ORIGINAL ARTICLE

Histopathological analysis of apoptotic cell count and its role in oral lichen planus

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ABSTRACT

Apoptosis is a process of genetically programmed cell death by which senescent, DNA-damaged and diseased cells are eliminated from the body. **Aim of the Study:** To identify and count the number of apoptotic cells in oral lichen planus (OLP) and correlate with the degree of keratinization, thickness of epithelium and thickness of lymphocytic infiltration of OLP. **Materials and Methods:** The study comprised 40 diagnosed cases of OLP. Sections were stained with hematoxylin and eosin to identify and count the number of apoptotic cells. Measurement of other histopathological parameter of OLP such as degree of keratinization, thickness of epithelium and thickness of lymphocytic infiltration was done by using stage micrometer and eyepiece graticule. Statistical analysis was done to understand the correlation between apoptotic cells and histopathological features of OLP. **Result:** The result showed that the number of apoptotic cells increased, with an increase in thickness of lymphocytic infiltration and degree of keratinization, but there was a decrease in the epithelial thickness. **Conclusion:** Further immunological and molecular studies are required for a stronger evidence in correlating apoptotic cell and histological parameters of OLP.

Key words: Apoptosis, oral lichen planus, keratinization, lymphocytic infiltration

INTRODUCTION

In recent years, an increase in the autoimmune disorders of mucocutaneous origin is noticed. Among the several diseases of this nature, lichen planus is one of the commonest.

Oral lichen planus (OLP) is a lymphocyte-mediated immunologic disorder in which the basal cells appear to be the targeted by T-lymphocyte. Lichen planus is histopathologically characterized by liquefaction degeneration of the basal layer associated with band-like inflammatory cell infiltration in the superficial lamina propria. The disease is lymphocyte-mediated; local release of cytokines is thought to interfere with lymphocyte function, as well as promote apoptotic death of basal keratinocyte.[1]

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/0973-029X.131900

Apoptosis is the process of elimination of cells that have completed their life cycle or on genetic damage have become useless or harmful for the organism.[2] Signaling for apoptosis occurs through multiple independent pathways that are initiated either from triggering event within the cell or from outside the cell by ligation of death receptor. All apoptosis-signaling pathways are activated by a family of cysteine protease (caspase) resulting in the formation of apoptotic bodies.[3]

It is reported that the basal cell degeneration that is an important histopathological finding observed in lichen planus is mediated through the process of apoptosis.[4] Only few studies are available in the literature, regarding the relation between apoptosis and other histopathological feature of OLP. So the present study is conducted to know the frequency of occurrence of apoptotic cells and their correlation with the different histopathological features of lichen planus.

MATERIALS AND METHODS

Forty cases of clinically and histopathologically diagnosed OLP were taken for the study. Sections of 4 μm were made from the archival wax blocks. Sections were stained with hematoxylin and eosin for histopathological identification of apoptotic bodies [Figure 1] and assessment of other

histopathological parameters such as amount of keratinization, thickness of epithelium, number of apoptotic bodies and lymphocytic infiltration [Figure 2].

Apoptotic cells were identified at the basal layer based on following criteria put forward by Bloor *et al*.[5]

- Cell shrinkage
- Chromatin condensation at the periphery of the nucleus
- Uniform eosinophilic cytoplasm
- Nuclear and/or cytoplasmic fragmentation
- Nuclear pyknosis
- Nucleolar disintegration.

Total number of apoptotic cells were counted and recorded, simultaneously the same area was measured for other histopathological parameter by using stage micrometer and eyepiece graticule. The obtained values were subjected to statistical analysis using Karl Pearson's correlation coefficient test.

RESULT

In the present study, mean, range, standard deviation, minimum and maximum number of apoptotic cells in relation to epithelial thickness, lymphocytic infiltration and amount of keratinization in OLP are as shown in Table 1.

The correlation between the number apoptotic cell and the epithelial thickness (P = 0.000) is highly significant [Figure 3]. The correlation of the number of apoptotic cell with lymphocytic infiltration (P = 0.039) is statistically significant [Figure 4]. The correlation of the number of apoptotic cell with amount of keratinization (P = 0.189) is statistically not significant [Figure 5] [Table 2].

