بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / حسام الدين محمد مغربي

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات : لا يوجد

Evaluation of Muco Adhesive Tacrolimus Patch on Caspase-3 Inducing Apoptosis in Oral Lichen Planus

(A Randomized Controlled Clinical Trial with Immuno Histochemical Analysis)

Thesis submitted to the Faculty of Dentistry, Ain-Shams University in partial fulfillment of the requirements for the doctoral degree in oral medicine

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2022

Acknowledgment

Thanks to God before and after. I would like to express my deepest gratitude to ***prof. Dr. Suzan Sief Allah Ibrahim***, Professor of Oral Medicine, Periodontology and Oral Diagnosis, Faculty of Dentistry, Ain Shams University, for her valuable directions and persistent kind supervision, help and care throughout this work.

Much of appreciation and gratitude is expressed to ***prof. Dr. Nivine Ibrahim Ragy***, Professor of Oral Medicine, Periodontology and Oral Diagnosis, Faculty of Oral and Dental Medicine, Cairo University, for her patient guidance, encouragement throughout this study and continuous support throughout my professional career.

Much of appreciation and gratitude are expressed to ***Ass. Prof. Ola Mohamed Ezzatt***, Assistant Professor of Oral Medicine, Periodontology and Oral Diagnosis, Faculty of Dentistry, Ain Shams University who was abundantly helpful, and offered invaluable support and guidance.

I am greatly indebted to ***Ass. Prof. Hala Ahmed El Kammar***, Assistant Professor of Oral Pathology, Faculty of Oral and Dental Medicine, Future University in Egypt, for supervising the histopathological and immunehistochemical analysis. Her role was axial and essential to get the results in a proper way.

My thanks to ***Ass. Prof. Asmaa Mohamed ElBakry***, Assistant professor of pharmaceuticals an industrial pharmacology for her help and support.

Lastly I would like to thank the patients who participated in the study for their volunteer character and appreciations to the value of medical research.

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List of Abbreviations

Anti-HCV: Anti-hepatitis C virus

ACEIs: Angiotensin converting enzyme inhibitors

AV: Aloe vera

BCL-2: B-cell lymphoma-2

BCL-KL: B-cell lymphoma-KL

BM: Basement membrane

CMC: carboxy methyl cellulose

C3: complement component 3

C4: complement component 4

CD8: Cluster of differentiation 8

CD4: Cluster of differentiation 4

CD95L: Fas Ligand

CD95: Fas

CS: Clinical Score

ER: Endoplasmic reticulum

Fas-L: Fas Ligand

FK506: Tacrolimus

GVHD: Graft versus host disease

HPA: hypothalamus pituitary adrenal axis

HCV: hepatitis C virus

HLA: Human leukocyte antigen

HLA-DR: Human Leukocyte Antigen- DR isotype

HPMC: Hydroxypropyl Methyl Cellulose

H&E: Hematoxylin and Eosin

IGM: immunoglobulin M

IL-2: Interleukin-2

IFN-α: Interferon gamma

ICAM-1: Intercellular Adhesion molecule 1

MMP-9: Matrix Metalloproteinase

MCL: Apoptosis regulator protein

MHC: Major histocompatibility complex

MMP: Matrix metalloproteinase

MAF: Mean area fraction

NIH: National institutes of health

NSAIDs: Non-steroidal anti-inflammatory drugs

NFAT: Nuclear factor of activated T cells

NF-KB: Nuclear factor kappa B

OLP: Oral Lichen Planus

OSCC: Oral squamous cell carcinoma

OLL: Oral lichenoid lesions

OLDR: Oral lichenoid drug reactions

OLCL: Oral lichenoid contact lesions

P53: Tumor suppressor gene

PKB: Protein kinase B

PDT: Photo dynamic therapy

RCT: randomized controlled trials

RNA: ribonucleic acid

RCA R: Regulators of complement of ABA receptor

RCA: Regulators of complement of ABA

SCC: squamous cell carcinoma

TIMP: tissue inhibitor metalloproteinase

TCR: T cell receptor

Treg: regulatory T cells

TH1: T helper 1

TH2: T helper 2

TNF: Tumor necrosis factor

TAA: Total Atrophic area

TNSIs: Tacrolimus non-steroidal inhibitors

TLC: Total lymphocytic count

VCAM-1: Vascular cell adhesion molecule 1

VAS: Visual analogue scale

WHO: world health organization

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INTRODUCTION

Oral lichen planus (OLP) is a T-cell–mediated and chronic inflammatory disorder affecting the oral mucosa, with a higher incidence in women and age range varies around the world. However, it is rare in children (**Drogoszewska et al,2014).** Moreover, smokers and patients who abuse alcohol have a higher prevalence of OLP **(Mozaffari et al,2016).**

