IMMUNOHISTOCHEMICAL STUDY OF INTERLEUKIN 17 IN PATIENTS OF ORAL LICHEN PLANUS

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ABSTRACT

Background: Lichen planus may affect the skin, mucous membranes (especially the oral mucosa), scalp, nails, and genitalia. Interleukin-17 is considered as an important ‘bridging molecule’ between the adaptive and innate immunity.

Aim of the study: This study aimed to evaluate immunohistochemical study of tissue IL17 in patients of oral lichen planus in comparison to apparently healthy individuals regarding as control.

Patients and methods: twenty four patients clinically diagnosed as OLP were enrolled in this study, another 12 healthy control. Histopathological sections stained by H and E and immunohistochemical staining of IL17 was done on all patients and controls.

Results: The results of this study showed high immunoexpression of IL17 in diseased patients compared to few sporadic cells in the control.

Conclusion: IL-17 is included in the pathogenesis of OLP, this was confirmed by its presence in higher concentration in immunohistopathology of lesions of OLP more than controls.

Keywords: Oral lichen planus (OLP), Interleukin 17, immunohistochemistry

I. INTRODUCTION:

Oral lichen planus is a chronic inflammatory disease affecting the oral mucosa and may have an immunological background. It affects 0.5% to 2.6% of the world population with variation between different countries, more common in females between 30 to 60 years of age but may affect younger adult and children. OLP has symptoms that ranges from mild burning sensation to severe pain and discomfort affecting patient's quality of life (1).

Impairment in the function of regulatory T-lymphocytes, keratinocytes and cell matrix communication has been reported in OLP. Deficit in growth factors like transforming growth factor β and protiens like fibronectins have been also reported[2]

IL-17 has emerged as a central player in the immune system. Although produced primarily by T cells from the adaptive immunity, IL-17 also plays a crucial role in innate immunity by triggering the production of numerous chemokines, resulting in the recruitment of neutrophil and macrophage to clear pathogens. Thus, IL-17 is considered as an important ‘bridging molecular’ between the adaptive and innate immunity [3].

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In addition, IL-17 has pleiotropic effects on different tissue cells and immune cells. It was demonstrated that IL-17 stimulates the production of various inflammatory mediators such as TNF-α, IL-1β, IL-6, IL-8, MCP-1, GM-CSF and matrix metalloproteases (MMP) in monocytes, epithelial cells, endothelial cells, keratinocyte and fibroblasts. In this way, IL-17, together with its down-stream molecules, is involved in the formation and maintenance of the local inflammatory microenvironment[4].

Interleukin 17 detected by immunohistochemistry was overexpressed in OLP especially in reticular lesion more than in normal mucosa [5].

We aimed in this work to evaluate immunohistochemical study of tissue IL17 in patients of oral lichen planus in comparison to apparently healthy individuals regarding as control.

II. PATIENTS AND METHODS:

This work was a randomized control study including 36 person (24 patients and 12 control), twenty four patients with clinically diagnosed as OLP were enrolled in this study. Their age ranged from 30 to 72 years. There were 20 females (55.55%) and 16 males (44.44%), they came to outpatient clinics of dermatology, venereology and andrology department at zagazig university hospitals from May 2018 to May 2019. Patients with history of topical or systemic therapy for treating oral lichen planus in the past 4 weeks prior to the study, bleeding tendency, patients on anticoagulant therapy, anemia who had fungal infection as oral candidiasis, who had active oral herpes simplex or history of recurrent oral herpes simplex, and pregnant or lactating women were excluded from the study.

Ethical approval: Institutional Review Board (IRB) approval was taken from Zagazig University and also informed written consent was taken from patients and/or their caregivers. We performed this study with respect to (Declaration of Helsinki), ethics code of World Medical Association regarding human studies.

Biopsy and histopathological examination

- Incisional biopsy and histopathological examination of the lesion with immunohistochemical assessment of tissue interleukin 17 was done.

Biopsy:

Incisional biopsies from patients and control were taken surgically at dental department from the oral lesion.

Biopsies were taken under complete aseptic condition.

Biopsy handling:

Each specimen of both groups was fixed in formalin and embedded in paraffin to form paraffin blocks. Serial sections will be obtained from each block and stained with the following:

Haematoxylin and eosin (H&E) for histological diagnosis

Stained section was examined under light microscope for histopathological examination of the lesion.

Histopathological evaluation of OLP slides by H&E showing the characteristic features of oral lichen planus lesion in the form of:

- Liquifactive degeneration of the basal cells accompanied by apoptosis of keratinocytes and band like lymphocytic infiltrate at the interface between the epithelium and subepithelial connective tissue.

