Efficacy of intralesional injection of autologous platelet rich plasma versus intralesional injection of corticosteroids on pain relief and ulcers healing in patients with erosive oral lichen planus

Randomized clinical trial

Protocol submitted to oral medicine, diagnosis and periodontology department at faculty of dentistry, Cairo University for partial fulfillment of Doctoral degree in Dentistry

(ORAL MEDICINE AND DIAGNOSIS)

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Administrative information

- *Title:*

Efficacy of intralesional injection of autologous platelet rich plasma versus intralesional injection of corticosteroids on pain relief and ulcers healing in patients with erosive oral lichen planus

Randomized clinical trial

- *Trial registration*:

It is intended to be registered in *Clinicaltrials.gov* registry database.

- *Funding*

Self-funded.

- *Roles and responsibilities*

1. Dr. Abdelhameed Hamid Mohammad Hijazi; (H.A.) PhD Candidate at Oral medicine, diagnosis and periodontology department, Cairo University.
   The primary investigator in the study (performing the interventions of the trial and collecting data)

2. Prof. Soheir Gaafar; (G.S.) Professor of Oral Medicine at Oral medicine, diagnosis and periodontology department, Cairo University.
   The main supervisor of the study.

3. Dr. Wessam Abdel-Moneim; (A.W.) Associate professor of Oral Medicine at Oral medicine, diagnosis and periodontology department, Cairo University.
   Outcomes assessor and Co-supervisor of the study.

Introduction

- *Background and rationale*

Lichen planus (LP) is a chronic mucocutaneous inflammatory disease that frequently affects the oral mucosa, it tends to affect mostly middle-aged white female patients. Oral lichen planus (OLP) occurs more frequently than the cutaneous form, it is estimated to affect 0.5% to 2% of the general population and tends to be more persistent and more resistant to treatment **(Edwards & Kelsch, 2002)**. Oral lichen planus (OLP) can present clinically in six different patterns: papular, reticular, plaque, atrophic, erosive and bullous, each have specific characteristics and can be found isolated or associated together. The most prevalent type of those is the reticular type, which is characterized by the presence of Wickham striae, which are typically symmetric, bilateral, asymptomatic, and mainly found in the buccal mucosa. The erosive form, despite being less frequent, presents greater clinical significance as the lesions are usually symptomatic, ranging from a minimal discomfort to episodes of severe pain **(Payeras et al., 2013).**

In OLP disease, antigen-specific and non-specific mechanisms both may be involved in its pathogenesis. Antigen specific mechanisms in OLP include antigen presentation by basal keratinocytes and antigen specific keratinocyte killing by CD8+ cytotoxic T-cells. Non-specific mechanisms include mast cell degranulation and matrix metalloproteinase (MMP) activation in OLP lesions. These mechanisms may act simultaneously to cause T-cell accumulation in the superficial lamina propria, basement membrane disruption, intra-epithelial T-cell migration, and keratinocyte apoptosis in OLP. It has been hypothesized that OLP chronicity may be due, to some extent, to deficient antigen-specific TGF-β1-mediated immunosuppression **(Sugerman et al., 2002).**

Topical corticosteroids have been considered the first-choice agent for the treatment of OLP, corticosteroids are especially helpful in the management of OLP because of their anti-inflammatory effect and anti-immunologic properties, but their prolonged use should be avoided because of the associated adverse effects **(Lodi et al., 2005). Lee et al., 2013** compared topical approach with intralesional injection in patients

with oral lichen planus and reported that intralesional injection with triamcinolone acetonide has significantly less side effects compared to topical application**.**

Platelet Rich Plasma (PRP) is a plasma concentrate of the patient's blood that predominantly contains platelets. In the PRP, apart from a high concentration of platelets, an increase in all coagulation factors may be recorded. Activated platelets release different growth factors that contribute to cell migration, proliferation, differentiation, angiogenesis, removal of tissue debris and regeneration of the appropriate type of tissues **(Pietrzak & Eppley, 2005).**

