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Evaluation of topical human platelet lysate versus topical clobetasol in management of methotrexate-induced oral ulceration in rheumatoid arthritis patients: Randomized-controlled clinical trial

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ABSTRACT

Background: There is no mainstay protocol for management of Methotrexate-induced oral ulcers; commonly used protocols are cessation of Methotrexate, folic acid treatment, corticosteroids or combination. A new era of oral ulcers management is represented by platelet concentrates. The current study assessed the effect of topical human platelet lysate compared to topical Clobetasol Propionate in management of methotrexate-induced oral ulceration in rheumatoid arthritis patients.

Methods: This randomized controlled clinical trial include 30 patients in two parallel groups (intervention - human platelet lysate, control - Clobetasol Propionate), with allocation ratio 1:1. Outcome measures were pain intensity using numerical rating scale, WHO scale for oral mucositis, measuring size of the largest ulcer and total number of oral ulcers.

Results: A statistically significant difference was detected between HPL and Clobetasol groups on comparing numerical rating scale, WHO mucositis scale, size and total number of oral ulcers throughout all visits. A considerable quick pain reduction and clinical improvement were noticed in HPL group compared to Clobetasol.

Conclusion: Human platelet lysate has superior effect when compared to one of the most potent topical corticosteroids, Clobetasol Propionate, in reducing pain and clinical signs of Methotrexate-induced oral ulcers in patients with rheumatoid arthritis.

1. Introduction

An interesting scope of health and dental care providers is adverse drug reactions. They are common and unfortunately increasing with continuous introduction and approval of new therapeutic modalities. Adverse drug reactions affect the oral cavity in different clinical presentations including xerostomia, dysgeusia, pigmentation, infections, tumors, keratosis and ulcers [1,2].

Drug-induced oral ulcers are debilitating and could further complicate the patient's condition. Management of such ulcers is complex and represents a real challenge. They don't respond properly to conventional treatments such as corticosteroids; dose modification or even drug cessation and exchange could be the only option [2,3].

Rheumatoid arthritis (RA) and Methotrexate, its gold standard therapy, is one famous example. Despite controlling RA and keeping the beast into cage for years; Methotrexate causes debilitating resistant oral ulceration as a common complication [4,5].

Methotrexate exerts its therapeutic effect via interfering with DNA synthesis through inhibiting key enzymes in the biosynthesis of purines and pyrimidines, thus targeting high turnover cells such as inflammatory and malignant cells. The anti-proliferative effect of Methotrexate is beneficial in management of RA; yet it causes oral ulcers due to targeting epithelial cells too [5,6].

There is no mainstay protocol for management of Methotrexate-induced oral ulcers; clinicians follow variable options. The commonly used protocols are cessation of Methotrexate, folic acid treatment, topical corticosteroids, systemic corticosteroids or a combination of two or more of them [7].