DISCUSSION

OLP is a chronic inflammatory condition that affects the oral mucous membrane with a variety of clinical presentation

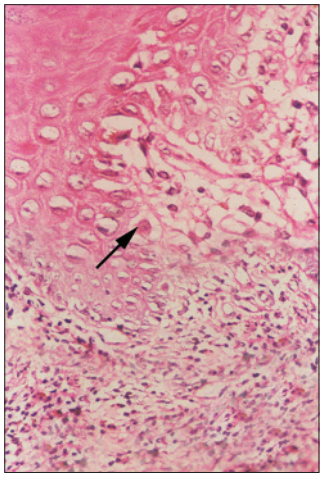

Figure 1: Photomicrograph of Oral lichen planus showing apoptotic cells (H&E stain, x400)

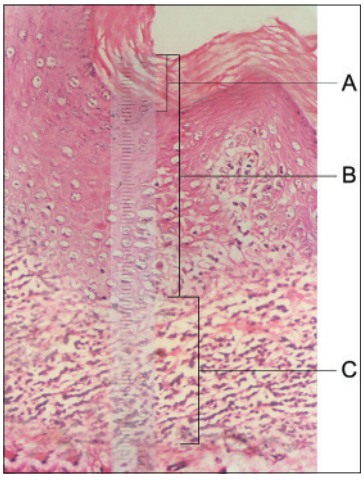

Figure 2: Photomicrograph of Oral lichen planus. (a) Degree of keratinization. (b) Epithelial thickness. (c) Thickness of lymphocytic infiltration (H&E stain, x200)

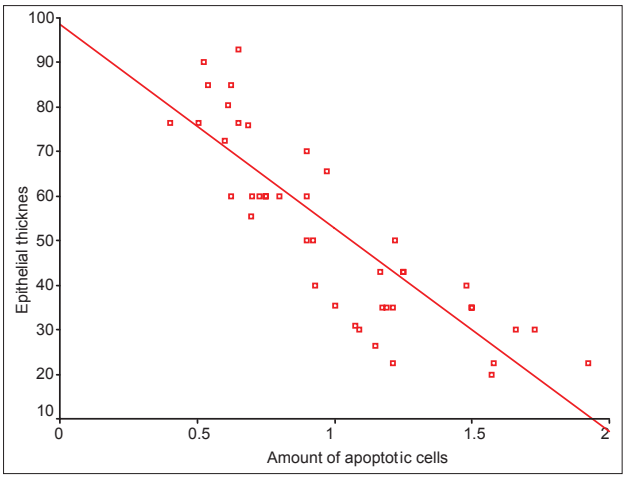

Figure 3: Relation between the apoptotic cell and the thickness of the epithelium

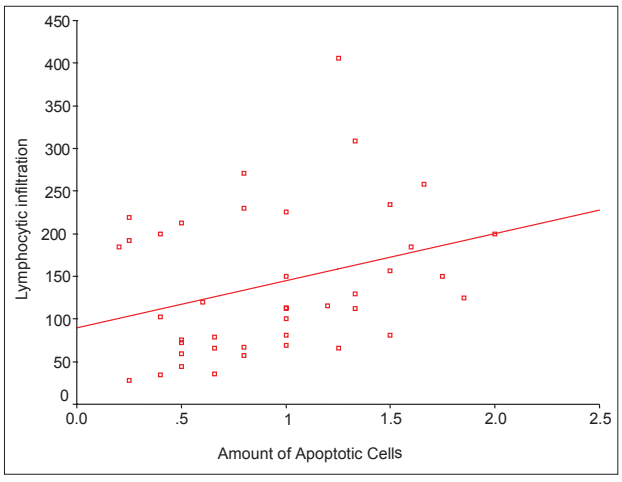

Figure 4: Amount of apoptotic cell increase along with increase in lymphocytic infiltration

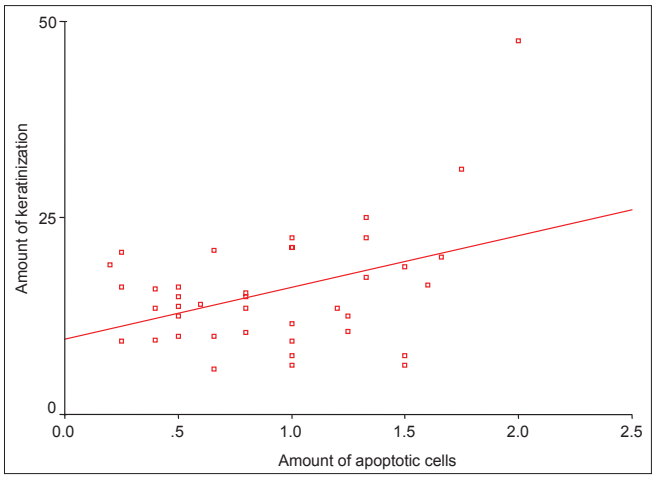

Figure 5: Relation between the apoptotic cell and the keratinization of the epithelium

Table 1: Mean, range and standard deviation of number of apoptotic cell, epithelial thickness, lymphocytic infiltration and amount of keratinization in OLP cases

	Number of apoptotic cell per micrometer area	Epithelial thickness in micrometer	Thickness of lymphocytic infiltration in micrometer	Degree of keratinization in micrometer
Mean	0.9157	49.6095	140.07	15.647
Standard deviation	0.4707	20.278	85.466	7.6702
Range	1.80	76.25	378.12	41.675
Minimum	0.20	20.00	28.12	5.825
Maximum	2.00	96.25	406.25	47.5