Growth factors, to be specific cytokines and chemokines, include platelet derived growth factors (PDGF-aa, -bb and -ab isomeric), transforming growth factors-β (TGF-β, - β 1 and - β 2 isomeric), interleukin-1, platelet-derived angiogenesis factor, vascular endothelial growth factor, epidermal growth factor, epithelial cell growth factor, platelet-derived endothelial growth factor, insulin-like growth factor, fibronectin and others are found in large numbers in alpha granules within platelets **(Dionyssiou et al., 2013)**

PRP injections because of the high concentration of growth factors has been used in various conditions, including chronic ulcers and wound healing **(Martinez-Zapata et al., 2012). Bolanča et al., 2016** reported beneficial effect of using PRP injections in lichen planopillaris resistant to intralesional corticosteroids. Another study conducted by **El-Komy et al., 2015** reported significant clinical improvement of resistant oral erosions in patients with pemphigus vulgaris after intralesional injection of PRP.

PRP injections had been used widely in the treatment of tendons injuries and in orthopedic surgeries to promote healing and regeneration **(Dohan et al., 2014)**. Also in temporomandibular joint disorders, PRP injections has proven to be effective in pain relief, reduction of the inflammatory signs in the joint **(Hegab et al., 2015)**. For oral surgeries and periodontal defect repairs also PRP had shown a significant role in tissue regeneration **(Mostafa et al., 2013; Hou et al., 2016).**

Recently **Loré et al., 2016** compared in a pilot study the effect of PRP gel with cyclosporine mouth wash and retinoic acid lotion in different OLP phenotypes; namely erosive, reticular and plaque type OLP, respectively. They concluded that treatment of OLP should take into consideration the phenotype of the disease, as PRP has a high concentration of growth factors it is advised to be used in erosive type, which proved to be effective when applied once weekly.

Rationale:

We are conducting this clinical trial to provide a safe alternative treatment for patients suffering from resistant erosive oral lichen planus; which had proved a promising regenerative potentials.

- *Hypotheses:*

The intralesional injections of platelet rich plasma has the same effect of intralesional injections of triamcinolone acetonide regarding pain relief and ulcers healing in patients with erosive oral lichen planus

- *Trial design allocation ratio,*

The study is a randomized clinical trial with parallel groups, exploratory framework.

Methods Participants, interventions, and outcomes

- *Study setting:*

Oral medicine clinic at the Faculty of dentistry, Cairo University.

- *Eligibility criteria:*

1. Inclusion criteria:

Patients presenting with a clinical picture that assumes the diagnosis of erosive oral lichen planus (bilateral, more or less symmetrical erosive lesions with lacelike network of slightly raised gray white lesions (reticular pattern), and a histological findings that confirms the diagnosis (liquefaction degeneration of the basal cell layer with irregular–saw teeth like rete pegs.

2. Exclusion criteria:

- Systemic disorders such as hematological diseases, severe cardiovascular diseases, treatment with any drugs that could cause Lichenoid reaction.
- Pregnancy or active breastfeeding
- Patients who had lesion/lesions with dysplasia
- Patients who received topical treatment for Oral Lichen planus in the last 2 weeks **(Lee et al., 2013)** or systemic treatment for OLP in the past 3 months.
- Platelet count < 150,000/mm3; Hgb < 11 g/dl.
- Immunosuppressed patients
- Patients receiving therapy with anticoagulants and use of non-steroidal anti-inflammatory drugs in the 5 days before taking the blood sample.

- *Interventions:*

PRP will be prepared in the dental clinic from autologous blood collected in the same visit according to **Mostafa et al., 2013**:

a. Twelve ml of peripheral blood will be drawn from the patient's antecubital fossa into a sterile citrated graduated plastic falcon tube (tube 1) containing anticoagulant sodium citrate (best support for platelet viability) in the ratio of 1/10 (12 ml blood and 1.2 ml sodium citrate), under complete sterile conditions.

b. Tube 1 will be shaken gently to enhance complete mixing of the blood with anticoagulant and left to sediment for 45 min. to obtain the best yield.

