Figure 1), consistent with a significant increase in COX-2 expression in the inflammatory infiltrates of OLP lesions ( $P < 0.001$ ; Figure 2B). As a negative control, the OLP section, in which the primary antibody against COX-2 was omitted, showed no staining, whereas intense staining of



**Figure 1** Increased COX-2 expression in OLP. COX-2 expression (brown staining in the cytoplasm) in OLP epithelium was greater than that in normal epithelium. Note intense COX-2 staining in OLP inflammatory infiltrates in the lamina propria. The OLP section, in which the primary antibody against COX-2 was omitted, showed no staining as a negative control. As a positive control for COX-2 expression, the OSCC specimen showed intense brown staining in the cancer cells and stromal inflammatory cells. Original magnification, 200 $\times$ . COX-2 = cyclooxygenase 2; OLP = oral lichen planus; OSCC = oral squamous cell carcinoma.

COX-2 expression was found in the OSCC specimen, which served as a positive control (Figure 1).

### Enhanced COX-2 expression in relation to the clinical severity of OLP

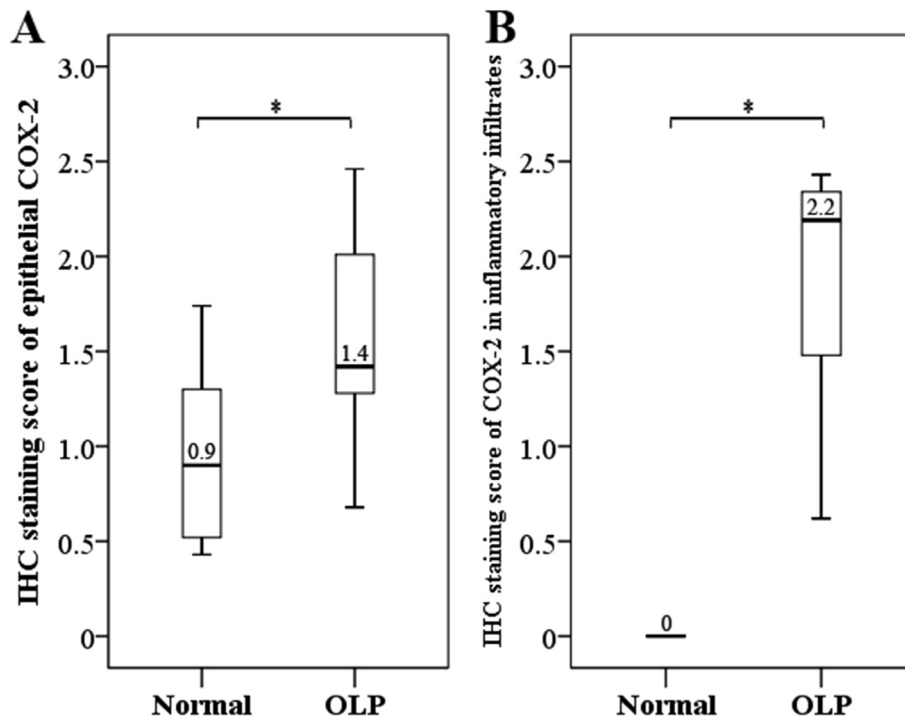
COX-2 staining in both OLP epithelium and inflammatory infiltrates was more intense in the higher clinical criteria scores (scores 3–5) than in the lower clinical criteria scores (scores 0–2; Figure 3). Moreover, significant and positive correlations were demonstrated between the clinical criteria score and COX-2 expression in both OLP epithelium and inflammatory infiltrates ( $r = 0.428$ ,  $P = 0.007$ , and  $r = 0.681$ ,  $P < 0.001$ , respectively; Figures 4A and 4B). A significant and positive correlation was found between the VAS score and COX-2 expression in OLP inflammatory infiltrates ( $r = 0.605$ ,  $P < 0.001$ ; Figure 4D), whereas no significant correlation was found between the VAS score and COX-2 expression in OLP epithelium (Figure 4C).

### Discussion

In this study, we demonstrated a significant increase of COX-2 expression in OLP epithelium compared with that in normal oral epithelium by immunohistochemistry. This finding is in line with the results of previous studies.<sup>12,18,19</sup> In addition to increased COX-2 expression in OLP

epithelium, we found intense COX-2 staining in OLP inflammatory infiltrates similar to the results of several studies.<sup>12,15,17–20</sup> However, all those studies have not yet shown the correlations between increased COX-2 expression in both OLP epithelium and inflammatory infiltrates and the clinical severity of OLP. Therefore, our correlation data in Figure 4 have further extended their results by showing significant and positive relationships between enhanced COX-2 expression in OLP epithelium and inflammatory infiltrates and OLP severity, represented by the clinical criteria and the VAS scores.

