Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology

journal homepage: www.elsevier.com/locate/jomsmp

Original research

Study of extrinsic apoptotic pathway in oral Lichen Planus using TNFR 1 and FasL immunohistochemical markers and TUNEL technique☆

Parviz Deyhimi*, Elham Arzhang

Dental Research Center, Department of Oral and Maxillofacial Pathology, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Keywords:
Lichen planus
Apoptosis
TUNEL technique
TNFR1
FasL

ABSTRACT

Objective: The exact pathogenesis of lichen planus (LP) is still unknown and there are some controversies concerning role of apoptosis in its creation. The purpose of the present study is to investigate extrinsic apoptotic pathway in oral lichen planus(OLP).

Methods: In the present cross-sectional study, the presence of apoptosis was investigated on 25 specimens of OLP and 6 specimens of normal oral mucosa using TUNEL technique and also pro apoptotic immunohistochemical (IHC) markers of FasL and TNFR1 in 4 areas of degeneration, basal and parabasal layers, and lymphocytic band. Then staining intensity distribution (SID) index was determined and the results were analyzed by Wilcoxon and Mann-Whitney tests (P-value < 0.05).

Results: There was no significant difference between SID of TUNEL in the areas of parabasal and lymphocytic band, but the difference was significant among other areas. There was significant difference between SID of TNFR1 in the areas of degeneration and lymphocytic band, basal and lymphocytic band and also parabasal and lymphocytic band, but there was no significant difference between other areas.

There was no significant difference between SID of FasL in the areas of degeneration and basal, degeneration and parabasal, and also basal and parabasal, but there was significant difference in other areas.

Conclusions: Apoptosis probably doesn't play a major role in destruction of basal cells in OLP. Destruction of basal cells in OLP is probably resulting from necrosis of these cells. Because of low level or lack of apoptosis, there is low probability of carcinomatous changes in OLP.

1. Introduction

There are several hypotheses about the pathogenesis of lichen planus(LP) including apoptosis that has been noticed as one of the possible mechanisms in recent years [1–14]. The apoptosis is activated through the intrinsic and extrinsic pathways. Both these pathways lead to the activation of caspase 3. The caspase 3 causes induction of DNA fragmentation which can be identified by different methods such as TUNEL technique [15]. The extrinsic apoptotic pathway begins by activation of cell death receptors in cell surface. The best known death receptors are Fas and TNFR1. The Fas/FasL system is one of the important mechanisms in induction of apoptosis. The T cytotoxic cells are the most substantial source of FasL expression [7,16,17]. TNF is a strong cytokine which acts via two receptors of TNFR1 and TNFR2. TNFR1 is expressed in all human tissues and is an important signaling receptor for TNF-α [18].

Neppelberg et al. [5] showed that in patients with oral lichen planus (OLP) in comparison with healthy people, there was a prominent expression of FasR/FasL which was more abundant in basal area rather than supra basal [5]. Sklavounou et al. [4] based on the reactivity of BcL2, showed that unlike infiltrative lymphocytes, the keratinocytes undergo apoptosis in OLP lesions [4].

On the other hand, the researchers such as Dekker [19], Bloor [20], Bascones- Ilundain [21,22], and Arreaza [23] indicated that the role of apoptosis in degeneration of basal layer of LP as ineffective. Bascones et al. according to their studies in 2005–2006 considered the role of apoptosis in pathogenesis of LP as small that from their viewpoint, above phenomenon has an effective role in carcinomatous transformation of LP [21,22].

In the present study, the TUNEL technique was applied in order to examine the role of apoptosis in OLP. Moreover, the effective markers in extrinsic apoptotic pathway including FasL and TNFR1 were used to determine the type of apoptotic pathway.

☆ This research with institutional bioethics approval and investigative proposal number 394513 has been performed by scientific and financial support of vice-chancellery for research of Isfahan University of medical sciences.

* Corresponding author.
E-mail address: Deihimy@dnt.mui.ac.ir (P. Deyhimi).

https://doi.org/10.1016/j.ajoms.2018.02.007
Received 13 November 2017; Received in revised form 14 February 2018; Accepted 15 February 2018

2. Materials and methods

In this cross-sectional study, 25 specimens of OLP and 6 specimens of normal oral mucosa were selected from department of oral and maxillofacial pathology, school of dentistry, Isfahan University of medical sciences, Iran. All of the OLP samples had histopathological characteristics of LP including dense lymphocytic infiltration band just under the epithelium and hydropic degeneration of the basal epithelial cells. Also the samples had sufficient surrounding normal appearing tissue for immunohistochemical(IHC) consideration.

The samples lacking adequate surrounding normal appearing tissue and containing severe mixed inflammation and also ulcerative surface were excluded from this study.