c. First Spin: Tube 1 will be centrifuged using a standard common bench centrifuge (model 800 D, maximum speed 4000 rpm) at speed of 500 rpm for 10 min, resulting into separation of the whole blood into 3 layers: a bottom layer comprised of red blood cell (RBCs), middle layer comprised of platelets and white blood cells (buffy coat), high concentration of platelets was found in the boundary layer between the two regions and top plasma layer of platelet poor plasma**.**

d. The upper plasma layer and middle layer (1-2ml of the top part of the RBC layer known as buffy coat), will be aspirated using a sterile Pasteur pipette and transferred to another plastic tube (tube 2), while the RBCs fraction will be discarded.

e. Second spin: Tube 2 will be centrifuged first at speed of 1000 rpm for 5 min, then elevated to 1500 rpm for 5 min more, as a result, the platelets will be concentrated at the bottom, leaving the PPP at the upper portion of the tube.

f. The upper layer PPP will be aspirated with Pasteur pipette and separated in tube 3, leaving nearly 1.5ml of serum with concentrated platelets (PRP) at the bottom in tube 2.

g. The sedimented PRP (1.5 ml) will be kept at room temperature and will be mixed well before use.

In control group, triamcinolone acetonide 40 mg (Kenacort A vial; *SmithKline Beecham Co.; Egypt*) will be used for intralesional injections once every week for 4 weeks **(Lee et al., 2013)**.

The injections in both groups will be applied after a field block with Mepevicaine 3% *Alexandria Co.; Egypt* anesthetic without vasoconstrictor.

An amount of 0.5 ml of each treatment will be injected per 1cm^2 of ulcerated mucosa using a 25 gauge needle.

- *Outcomes*

Primary outcome:

Pain self-assessed by the patient using an 11-point (0-10) numerical rating scale, in which (0 = no pain) and (10 = worst possible pain) **(Seymour, 1982)**

Secondary outcome:

1- Clinical picture assessed by Thongprasom sign scoring **(Thongprasom et al., 1992)**, the measures will be recorded using a calibrated squared grid as following:

Score 0= no lesions, normal mucosa

Score 1= mild white striae, no erythematous area

Score 2= White striae with atrophic area less than 1 cm^2

Score 3= White striae with atrophic area more than 1 cm^2

Score 4= White striae with erosive area less than 1 cm^2

Score 5= White striae with erosive area more than 1 cm^2

2- Remission time according to **Conrotto et al., 2006**: the measures will be recorded using a binary scale [(Yes/Stable or No/Not stable): Yes indicates signs score more than 1; No indicates signs score equals 1 or less.

Baseline characteristics will be recorded by the primary investigator (*H.A.*) and the assessor (*Dr. A.W.*) who will be blinded, before the treatment using the previous scale and score, the difference in each outcome will be recorded at the beginning of each visit during the trial and in the follow up visits.

- *Participant timeline*

A biopsy of the lesion will be performed for histopathologic examination for the confirmation of the diagnosis of the new undiagnosed patients, after that recruited patients will be assigned to one of the groups (intervention or control), after that, all participants in intervention group should provide a recent complete blood picture to confirm that their platelets count is over 150000, then a pre-treatment records will be obtained for the lesions.

After that each week the same treatment protocol for each group will be followed for consecutive 4 weeks, in total 4 injections will be injected for each participant.

After the last injection the patients will be followed up for 1 week to obtain the endpoint measures.

All participants will be followed up by the primary investigator *H.A.* every 2 weeks for 3 months from the last visit to report flare episodes of the disease.

- *Sample size:*

The aim of this study is to compare between the intralesional injection of autologous platelet rich plasma in treatment of patients with erosive type of oral lichen planus and the intralesional injection of corticosteroids. Based on previous studies by **Xia et al., 2006**, a total sample size of 8 (4 in each group) will have 90% power to detect a difference in the VAS means between the 2 groups of 49.69 (the difference between the intervention group with mean of 8.33 and the control group with mean of 49.69) assuming that the intervention group standard deviation is 10.11 and the control group standard deviation is 12.85 using t-test with a 0.05 two-sided significance level. The number is increased to a total sample size of 10 to allow for the use of non-parametric test. To allow for 25% losses, the sample was further increased to **14 (7 participants in each group)**. Sample size estimation was performed by nQuery statistical package.

- *Recruitment*:

Patient's database from the department of oral medicine, diagnosis and periodontology will be filtered by their condition, eligible subjects will be contacted to ask for follow up visit.