OLP: Oral lichen planus

Table 2: Statistical analysis of comparison between number of apoptotic cell and other histopathological parameters in OLP cases

	r value	*P* value	Significance
Number of apoptotic cells versus epithelial thickness	−0.880	0.000	Highly significant
Number of apoptotic cells versus lymphocytic infiltration	0.327	0.039	Significant
Number of apoptotic cells versus degree of keratinization	0.212	0.189	Nonsignificant

$P \leq 0.05$; OLP: Oral lichen planus

including reticular, papular, plaque-like, atrophic and ulcerative lesions. OLP affects about 0.1-4% of the population, it is a disease of the middle-aged and is more common among women.[6]

A large body of evidence supports a role for immune dysregulation in the pathogenesis of OLP, specifically involving the cellular arm of the immune system. The inflammatory infiltrate consists primarily of T-cell and macrophages.[6] Local release of cytokines is thought to act on lymphocytes and infiltrating cluster of differentiation (CD) 8 + lymphocyte secreting granzyme-B around keratinocyte, thereby inducing keratinocyte nuclear injury and apoptosis.[6]

Some investigators have studied the number of apoptotic cells in hematoxylin and eosin-stained sections and compared it with the number of apoptotic nuclei identified by *in situ* end labeling (ISEL) in OLP and normal buccal epithelium. Their result showed that higher number of apoptotic cells could be seen in hematoxylin and eosin stained sections than ISEL.[5] OLP sections stained with the hematoxylin and eosin is favorable to appreciate the apoptotic cells.

We could observe 0.9157 apoptotic cells/μm area of epithelium including basal and suprabasal layer of OLP.

Bloor *et al*., and few other investigators did studies and stated that the rate of apoptosis appears to be increased in OLP epithelium compared with normal epithelium. Some author suggested that approximately one apoptotic cell was visualized per millimeter of basal length. When the third dimension is also taken into account, the rate of apoptosis per square millimeter of epithelium could in fact be high. Thus, number of apoptosis in single section may belie their significance in the disease process.[5]

Our study showed that the number of apoptotic cell increased with an increase in thickness of lymphocytic infiltration and degree of keratinization, but the epithelial thickness was reduced. Dekker *et al*., stated that bcl-x was negative to weak in normal buccal mucosa and inflamed gingiva and moderate in OLP. This overexpression of bcl-x found in keratinocytes may be related to the process of hyperkeratinization.[7] This could be probably explaining our observation of increase in the number of apoptotic cells with hyperkeratinization.

Bloor *et al*., reported that the alteration in epithelial thickness was due to imbalance between cellular proliferation and apoptosis.[5] Neppelberg *et al*., stated that as the number of apoptotic cells at the basal degeneration area increased, a decrease in epithelial thickness was observed.[8] This observation is consistent with that of our study.

Bloor *et al*., proved that both ISEL and histological count reveal significantly more apoptosis in the area of dense lymphocytic transgression of the epithelium/connective tissue junction. These findings are in accord with the generally held view of a causal role of lymphocyte and their cytokines in the induction of epithelial apoptosis.[5] In our study, we have also observed that the number of apoptotic cells was more in those areas where the lymphocytic infiltration was dense.

Identification of apoptotic cells is a great task in the presence of dense lymphocytic infiltration and eosinophilic coagulum. The measurement of apoptotic cells and other histological parameters can only be two dimensional under

light microscope. The assessment of epithelium thickness is difficult due to basal degeneration and also quantitative assessment of lymphocytic infiltration is difficult due to the variation in density from place to place. So further immunological and molecular studies are required to analyze and interpret the correlation of the number of apoptotic cells and other histopathological parameters of OLP.

CONCLUSION

In the present study, measurement of epithelial thickness, lymphocytic infiltration and amount of keratinization were done with the help of eyepiece of graticule and stage micrometer using light microscope; only linear measurement was possible.

Our study results showed that the number of apoptotic cells increased, with an increase in thickness of lymphocytic infiltration and degree of keratinization but the thickness of the epithelium was reduced.

Further advanced studies are required for a stronger evidence in correlating apoptotic cell count with other histological parameters of OLP.

ACKNOWLEDGMENT

I express my gratitude to Dr. Vidya M and Dr. Maji Jose for their kind support and encouragement throughout my course. I also thank Yenepoya University for its consistent support during my study.

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How to cite this article: Doddawad VG. Histopathological analysis of apoptotic cell count and its role in oral lichen planus. J Oral Maxillofac Pathol 2014;18:42-5.

Source of Support: Nil. **Conflict of Interest:** None declared.