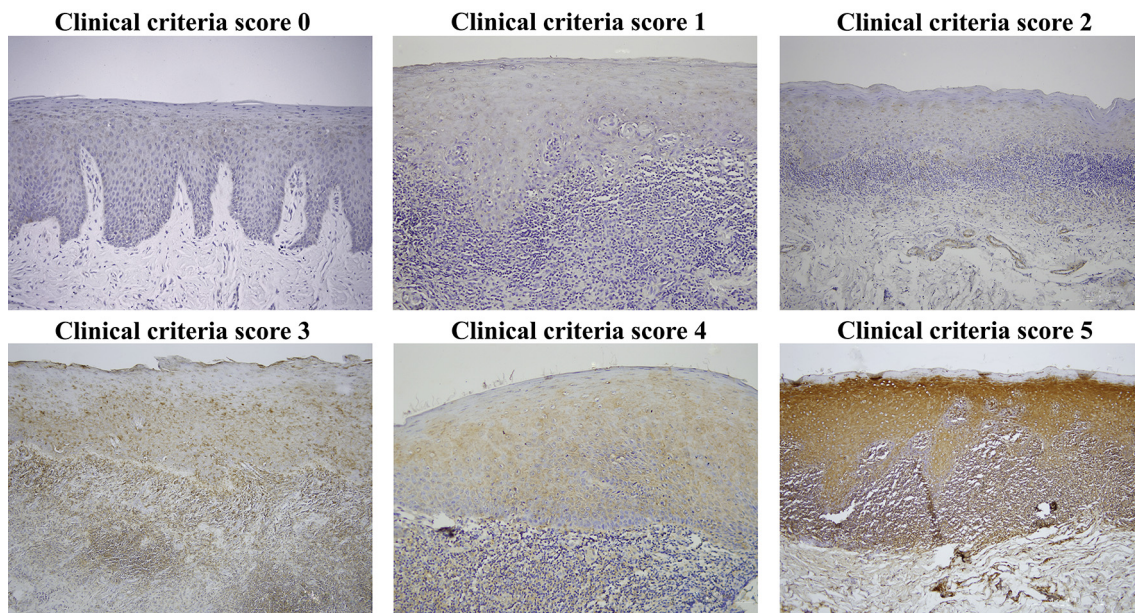
Clinically, burning sensation or pain symptoms in patients with OLP may be explained by high levels of COX-2 expression in both OLP epithelium and inflammatory infiltrates accumulating in the OLP lamina propria because an important function of the COX-2 enzyme is to produce PGE<sub>2</sub>, which is considered to be one of the pain mediators. In the chronic ulcerative/erosive lesions of OLP, elevated PGE<sub>2</sub> levels can probably sensitize peripheral sensory nerves, causing pain signals to be readily and more frequently transmitted to the brain,<sup>26,27</sup> resulting in more pain and higher VAS scores in the ulcerative/erosive type of OLP than those in the other types. Accordingly, a significant and positive correlation between intense COX-2 staining in OLP inflammatory infiltrates and the VAS score in Figure 4D is consistent with the aforementioned explanation and with the findings from a previous study<sup>16</sup> that revealed significantly higher PGE<sub>2</sub> levels in the ulcerative/erosive type than in the atrophic type of OLP.



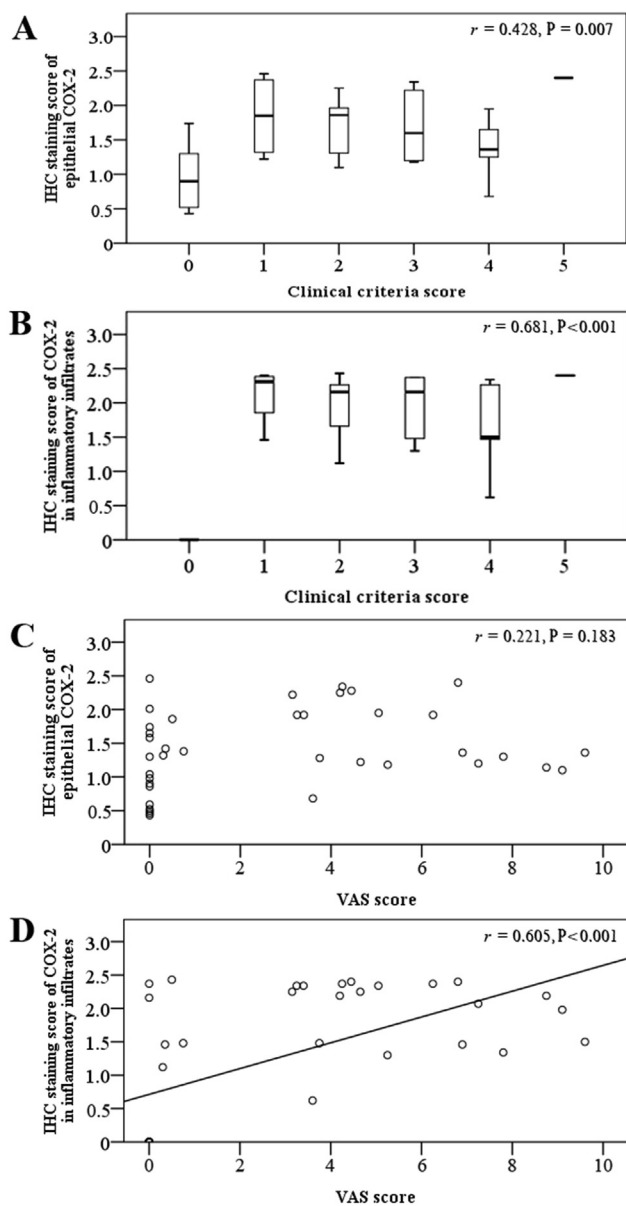
**Figure 2** Significant increase in COX-2 expression, as evaluated by the IHC staining score in both (A) OLP epithelium and (B) inflammatory infiltrates. The box-plot graphs demonstrated a significantly higher COX-2 expression in OLP epithelium and inflammatory infiltrates than in normal oral mucosa. The horizontal lines in each box plot represent the 25<sup>th</sup> percentile, 50<sup>th</sup> percentile, and 75<sup>th</sup> percentile of COX-2 expression. \* $P < 0.001$ . COX-2 = cyclooxygenase 2; IHC = immunohistochemical; OLP = oral lichen planus.