At first, sections (4–5 μm) were prepared from the paraffin blocks and then deparaffinization and rehydration in 5 descending stages were performed on them. Next following stainings were done:

2.1. TUNEL (TdT-mediated dUTP-biotin nick end Labeling) technique

After washing with phosphate buffer saline (PBS), the slides were fixed with 4% paraformaldehyde and then incubated in Tween solution and after washing with PBS, incubated in Terminal Deoxynucleotidyl Transferase(TDT) end labeling solution at ambient temperature. Then, after washing with PBS, the samples were incubated with blocking buffer at room temperature, then incubated in 3% H_2O_2 and after washing with distilled water (DW), incubated in TUNEL (Merck 17-141; Darmstadt, Germany) at 37 °C. Thereafter incubation of secondary antibody (Novolink polymer RE7140-K; Novocastra[Leica], Newcastle, UK) was performed in two phases: At first incubation was carried out with post primary solution and then polymer + HRP solution. Next the secondary antibody was washed with PBS and incubated with diaminobenzydine (DAB). Afterwards, hematoxylin staining was performed and finally samples were mounted.

Natural pulmonary tissue of mouse was used as positive control and the same samples were used as negative control by eliminating TDT.

2.2. TNFR1 IHC staining

The samples were incubated in Tris-EDTA (pH = 9) in microwave for antigen retrieval. The samples were then placed in 3% H_2O_2 to block endogenous peroxidase activity and then were washed with PBS. Next, the Anti-TNF Receptor 1 polycolonal primary antibody (TNFR1 ab 19139; Abcam, Sanfrancisco, California, USA) at 1/25 dilution was added and samples were incubated at ambient temperature. They were then incubated with Polymer Envision secondary antibody (Envision K4061; DAKO, Glostrup, Denmark) at room temperature. After placing the samples in DAB, opposite hematoxylin staining was performed and then the samples were mounted. Prostate adenocarcinoma was used as positive control, and the same samples were used as negative control by eliminating the primary antibody.

2.3. FasL IHC staining

Incubation was performed in Tris EDTA and H_2O_2 the same as TNFR1. After washing with PBS, Anti-Fas Ligand polyclonal antibody (Anti FasL ab2440; Abcam, Sanfrancisco, California, USA) at 1/100 dilution were added to the tissue and incubated at room temperature. Then, the samples were incubated with the secondary antibody (Biogenex QD430-XAKE; Sanfrancisco, California, USA), including a super enhancer and SS Label solution at room temperature. Next, the samples were incubated in DAB the same as TNFR1, stained with hematoxylin and finally the mounted. Breast adenocarcinoma was used as positive control, and the same samples were used as negative control by eliminating the primary antibody.

2.4. Evaluation of the samples

The samples were observed by optical microscope of Olympus Bx41TF Tokyo, Japan. The cells were analyzed in 4 areas of basal layer degeneration, basal and parabasal layers of the surrounding area of degeneration and lymphocytic band.

The rate or percentage of cellular staining expression for TUNE, FasL, and TNFR1 was estimated by counting stained brown cells for 100 cells in 10 high power fields with 400 magnification. The results were recorded as a semi- quantitative and ranked as scores: 1+ = 1–25%, 2+ = 26–50%, 3+ = 51–75% and 4 + > 75% of cells.

Furthermore, the rate of staining intensity (ranging from negative to deep brown) was evaluated as scores: 0 = negative, +1 = very low, +2 = low, +3 = moderate and 4 = high.

Finally staining intensity distribution (SID) index was obtained via multiplying the two scores of the staining expression and intensity of each sample [24,25]. After data collection, the collected data were analyzed by means of statistical software SPSS20 with statistical tests of Kruskal-Wallis, Mann-Whitney, and also Wilcoxon and Friedman at the significant level of 0.05.

3. Results

In study of TUNEL staining, it was observed that the staining expression and intensity rates of TUNEL was zero in basal and parabasal layers of normal oral mucous membrane. Results of TUNEL staining in the specimens of LP are presented in Table 1 (Fig. 1).

According to Mann-Whitney test performed on SID index of TUNEL in each two different areas, there was no significant difference between parabasal and lymphocytic band areas (PV = 0.525), but there was significant difference between each two following areas: degeneration and basal (PV < 0.001), degeneration and parabasal (PV = 0.008), basal and parabasal (PV < 0.001), degeneration and lymphocytic band (PV = 0.10), and also basal and lymphocytic band (PV < 0.001). Also according to this test, there was no significant difference between normal and lesional basal area; however, there was significant difference between normal and lesional parabasal area.

In study of TNFR1 staining, it was observed that the staining

Table 1
Results of TUNEL staining expression and intensity.

Ranking of staining expression/intensity	Number(percentage)of positive samples							
	Degeneration area		Basal layer		Para basal layer		Lymphocytic band	
	expression	Intensity	expression	Intensity	expression	Intensity	expression	Intensity
0	0	0	21(84%)	21(84%)	2(8%)	2(8%)	0	0
1+	16(64%)	10(40%)	4(16%)	4(16%)	19(76%)	17(68%)	25(100%)	19(76%)
2+	9(36%)	13(52%)	0	0	4(16%)	6(24%)	0	5(20%)
3+	0	2(8%)	0	0	0	0	0	1(4%)
4+	0	0	0	0	0	0	0	0
total	25	25	25	252	25	25	25	25