Expression of COX-2 and Bcl-2 in 50 patients of Lichen Planus

Gunjot Kaur, KS Chahal, RK Sharma, Arun Puri and Permeet Kaur Bagga

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Abstract
Lichen Planus is subacute or chronic inflammatory immune mediated disease that may involve skin, mucous membranes, hair follicles and nails. Lichen Planus is characterized by epidermal thickening, liquefaction, degeneration of basal cell layer, appearance of civatte bodies and dense lymphocytic infiltrate in a band like pattern along dermoepidermal junction. Bcl-2 plays important role in cellular protection against apoptosis. COX-2 plays important role in inflammation, tumor growth, invasion and inhibition of apoptosis. In this study 50 cases of clinically and histopathologically proven Lichen Planus were studied for expression of Bcl-2 and COX-2 by immunohistochemical staining. The results were compiled and analysed statistically.

Keywords: Lichen Planus, Bcl-2, COX-2

Introduction
Normal skin constitute of epidermis, epidermal basement membrane, papillary, reticular dermis with related adnexa, subcutis, subcutaneous fat. The skin has limited number of reaction patterns with which it can respond to various pathological stimuli, clinically different lesions may show similar histological patterns. [1]

Lichen Planus is a subacute or chronic dermatosis that may involve skin, mucous membranes, hair follicles and nails. [2] The name “lichen” refers to the lichen plant which grows on rocks or trees, and “planus” means flat. Cutaneous lichen planus affects men and women equally, but oral lichen planus affects women twice as often as men. Lichen planus happens most often in middle aged adults. Lichenoid reactions are clinically and histologically similar to lichen planus with identifiable etiological factors.

Lichen Planus is characterized by shiny, itchy, violaceous, flat-topped polygonal papules which retain the skin lines and which vary in size from pinpoint to a centimetre or more and they may be closely aggregated or widely dispersed. White lines, known as Wickham's striae [3] may traverse the surface of the papules. Linear lesions often appear along scratch marks or in scars (Koebner phenomenon), while annular lesions may be formed either by groups of papules arranged in rings or by single, large papules clearing in the centre and leaving an active margin.

Malignant transformation of LP remains a very controversial issue. [4] Some reported cases diagnosed originally as LP on clinical and histological grounds were probably epithelial dysplasia (lichenoid dysplasia) that progressed subsequently to overt squamous cell carcinoma (SCC). [5] The OLP lesions are consistently more persistent than the dermal lesions and have been reported to carry a risk of malignant transformation to oral squamous cell carcinoma (OSCC) of 1-2% (reported range of malignant transformation 0-12.5%). [6]

There is a contributory role for apoptosis associated proteins in the pathogenesis of lichen planus. The relation between the two representative histological features of lichen planus (i.e. the liquefaction degeneration and civatte bodies) and apoptosis has been reported and suggested that the civatte bodies represent non-phagocytised apoptotic cell fragments. COX-2 enzyme is not present in healthy tissues. It is an inductive enzyme with a considerably increased production during pathologic conditions such as malignancies and inflammation. [7] The cyclooxygenases (COXs) are a family of enzymes, which catalyze the rate limiting step of prostaglandin biosynthesis. This family of enzymes contains three members: ubiquitously expressed COX-1, which is involved in homeostasis; the inducible
COX-2 isoform, which is mainly up regulated during both inflammation and cancer. COX-2 modulates cell proliferation and apoptosis mainly in solid tumors, that is, colorectal, breast, and prostate cancers, and, more recently, in hematological malignancies. Overexpression of COX-2 is important in tumor growth, invasion, metastasis, angiogenesis and inhibition of apoptosis. COX-2 acts independently of Bcl-2 and in some tumors like lymphoma while it is Bcl-2 dependent in some others. The Bcl-2 oncogene is located at chromosome 18q21 but the translocation (14:18) juxtaposes the gene to the immunoglobulin heavy chain at chromosome 14. This creates the so called bcl-2/ IgH fusion gene resulting in high level of bcl-2 protein. The Bcl-2 protein is found to be located in the periphery of mitochondria on the perinuclear membrane and the endoplasmic reticulum. Bcl-2 was the first anti-apoptotic gene to be identified, is a member of large family of homodimerizing and heterodimerizing protein, some of which inhibits apoptosis such as bcl-2, bcl-xl,mcl-1 where as others favour apoptosis such as bax, bad, bak, bcl-x58. The family of gene contains two functionally antagonistic groups:

1. Cell death suppressors as bcl-2, bcl-x and mcl-1
2. Cell death promoters such as bax, bcl-xl, bak and bad.

Bcl-2 protein expression blocks apoptotic cell death. The over expression of bcl-2 protein suppresses the DNA fragmentation that occurs in apoptosis and is associated with decreased level of cytosolic calcium and increased level of mitochondrial calcium. This indicates the role of bcl-2 protein in the regulation of intracellular Ca2+ distribution. Bcl-2 protein expression is mainly observed in cell populations with a long life and/or proliferating ability such as duct cells in exocrine glands, basal keratinocytes, cells at the bottom of colon crypts, and neurons. In the skin of both adult and embryo and also embryonic kidney and cartilage, bcl-2 expression was observed in cells which were undergoing morphological transition from undifferentiated stem cells to committed precursor cells. These observations support the view that the bcl-2 gene may have an important role in cell development, maturation, and the path to terminal differentiation.

Bcl-2 is anti-apoptotic gene; it prolongs the survival of cells in the absence of required growth factors by inhibiting apoptosis. Low expression of this protein promotes apoptosis of basal keratinocytes and increased expression inhibits apoptosis in lymphocytes that strengthens the cell mediated immune process.

The IHC score is calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score) as:

Staining Intensity Score:

Score 0- Negative
Score 1- weak
Score 2- moderate
Score 3- strong

Quantity Score:

Score 0- No staining
Score 1- 1-10% positive cells
Score 2- 11-50% positive cells
Score 3- 51-80% positive cells
Score 4- 81-100% positive cells

Subjects and Methods

The present study was conducted on 50 patients of lichen planus. Biopsy was taken from the area involved (lesional area) in the Department of Dermatology, Government Medical College Amritsar.

Technique

All biopsies taken were fixed in buffered formalin 10% and embedded in paraffin. Tissue sections were first stained with routine haematoxylin and diagnosis was made. Then IHC staining was done as follows:

Immunohistochemical demonstration of Bcl-2 and COX-2 in formalin fixed and paraffin embedded tissue section of cases using primary antibody to Bcl-2 and COX-2.

Material

1. Primary antibody- mouse monoclonal antihuman, clone 124, clone 4H12, USA/DAKO/Novoceastra/Biosystems.
3. PBS phosphate buffer saline
4. Positive control sections as per specification of kit
5. Negative control sections will be provided by omission of primary antibody.
6. Target antigen retrieval as per specification of kit
7. Avidin Biotin method of IHC

Method

1. 3-5 sections were cut mounted on slides. Slides were dried overnight at 37c, dewaxed in xylene and hydrated through descending concentrations of alcohol.
2. Endogenous peroxidase activity was blocked by adding freshly prepared 0.3% H2O2 in methanol for 10 minutes followed by three washing of in PBS.
3. Antigen retrieval was done as specification of kit.
4. Enzyme conjugate was added and incubated for 30 minutes.
5. One table of DAB was dissolved in reagent and incubated on the sections for 6-8 minutes.
6. Sections were washed with deionised water for 3 minutes.
7. Haematoxylin counterstaining was done for 2-5 minutes and dehydration was done through ascending concentration of alcohol.
8. Excess water was removed and mounting was done.
9. The most frequent site of immunolabeling for Bcl-2 is at band like infiltrate at dermoeipidermal junction and in submucosa and for COX-2 is band line inflammatory infiltrate and in the basal layer and in the submucosa.

