Immunohistochemical Expression of Yes-Associated Protein-1 (YAP-1) in Cutaneous Lichen Planus

The Departments of Dermatology & Veneral Diseases* and Pathology**, Faculty of Medicine, Tanta University

Abstract

Background: Lichen planus is a chronic muco-cutaneous inflammatory disease that affects skin, mucous membranes, scalp, and nails. The exact pathogenesis of the disease remains unclear but cell-mediated cytotoxicity is regarded as a major mechanism of pathogenesis of lichen planus. Yes-Associated Protein (YAP-1) was originally identified in Drosophila as a key component of the Hippo pathway, which regulates cell proliferation, apoptosis and migration.

Aim of Study: The aim of this work was to study YAP-1 expression in cutaneous lichen planus.

Patients and Methods: This study was carried out on 20 patients with cutaneous LP and 10 normal skin specimens served as controls. All specimens were examined by H&E, and by immunohistochemistry for YAP-1 expression.

Results: There was statistically significant increase of YAP-1 expression in lesional skin of lichen planus patients in comparison to normal skin of healthy controls.

Conclusion: Increased expression of YAP-1 was found in cutaneous lichen planus in comparison to normal skin suggesting a possible role of YAP-1 in lichen planus pathogenesis.

Key Words: YAP-1 – Immunohistochemistry – Lichen planus.

Introduction

Lichen Planus (LP) is chronic inflammatory immune mediated disease that may affect the skin, mucous membranes, hair follicles or nails. Although its pathogenesis is still unknown, there is evidence that immunological mechanisms, infections, genetic predisposition, allergic reactions to drugs, psychogenic disorders and some chronic and endocrine diseases may play important roles [1].

Yes-Associated Protein-1 (YAP-1) was originally identified in Drosophila. Its molecular weight is 65kDa, so it is also called YAP65 [2]. It is a key component of the Hippo signaling pathway that plays an important role in organ size control, cell proliferation and apoptosis [3]. YAP-1 was originally identified and named by its ability to associate with the SH3 domain of the tyrosine kinase YES-1. In recent years, it has been found to be up-regulated in many malignant tumors, such as liver cancer, breast cancer, lung cancer, gastric cancer and colon cancer. Studies have shown that it may be through inhibiting apoptosis, regulating cell cycle, promoting the migration and invasion abilities and inhibiting stem cell differentiation that YAP is involved in the occurrence and development of many tumors, so it is considered as an oncogene [4].

Aim of the work was to study the immunohistochemical expression of YAP-1 in cutaneous lichen planus.

Patients and Methods

This study was conducted on 20 patients with cutaneous lichen planus and 10 healthy individuals served as controls. They were selected from the outpatient Clinic of Dermatology and Venereology Department, Tanta University Hospitals between February 2017 and January 2018. Ethical approval was obtained from Ethical Committee before the commencement of the study. All patients included in the study were either newly diagnosed or did not receive treatment at least one month before biopsy taking. Patients with any other dermatological disease and pregnant and lactating females where excluded from the study.

All participants were subjected to complete careful history taking, thorough general and dermatological examinations, routine laboratory in-
Immunohistochemical Expression of YAP-1 in Lichen Planus

Results

Clinical results: The clinical characteristics of the studied groups were summarized in Table (1).

Laboratory results: Routine laboratory investigations were normal. Results of hepatitis C tests were summarized in Table (1).

Haematoxylin & Eosin Results were illustrated in Figs. (1,2).

Immunohistochemical results were illustrated in Table (2) and Figs. (3,4).

A- Normal control skin specimens: YAP-1 expression was detected as weak expression (+1) limited to basal cell layer of normal epidermis in 12 specimens (60%), while the remaining 8 specimens (40%) were negative (Table 2, Fig. 3).

B- Lichen planus specimens: YAP-1 expression in the epidermis and dermis of cutaneous lichen planus specimens showed moderate expression (+3,+4) in 16 specimens (80%) and strong expression (+6,+9) in 4 specimens (20%). (Table 2, Fig. 4).

There was statistically significant increase of YAP-1 expression in cutaneous lichen planus patients when compared to normal skin control ($p<0.001$), (Table 2).

Table (1): Comparison between the two studied groups according to demographic data.