T lymphocytes are predominantly found in the band-like inflammatory infiltrates of OLP lesions.<sup>28</sup> One of the adverse outcomes for T lymphocyte infiltration is degradation of basal lamina by production and secretion of

matrix metalloproteinase 9.<sup>5,29</sup> The basement membrane destruction can thus lead to infiltration of T lymphocytes into the OLP epithelial layer, resulting in direct cell-to-cell contacts between epithelial cells and T lymphocytes that



**Figure 3** Various degrees of COX-2 expression in different clinical criteria scores. The representative section from each clinical criteria score (0–5) is shown for COX-2 staining. Note the most intense COX-2 staining in OLP epithelial cells and inflammatory infiltrates for the clinical criteria score 5. Original magnification, 200 $\times$ . COX-2 = cyclooxygenase 2; OLP = oral lichen planus.



**Figure 4** Correlation data between COX-2 expression in (A and C) OLP epithelium and (B and D) inflammatory infiltrates, as assessed by the IHC staining score on the y-axis, and (A and B) the clinical criteria score and (C and D) the VAS score on the x-axis. Note the significant and positive correlations found between (A and B) COX-2 expression in OLP epithelium and inflammatory infiltrates and the clinical criteria score, and between COX-2 expression in OLP inflammatory infiltrates and the VAS score (the regression line in D). COX-2 = cyclooxygenase 2; IHC = immunohistochemical; OLP = oral lichen planus; VAS = visual analog scale.

can lead to apoptosis of OLP epithelial cells.<sup>7,30</sup> Increased epithelial apoptosis was clinically observed as the atrophic and the ulcerative/erosive type of OLP,<sup>31</sup> in which they were scored as 2, 3, 4, and 5 according to the clinical criteria score. Therefore, an increase in COX-2 expression in OLP epithelium in the clinical criteria scores 3, 4, and 5 (Figure 3) suggests that overwhelming COX-2 expression in

these scores may cause a nonhealing wound in OLP epithelium. This speculation is consistent with the previous finding that demonstrated an increase in epithelial apoptosis in the ulcerative/erosive type of OLP.<sup>31</sup> Interestingly, COX-2 was also weakly expressed in the suprabasal cell layers of normal oral epithelium (Figure 1), which is in line with the findings from other studies in normal oral epithelium<sup>32,33</sup> and in normal epidermis.<sup>34</sup>

To accurately compare clinical severities and interventions among different OLP studies, a single, well-accepted clinical criterion is required. A recent meta-analysis study has indicated that 25% of OLP studies used Thongprasom et al's<sup>35</sup> clinical criterion to assess OLP severity and treatment efficacy. Therefore, this criterion was selected to evaluate OLP severity, and a significant correlation was found between increased COX-2 expression in OLP epithelium and inflammatory infiltrates and the clinical criteria score. In conclusion, enhanced COX-2 expression in both OLP epithelium and inflammatory infiltrates is significantly correlated with OLP severity. Moreover, a strong correlation between COX-2 staining in OLP inflammatory infiltrates and the VAS score suggests that additional COX-2 expression in OLP inflammatory infiltrates may play a causative role in burning sensation or pain in patients with the atrophic or ulcerative/erosive type of OLP.

## Conflicts of interest

The authors have no conflicts of interest relevant to this article.

## Acknowledgments

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