Results and interpretation of IHC Staining

Positive control tissue showed coloured end product at the site of target antigen.

Negative control tissue sections did not have the above colour since there was no antigen antibody reaction.

Figure 1-4 shows results of IHC.

Results

The present study was conducted on 50 patients of Lichen Planus, received in the Department of Pathology,
Bcl-2 immunohistochemistry

**Qualitative and quantitative score of Bcl-2 in keratinocytes**

According to IHC scoring system, qualitative score of Bcl-2 in basal layer keratinocytes was calculated. Out of 50 cases, 29 cases showed score 1 (weak staining), 12 cases showed score 2 (moderate staining), 8 cases showed score 0 (no staining) and score 3 (strong staining) is seen in 1 case. Quantitative score of Bcl-2 in basal layer keratinocytes was calculated on the basis of percentage of stained keratinocytes. 21 cases showed score 1-10% positive cells, 14 cases showed score 2-11-50% positive cells. 8 cases showed score 0- no cell staining. 7 cases showed score 3-51-80% positive cells.

**Qualitative and quantitative Bcl-2 score in dermal lymphocytes**

According to IHC scoring system, qualitative score of Bcl-2 in dermal lymphocytes was calculated. Out of 50 cases, 29 cases showed score 1 (weak staining), 13 cases showed score 2 (moderate staining), 1 case showed score 0 (no staining) and score 3 (strong staining) is seen in 4 case. Quantitative score of bcl-2 in dermal lymphocytes was calculated on the basis of percentage of stained dermal lymphocytes. 28 cases showed score 1-10% positive cells. 18 cases showed score 2-11-50% positive cells. 1 case showed score 0- no cell staining. 3 cases showed score 3-51-80% positive cells.

<table>
<thead>
<tr>
<th>Qualitative Bcl-2 score in Keratinocytes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Chi-square value</td>
</tr>
<tr>
<td>Negative</td>
<td>41.814</td>
</tr>
<tr>
<td>Weak</td>
<td>12.5%</td>
</tr>
<tr>
<td>Moderate</td>
<td>7</td>
</tr>
<tr>
<td>Strong</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Table 1: Shows comparison between Qualitative Bcl-2 score in keratinocytes and Qualitative Bcl-2 score in dermal lymphocytes

Table 2: Shows comparison between Quantitative Bcl-2 score in keratinocytes and Quantitative Bcl-2 score in dermal lymphocytes

Table 3 shows comparison between Qualitative Bcl-2 score in keratinocytes and Quantitative Bcl-2 score in dermal lymphocytes whereas Table 4 shows comparison between Quantitative Bcl-2 score in keratinocytes and Quantitative Bcl-2 score in dermal lymphocytes.

**COX-2 immunohistochemistry**

**Qualitative and quantitative COX-2 score in keratinocytes**

According to IHC scoring system, qualitative score of COX-2 in keratinocytes was calculated. Out of 50 cases, 30 cases showed score 1 (weak staining), 17 cases showed score 2 (moderate staining), 2 cases showed score 0 (no staining) and score 3 (strong staining) is seen in 1 case. Quantitative score of COX-2 in basal layer keratinocytes was calculated on the basis of percentage of stained keratinocytes. 23 cases showed score 1-1-10% positive cells. 17 cases showed score 2-11-50% positive cells. 2 cases showed score 0- no cell staining. 8 cases showed score 3-51-80% positive cells.

**Qualitative and quantitative COX-2 score in dermal lymphocytes**

According to IHC scoring system, qualitative score of COX-2 in dermal lymphocytes was calculated. Out of 50 cases, 29 cases showed score 1 (weak staining), 21 cases showed score 2 (moderate staining). Quantitative score of COX-2 in dermal lymphocytes was calculated on the basis of percentage of stained dermal lymphocytes. 26 cases showed score 1-1-10% positive cells. 24 cases showed score 2-11-50% positive cells.