<table>
<thead>
<tr>
<th></th>
<th>Lichen Planus Group (n=20)</th>
<th>Control Group (n=10)</th>
<th>Test of $\chi^2$- sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>5</td>
<td>50.0</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>5</td>
<td>50.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. - Max.</td>
<td>30-55</td>
<td>30.0-60.0</td>
<td>F= 0.168</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>47.60±8.62</td>
<td>44.80±9.80</td>
<td>1.842</td>
</tr>
<tr>
<td>Median</td>
<td>50</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Smoker</td>
<td>4</td>
<td>20.0</td>
<td>6.667</td>
</tr>
<tr>
<td>Duration (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min. - Max.</td>
<td>Few days 13 ys</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.20±4.52</td>
<td>1.821</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.50</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>10.0</td>
<td>8.523 *</td>
</tr>
<tr>
<td>Hapatitis C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>20.0</td>
<td>3.069 *</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
<td>80.0</td>
<td>13.303 &lt;0.001 *</td>
</tr>
</tbody>
</table>

*: Statistically significant at $p<0.05$.

Table (2): Comparison between the two studied groups according to intensity of YAP-1 immunohistochemical expression.

<table>
<thead>
<tr>
<th>Intensity of YAP-1 Expression</th>
<th>Lichen Planus specimens (n=20)</th>
<th>Normal skin specimens (n=10)</th>
<th>Test of sig.</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. - Max.</td>
<td>3.0-9.0</td>
<td>0.0-1.0</td>
<td>H=</td>
<td>0.007 *</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.0±2.0</td>
<td>0.60±0.52</td>
<td></td>
<td>24.441*</td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MCP: $p$-value for Monte Carlo correction of Chi square test for comparing between the two groups.

*: Statistically significant at $p<0.05$. 

Staining pattern: Five fields at (400 magnification) were randomly chosen for each section. The cells with clearly defined staining compared with the positive control were counted, and the count was divided by the total number of cells in each field. The mean of the five fields was estimated for each sample. Each field was evaluated for the proportion of stained cells and staining density. The proportion of stained cells was assessed staining intensity was graded as follow:

• 0 (absent): No stained cells.
• +1 (mild): <25% stained cells.
• +2 (moderate): 25-50% stained cells.
• +3 (strong): >50% stained cells.

Statistical analysis: Statistical presentation and analysis of the present study was conducted, using mean value and standard deviation and chi-square test by SPSS V.16. A significant difference was considered at $p\leq0.05$.

vestigations, written informed consent and digital photographing of the lesions. Then, punch skin biopsies (4mm) were obtained from cutaneous lichen planus lesions. In addition, normal skin specimens from matched healthy individuals will be obtained during plastic operation to serve as control. Specimens were fixed in formalin and imbedded in paraffin blocks and 2 sections were prepared from each block for:

1- Haematoxylin & Eosin staining to confirm the clinical diagnosis.

2- YAP-1 immunohistochemical staining using the standard streptavidin-peroxidase complex immunohistochemical method. Primary antibody: Rabbit Monoclonal anti Yap-1 antibody (catalogue No, GTX633541, from lab vision, Neo-markers) and at used at 1:100 dilution. Secondary antibody: Goat anti-rabbit IgG and used according with the manufacturer’s instructions.

Laboratory investigations, written informed consent and digital photographing of the lesions. Then, punch skin biopsies (4mm) were obtained from cutaneous lichen planus lesions. In addition, normal skin specimens from matched healthy individuals will be obtained during plastic operation to serve as control. Specimens were fixed in formalin and imbedded in paraffin blocks and 2 sections were prepared from each block for:

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Results

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Haematoxylin & Eosin Results were illustrated in Figs. (1,2).

Immunohistochemical results were illustrated in Table (2) and Figs. (3,4).
Fig. (1): Lichen planus specimen showed orthokeratosis, hypergranulosis, acanthosis, saw toothed rete ridges, and hydrobic degeneration of basal cells. The upper dermis showed band like inflammatory infiltrate, (H&E x 100).

Fig. (2): Hypertrophic lichen planus specimen showed hyperkeratosis, hypergranulosis irregular acanthosis and papillomatosis. (H&E x 200).

Fig. (3): Normal control skin specimen showing (+1) mild immunohistochemical expression of YAP-1 in the epidermis, (immunohistochemistry x 200).

Fig. (4): Lichen planus specimen showing (+3) strong immunohistochemical expression of YAP-1 in both the epidermis and dermis, (immunohistochemistry x 200).

Discussion

In this study, the incidence of lichen planus was higher in females (70%) than males (30%) with a ratio 7:3. This result was in agreement with the result of Shiohara et al., [5] who reported that, the incidence of lichen planus was higher in females than males. However, Gorouhi et al., [6] recorded no sex predilection among lichen planus patients.

In the present study, the age of lichen planus patients ranged from 30 to 55 years. The high incidence of lichen planus among adults in our study was in agreement with the result of Black MM [7]. On the other hand, Pandhi et al., [8] reported lichen planus in childhood.