Immunohistochemical staining using monoclonal antibodies:

Sections 3–5 µm thick were cut from formalin-fixed, paraffin-embedded blocks, mounted on positive charged slides, were deparaffinized in xylene for 30 min and rehydrated in descending grades of alcohol. The slides were rinsed in distilled water for 5 min. The mounted sections were immersed and boiled in ready to use Dako target retrieval solution (PH 6.0) in a microwave for 20 min, and then washed in phosphate buffered saline (PBS) (pH 7.3). Thereafter, blocking of endogenous peroxidase activity with 3% H2O2 in methanol was carried out. The slides were then incubated overnight using primary antibodies of IL 17 (rabbit anti-human, Sigma St Louis, MO,U.S.A).
Incubation with horseradish peroxidase (HRP) conjugated anti-rabbit secondary antibody (Cat# K4010, Dako, Carpinteria, CA) and visualized using 3,3'-Diaminobenzidine (DAB) chromogen solution. Finally, sections were counterstained with Mayer’s hematoxylin and washed with distilled water and PBS. The slides were rinsed gently in an ascending grade of alcohol (2-5) min, then in xylene for 3 changes and finally mounted with a cover slip [6].

- Positive and negative controls were stained at the same setting with our selected slides.
- Evaluation of immunostaining was performed, and classified as following
  - 0 (no staining)
  - 1 (weak intensity)
  - 2 (medium intensity)
  - 3 (high intensity)

**Statistical analysis**

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 18.0.

### III. RESULTS

Demographic data of the studied groups were matched regarding age and sex.

The severity of immunohistochemical staining of IL17 was evaluated as following according to the pathologist experience: 0 (no staining), 1 (weak intensity), 2 (medium intensity) and 3 (high intensity), all cases in both groups had IL17 cytoplasmic staining.

There was a statistically significant difference between the Group A and Group B in the severity of IL-17 (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A (OLP) (n=24)</th>
<th>Group C (Control) (n=12)</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity of staining:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (no staining)</td>
<td>0</td>
<td>10</td>
<td></td>
<td>83.3</td>
</tr>
<tr>
<td>1 (weak intensity)</td>
<td>9</td>
<td>2</td>
<td>28.3</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>2 (medium intensity)</td>
<td>11</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3 (high intensity)</td>
<td>4</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure (1):** Immunohistopathology of normal oral mucosa (control) shows scanty cytoplasmic staining of IL17 in epidermal keratinocytes and absent inflammatory cells (immunostain ×200).
IV. DISCUSSION:

Oral lichen planus is a chronic disease and rarely undergoes spontaneous remission; it may cause significant pain and morbidity. OLP most commonly affects middle aged adults of both sexes, with a slight female predominance, and without any apparent racial predilection [7].

Histopathological confirmation is also important before starting any treatment, as a common cause of therapy failure is inappropriate diagnosis [8].

This study aimed at evaluation of immunohistochemical study of tissue IL17 in patients of oral lichen planus in comparison to apparently healthy individuals regarding as control.

In this study, Regarding Immunohistochemical study of IL 17 in OLP lesions (done on 36 subjects 24 OLP patients and 12 healthy controls) showed that there was statistically significant increase in immunoexpression of IL17 in OLP patients compared to few sporadic cells in the controls.

In similar study Lu et al. 2014, [5] had the same results that abundant IL-17 positive stainings in the cytoplasm of the infiltrated lymphocytes in the lesions of both erosive and reticular OLP, but only a few sporadic IL-17+ cells in the normal oral mucosa. In addition, erosive OLP lesions contained a significantly increased number of IL-17+ cells compared to the reticular OLP lesions.

In similar study done by Shen et al., [9] on 42 patients showed that IL 17 immunoexpression was higher in OLP patients compared to controls.

Similar results in both Lu et al [5] and Shen et al [9] and the present study clarify the high concentration of IL-17 immunoexpression in OLP lesions compared to control, so IL-17 may have important role in the pathogenesis of OLP.

In a DNA microarray study, IL-17A gene was identified to be up-regulated by over sevenfold in OLP lesions compared with the normal oral mucosa. Subsequently, IL-17+CD4+T cells, termed as Th17 cells, were found to present in OLP lesions, especially in atrophic–erosive form [10].

In addition Piccinni et al., 2014 [11] showed increased mRNA levels of IL-17 in both biopsies of erosive OLP lesions and infiltrated CD4+T-cell clones from erosive OLP lesions.

Another study showed elevated IL-17 concentrations in the saliva of patients with erosive OLP compared with reticular lichen planus and healthy controls. This could be linked to disease severity. Serum IL-17 concentrations were highest in erosive lichen planus, followed by nonerosive lichen planus and controls. It was also noted that the decreased oral bacterial diversity and richness correlated negatively with the IL-17 values. This loss of the normal oral bacteriome could pave the way for opportunistic pathogens, which can trigger the innate and adaptive immune systems, leading to a disease flare [12].

V. CONCLUSION

IL-17 is included in the pathogenesis of OLP, this was confirmed by its presence in higher concentration in immunohistopathology of lesions of OLP more than controls. This may lead dermatologists to try biological therapy targeting IL17 such as Secukinumab (Cosentyx) and ixekizumab (Taltz). Both are monoclonal antibody, they bind and inhibit IL-17A and IL-17A/F.
REFERENCES