Those who agree to be enrolled in the trial will be asked to sign an informed consent, then all participants will be allocated randomly to either study groups according to the computer generated sequence.

Allocation

- *Sequence generation*

Simple randomization using computer based sequence generation software will be used after patients' consent of enrollment.

- *Allocation opaque, sealed Concealment are assigned mechanism*

No concealment will be used in this trial, as the interventions can't be blinded from the patients or the investigator (too obvious to know).

- *Implementation*

The primary investigator *H.A.* will be responsible for the sequence generation, allocation and enrollment of the participants.

- *Blinding*

Dr. A.W. the assessor of outcomes will be blinded; as she will NOT be involved of any step during patients' allocation or during treatment delivery.

No circumstances will make unblinding permissible.

- *Data collection, management, and analysis Data collection methods*

Data collected by the primary investigator *H.A.* and the assessor *Dr. A.W.* will be revised by the main supervisor (*Dr. G.S.*) at the end of the trial after 4 weeks and after 3 months of follow up.

Patients will be followed up every 2 weeks for 3 months after the last visit to check flare ups of the disease, the primary investigator *H.A.* and the assessor *Dr. A.W.* are responsible for the follow up records.

- *Data management*

Each participant will have 2 separate files, one with the primary investigator *H.A.* and the second will be with the assessor *Dr. A.W.*, rather than the original administrative file in the department of oral medicine.

- *Statistical methods:*

Data will be analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 23 (SPSS Inc., Chicago, IL). Numerical data will be described as mean and standard deviation or median and range, as appropriate. Comparisons between the intervention and the control group will be performed using the Mann-Whitney test. A p-value less than or equal to 0.05 will be considered statistically significant. All tests will be two tailed.

Monitoring

- *Data monitoring*

Data will be recorded directly by the assessor (*Dr. A.W.*) and another reading will be recorded by the primary investigator (*H.A.*) of the trial, then reviewed by the main supervisor (*Dr. G.S.*) with no involvement of other parties in the process.

- *Harms*

Short term use of the treatments of the trail will cause minimum side effects according to published evidence, although exaggerated pain or flare up of the disease after starting the treatment will be recorded and the adverse effect will be dealt as following:

1- Pain after injections: the patient will be informed about the probability of exaggerated pain feeling at the injection site, analgesics will be prescribed for each patient Ibuprofen 400 mg (Brufen tablet; *Kahira Pharmaceuticals & Chemical industries Co.; Cairo*), when needed
2- Flare ups of the lesions; the patient will be shifted to another treatment modality

- *Auditing*

The auditing will be held every week for 4 weeks from the start of the trial and every 2 weeks for 3 months of treatment free follow up period, pain reporting using numerical rating scale and photos of the lesions will be obtained in every visit by the assessor *Dr. A.W.* and the primary investigator *H.A.* separately to allow inter-assessor reliability check.

Ethics and dissemination

- *Research ethics approval*

It is planned to get the approval from committee of Ethics at the Faculty of Dentistry, Cairo University

- *Protocol amendments*

Amendments will be revised and approved by the supervisors *Dr. G.S.* and *Dr. A.W.*, then registered to *Clinicaltrials.gov* registry database.

- *Consent or assent*

The primary investigator *H.A.* will obtain informed consents from each participant.

- *Confidentiality*

The patients file will only be reviewed by the primary investigator (*H.A.*), assessor (*Dr. A.W.*)- Who will be blinded of the treatment received- and the supervisors (*Dr. G.S. and Dr. A.W.*) of the trial.

- *Declaration of interests*

We declare no financial interests for the overall trial.

- *Access to data*

Primary investigator (*H.A.*) and supervisors (*Dr. G.S. and Dr A.W.*) of the study only will have access to patient records (except the assessor *Dr. A.W.* who will be blinded) and health information.

- *Ancillary and post-trial care*

The primary investigator *H.A.* will be held responsible for the post trial care and compensation for any harm from the trail participation.

- *Dissemination policy*

Study results will be published in an international journal and contacts of the primary investigator *H.A.* as well the supervisors *Dr. G.S. and Dr. A.W.* will be listed in the published paper.

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