In this study, there is no significant relation between smoking and development of lichen planus. This result was in agreement with the result of Mansur et al., [9].

In the present study, the duration of lichen planus ranged from few days to 13 years. This result was in agreement with the result of Parihar et al., [10] who reported that the duration of lichen planus ranged from few days after diagnosis to several years.

In this work, positive family history of lichen planus was detected in only 10% of patients. This result matches with that of James L et al., [11] who found that lichen planus occurs in up to 10% of first-degree relatives of affected patients. In contrast Sandhu et al., [12] reported that there was no significant relation between family history and development of lichen planus.

The current study revealed that the most common clinical type among cutaneous lichen planus patients was classic LP with a percentage 20% of all studied patient with lichen planus followed by hypertrophic (15%), atrophic (10%) and actinic...
Our results were in accordance to Bhattacharya et al., [13] who suggested that the classic type of lichen planus was the most commonly observed.

In the current study, HCV antibody was positive in 16 patients (80%) which may insulate the relation between HCV infection and the development of lichen planus. This result matches with that of Bagan et al., [14] who found that the prevalence of HCV antibodies in patients with mucosal and cutaneous lichen planus is significantly higher than that in control populations. However, Cribier et al., [15] could not demonstrate a statistically significant association between HCV and lichen planus.

In the current study, the immunohistochemical expressions of YAP-1 in normal control skin specimens were weakly detected as nuclear and cytoplasmic expression limited to the basal cell layer of epidermis. On the other hand, moderate and strong YAP-1 immunohistochemical expressions were detected in the epidermis and dermis of cutaneous lichen planus specimens. YAP-1 expression was up-regulated in cutaneous lichen planus specimens in comparison to normal control skin.

Our results were in agreement with Jia et al., [16] who evaluated expression of YAP in cutaneous lichen planus lesions in comparison to healthy controls and found that YAP-1 up-regulated in LP patients (p<0.05) [16].

The role of YAP-1 in lichen planus is still unclear. YAP-1 protects cells against apoptosis through YAP-1 regulated apoptosis factors [17]. Since apoptosis is very important mechanism in lichen planus pathogenesis; as evidenced by cytotoxic T-cell infiltration into the epidermis with subsequent hydropic degeneration of basal cells [18], it is likely to suggest that YAP-1 may lay a role in disease pathogenesis through facilitation of basal keratinocyte apoptosis.

From this study, it could be concluded that: Immunohistochemical expression of YAP-1 was increased in patients with lichen planus when compared to controls. This may suggest a possible role of YAP-1 in disease pathogenesis and may open new insights for effective therapeutic approaches for lichen planus.

References


التعبير المناعي الكيميائي لبروتين (YP1)
في الحزاز المنبسط الجلدي

مقديمة البحث: بعد الحزاز المنبسط مرض مناعي مزمن غير معلوم السبب قد يصيب الجلد أو العضلات الداخلية أو كلاهما معاً ويجد عوامل كثيرة قد تساعد في حدوثه كالعوامل المناعية والوراثية وأيضاً البيئية. وله من المثير تحوّل الحزاز المنبسط إلى سرطان في الخلايا الحرشفية غير معتمداً على نوع الجلد أو حتى طريقة علاجه.

لقد تم اكتشاف الحزاز 1 في درسوفييا ويجب أن له دور كبير في تكاثر الخلايا وهجرتها وأيضاً في وفاتها. حيث يتم تنشيطه في نواة الخلايا الحرشفية ويزداد نشاطه لذلك قد أتى بإشرافه في الخلايا الحرشفية للجلد وفي الفم.

الهدف من البحث: إن الهدف من هذه الدراسة هو تقييم مستوى الحزاز 1 في عينة جلدية مأخوذة من مرضى مصابين بالحزاز المنبسط الجلدي والقارور بالمقارنة بالأشخاص غير مصابين.

المرضى وطريق البحث: لقد أجريت هذه الدراسة على عشرين فرد مصاب بمرض الحزاز وعشرين آخر مصاب بسرطان الخلايا الحرشفية بالإضافة إلى عشرين فرد كمجموعة ضابطة.

خضعت المجموعات موضوع الدراسة إلى:
- أخذ التاريخ المرضي كامل.
- فحص عام شامل وفحص جلدي.
- عمل التحاليل الروتينية وتحليل فورسات.
- صبغ العينات بصبغ البيبيتونكسلين والألوين.
- صبغ العينات بصبغة مناعية نسيجية كيميائية لتعبير الحزاز 1.

خلاصة نتائج الدراسة الحالية كانت على النحو التالي:

ارتفاع مستوى الحزاز 1 في أنسجة مرضى الحزاز عن فردي مصاب بالمرض السلوكي المضابط.