(Table 3 shows comparison between Qualitative COX-2 score in keratinocytes and Qualitative COX-2 score in dermal lymphocytes whereas Table 4 shows comparison between Quantitative COX-2 score in keratinocytes and Quantitative COX-2 score in dermal lymphocytes).
Table 3: Shows comparison between Qualitative COX-2 score in keratinocytes and Qualitative COX-2 score in dermal lymphocytes

<table>
<thead>
<tr>
<th>Qualitative COX-2 score in keratinocytes</th>
<th>Qualitative COX-2 score in dermal lymphocytes</th>
<th>Total</th>
<th>Chi-square value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Weak</td>
<td>2</td>
<td>100.0%</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>Weak</td>
<td>0</td>
<td>0.0%</td>
<td>5</td>
</tr>
<tr>
<td>Moderate</td>
<td>Strong</td>
<td>17</td>
<td>100.0%</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 4: Shows comparison between Quantitative COX-2 score in keratinocytes and Quantitative COX-2 score in dermal lymphocytes

<table>
<thead>
<tr>
<th>Quantitative COX-2 score in keratinocytes</th>
<th>Quantitative COX-2 score in dermal lymphocytes</th>
<th>Total</th>
<th>Chi-square value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No staining</td>
<td>1-10% positive cells</td>
<td>2</td>
<td>100.0%</td>
<td>13</td>
</tr>
<tr>
<td>11-50% positive cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51-80% positive cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2</td>
<td>100.0%</td>
<td>23</td>
</tr>
</tbody>
</table>

Discussion

In the study, we have made an effort to study the immunohistochemical expression of Bcl-2 and COX-2 in epidermal keratinocytes and dermal lymphocytes of lichen planus. The age and gender most affected in our study were women and the age group of 31-50 years. This was in accordance with Ahmad M et al. 2003 [9] who conducted study on 20 patients and observed 15 patients between 3rd to 4th decade with a maximum incidence in 3rd to 4th decade. Unbar TD et al. 2011 [10] and associates in their study conducted on 50 patients found the most of patients with lichen planus were in the age group of 20-50 years. Cutaneous Lichen planus occurs at similar frequencies in men and women, but women are likely to develop oral lichen planus and lichen planopilaris. Present study is in accordance to Soma Susan Varghese and colleagues 2016 [11] conducted study on 122 patients of LP and found more prevalence in females than males. McCartan and Healy 2008 [12] identified 45 studies and concluded the predominance in women to men is 3:1. In our study duration of disease of all the cases is up to 3 years. Largest group of patients is less than 1 year followed by less than 6 months followed by more than 1 year up to 3 years. This was in accordance with Anbar TD et al. 2011 [10] who conducted study on 50 patients of lichen planus and showed the duration of disease varied from 1 month to 3 years. The most common site of involvement in our study is buccal mucosa followed by scalp, limbs, back, abdomen, forehead, palm, buttock, foot, forehead, knee, thigh, waist etc.