OLP occurs in a wide range of clinical forms, usually bilateral, or less frequent unilateral. There are six types of OLP lesions: Reticular, papular, plaque-like-white forms, atrophic (erythematous), erosive (ulcerated) and bullous-red forms. Clinical types of OLP may occur alone or in various combinations (**Canto et al, 2010; Cheng et al, 2016).**

Microscopically, OLP is characterized by dense sub epithelial lymphocytic infiltrate and degeneration of basal keratinocytes **(Roopashree et al,2010)**. Apoptotic keratinocytes (Civatte/Colloid bodies) are seen by

light microscope as shrunken cells with condensed nuclei and eosinophilic cytoplasm **(Tobon et al,2004)**.

Factors which are involved in the induction of apoptosis in OLP includes tumor suppressor gene P53, B cell lymphoma BCL-2 family protein, Fas/FasL pathway, proteases of the matrix metalloproteinase-9 (MMP-9) and caspase-3 **(Sugerman et al,2000)**.

Caspases are cysteine-proteases and are required for programmed cell death. Two principal pathways to caspase activation are identified extrinsic and intrinsic one; The extrinsic pathway is triggered by attachment of "death receptors"; Tumor Necrosis Factor [TNF] family, TNF-1and FasL on the cell surface. The intrinsic pathway is triggered by various forms of stress, including inadequate cytokine support and different types of intracellular damage **(Adams,2003)**.

Different medications have been used in the form of topical or systemic agents for the treatment of OLP including corticosteroids, immunosuppressive, retinoids and immunomodulatory agents **(Sonia et al,2015)**. However, various treatment protocols have been developed to control symptomatic OLP but a permanent cure is not yet available **(Sun,2019)**.

Reviews concerning OLP therapy recommend high potency topical corticosteroids as the treatment of choice, and indicated clobetasol propionate to be the most effective topical steroid, however refractory lesions to steroids needed alternative medications **(Lodi et al,2005; Mahdi,2019)**.

Topical immunomodulators, including both tacrolimus and pimecrolimus, are recent modalities to the therapeutic management of OLP, adverse effects of tacrolimus were generally minor and transient and didn't affect continued medication (**Su et al, 2022)**.

Tacrolimus, is a potent immunosuppressant macrolide antibiotic produced by *Streptomyces Tsukubaensis* **(Greaf et al ,1999).** It inhibits the first phase of T-cell activation, inhibiting the phosphatase activity of calcineurin **(Gupta et al,2003)**.

Topical 0.1% tacrolimus gel was effective in the treatment of symptomatic oral lichen planus, and its systemic absorption was minimum and the serum concentration were low or even un detected **(Sosa, 2019)**.

The use of currently available topical formulations has few limitations like reduced patient compliance and salivary washout, in contrast, muco-adhesive buccal film which provided the advantage of advanced retention time, increased patient compliance, prolonged release of the drug and better bioavailability at site of action **(Ashwathy et al, 2019)**.

Based on the previous mentioned mechanism of action of tacrolimus and advantages of muco-adhesive patch, we hypothesized that tacrolimus loaded in muco-adhesive patch could improve clinical results and patient compliance more the triamcinolone acetonide topical gel in management of symptomatic OLP.

To the best of our knowledge, there is one published preclinical study concerning the use of mucoadhesive patches for local delivery of clobetasol to oral mucosa **(Colley,2018)** and one study demonstrating the development and characterization of muco-adhesive patches as a carrier for tacrolimus for oral mucosal delivery **(Ashwathy et al, 2019).**

Thus, the aim of this randomized controlled clinical trial was to evaluate the clinical efficacy of tacrolimus 0.1% in muco-adhesive patch compared to tacrolimus or corticosteroids in gel form for symptomatic oral lichen planus and to investigate their effect on the expression of caspase 3 in oral lichen planus as an early marker of apoptosis using immune histochemical analysis.

AIM OF THE STUDY

The aim of this study was:

1- To evaluate the clinical efficacy of tacrolimus 0.1% in mucoadhesive patch compared to tacrolimus or corticosteroids in gel forms for symptomatic oral lichen planus **(Primary Objective).**

2- To investigate the effect of topical tacrolimus or corticosteroid on the expression of caspase-3 in oral lichen planus lesions as an early marker of apoptosis using immunohistochemical analysis **(Secondary Objective).**

REVIEW OF LITERATURE

Lichen planus is an inflammatory immune mediated vesiculo-bullous muco-cutaneous disease affecting skin and mucous membranes, it could also