Bcl-2 score

In the present study Bcl-2 scoring in epidermal...
keratinocytes and dermal lymphocytes was done both quantitatively and qualitatively. This study is in accordance with Abdel-Latif AM et al. (2009) [13] studied 25 biopsy specimens (15 cutaneous lichen planus and 10 oral lichen planus) using immunochemical methods for expression of Bcl-2 protein. Bcl-2 was expressed in inflammatory infiltrate in 15 cases of lichen planus (60%), showing mild expression in 12 cases (48%). Gonzalez-Moles MA et al. (2006) [14] studied the expression of bcl-2 in 51 patients with OLP and 26 controls for immunohistochemical analysis to quantify expression of the protein under study (−:0%, +:<10%, ++:10-25%, +++:26-50%, ++++:>50% positive cells). Bcl-2 was expressed in inflammatory infiltrate in 34 cases (72.3%) and was positive in <25% lymphocytes in 14 of these (29.7%). Rahman AH et al. (2008) [15] performed on ten histologically diagnosed lichen planus (group 1) together with ten oral mucosa biopsies taken from normal healthy subjects as controls(group 2). Bcl-2 protein was weakly expressed in 3/10 (30%) of group 1 cases in a little number of basal and suprabasal keratinocytes. Nofal E et al. (2008) [16] conducted study on 10 patients with generalized cutaneous lichen planus. Five control specimens were taken from healthy subjects. Dermal lymphocytes were negative for Bcl-2 expression in all specimens of control skin. On the other hand, dermal lymphocytes showed strong Bcl-2 expression in all cases of lichen planus. As observed in the current study results, the maintenance of a band-like lymphocytic inflammatory infiltrate across the entire corium-epithelial junction, as observed in LP, may be associated with the overexpression of the Bcl-2 protein and the capacity that it imprints on these cells to maintain an inflammatory response without undergoing apoptosis. This inflammatory response passes through the production of COX-2 and its metabolic products derived from arachidonic acid, not only in the infiltrate, but along the basal layer, and even in upper-epithelial layers. Both molecules have also been associated with genetic alterations that lead to malignancy. The Bcl-2 protein expressed in the infiltrating lymphocytes may enable them to escape from apoptosis and prolong survival. The low expression of bcl-2 promotes apoptosis of basal keratinocytes. Quantitative score of COX-2 in basal layer keratinocytes was calculated on the basis of percentage of stained keratinocytes. Cortes DA et al. 2010 [17] studied 40 samples from oral lichenoid diseases patients and classified according to their clinical (C1-papular lesions, C2-papular and other lesions) and histological features and immunohistochemical procedure was performed for COX-2 expression. Epithelial COX-2 over expression was observed in 24 cases (54.5%) and inflammatory COX-2 over expression was observed in 14 cases (31.8%). The aim of study was to analyze the COX-2 expression in different subtypes of oral lichenoid diseases because of its potential to be the marker of altered behaviour. Alven J Arreaza 2014 [18] compared the expression of bcl-2 and cox-2 in oral lichen planus and oral lichenoid reactions. 65 cases were studied and 34 were diagnosed as oral lichenoid reactions and 31 as oral lichen planus. Bcl-2 and COX-2 expression was studied. Results showed that 18/34 (53%) samples of oral lichenoid reactions were positive for COX-2 whereas 25/31 (81%) cases of oral lichen planus were positive for COX-2. Bcl-2 protein expression 26/34 (76%) were positive in oral lichenoid reactions and 30/31 (97%) were positive in oral lichen planus. COX-2 overexpression is an early event in epithelial carcinogenesis, postulating that the increased levels of COX-2 and EGFR (epidermal growth factor) in premalignant lesions constitute a mechanism for ‘field carcinogenesis’. Qualitative comparison between Bcl-2 scoring in keratinocytes and dermal lymphocytes was done and the difference was found in weak category and overall the p value was significant (p<0.05). Quantitative comparison between Bcl-2 scoring in keratinocytes and dermal lymphocytes was done and the difference was found in 1-10% and p value was significant (p<0.05). Similar comparisons were done in qualitative category of COX-2 expression in dermal lymphocytes and keratinocytes and also the quantitative category and p value was significant is the former sub-category (tables 3 and 4).

Conclusion

Bcl-2 molecule might not have a significant role to play in the anti apoptotic mechanism in lichen planes epidermis. However in the dermal lymphocytes, the overexpression of Bcl-2 by its anti apoptotic action strengthen the cell mediated immediate immunity leading to increased lymphocyte survival which maintains chronic prolonged inflammation in lichen planus thus may have an important role in maintenance of lesions of lichen planus. Enhanced COX-2 expression is correlated to clinical severity and malignant transformation.

COX-2 is related to epithelial destruction and activation of carcinogenesis mechanisms via continuation of inflammation.

References