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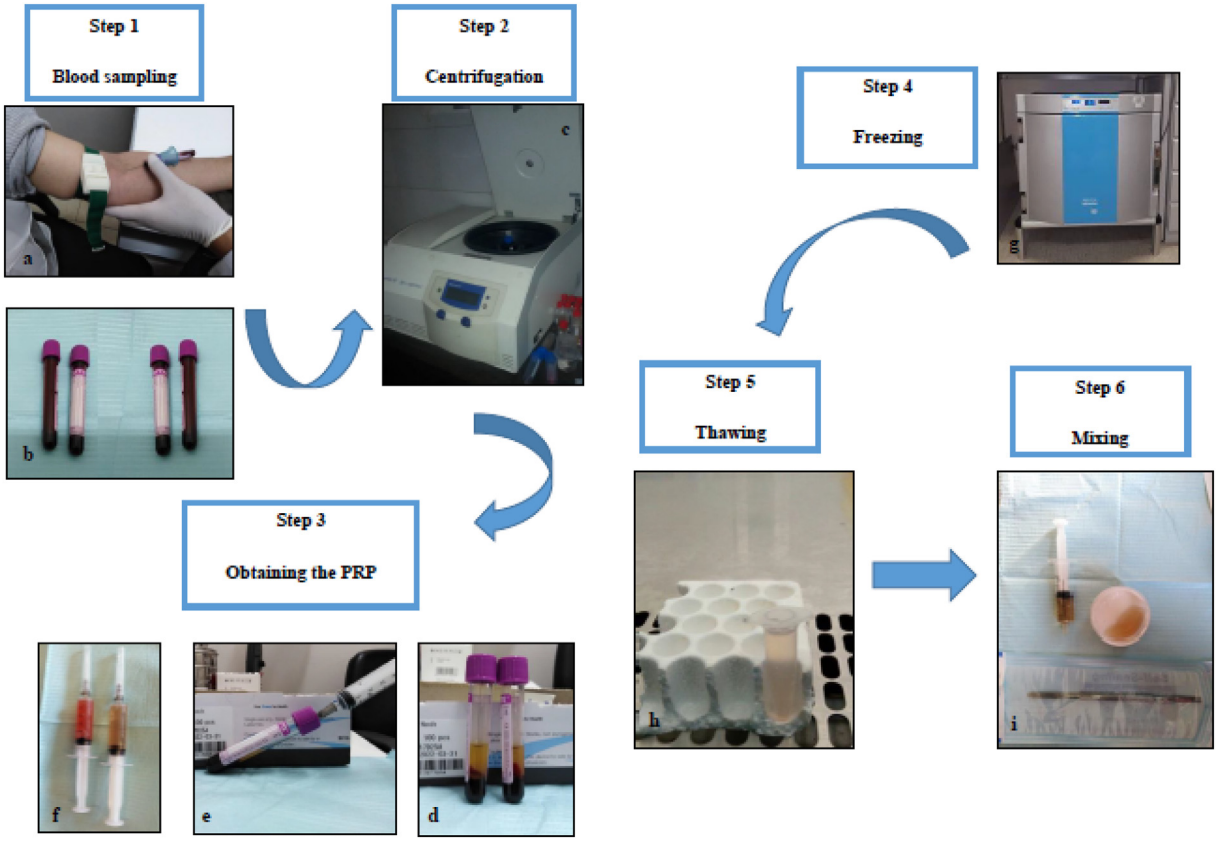

Fig. 1. HPL preparation and mixing with orabase, a- drawing the blood sample, b- blood in anti- coagulation tubes, c- centrifuge used, d- tubes after centrifugation, e- separating the PRP, f- PRP sample ready to be freezed, g- (−80) freezer, h- thawing the HPL, i- mixing.

Fig. 2. Clobetasol Propionate 0.05% and HPL packed in identical labelled containers.

Topical corticosteroids, with the super potent Clobetasol on top of list, are the mainstay of managing chronic oral ulcers including Methotrexate-induced oral ulcers [8]. However, the results in managing drug-induced ulcers are not always satisfying [1].

A new era of oral ulcers management is represented by platelet concentrates, among which platelet lysate is an emerging form that allow the use of growth factors without the platelet cell wall. The formula was first developed to treat oral ulcers of chronic graft versus host disease (GVHD) with promising results and avoiding corticosteroids side effects [9]. These results opened the door in front of researchers to evaluate its beneficial use in chronic oral ulcers treatment regardless their etiology.

The current study was conducted to evaluate the effect of topical human platelet lysate (HPL) compared to topical Clobetasol Propionate in management of methotrexate-induced oral ulceration in RA patients.

2. Materials and methods

2.1. Trial design and participants

This trial is a randomized controlled clinical trial that include 30 RA patients treated with Methotrexate and suffering from methotrexate-induced oral ulceration in two parallel groups (intervention - HPL, control - Clobetasol Propionate), with allocation ratio 1:1. Patients suffering from systemic disease known to cause oral ulceration; salivary gland diseases; malignancies and patients treated with corticosteroids at the time of setting were excluded. This clinical trial followed the principles of the Helsinki Declaration and was approved by the Research ethics committee of Faculty of Dentistry, Cairo University (Code: 15637).

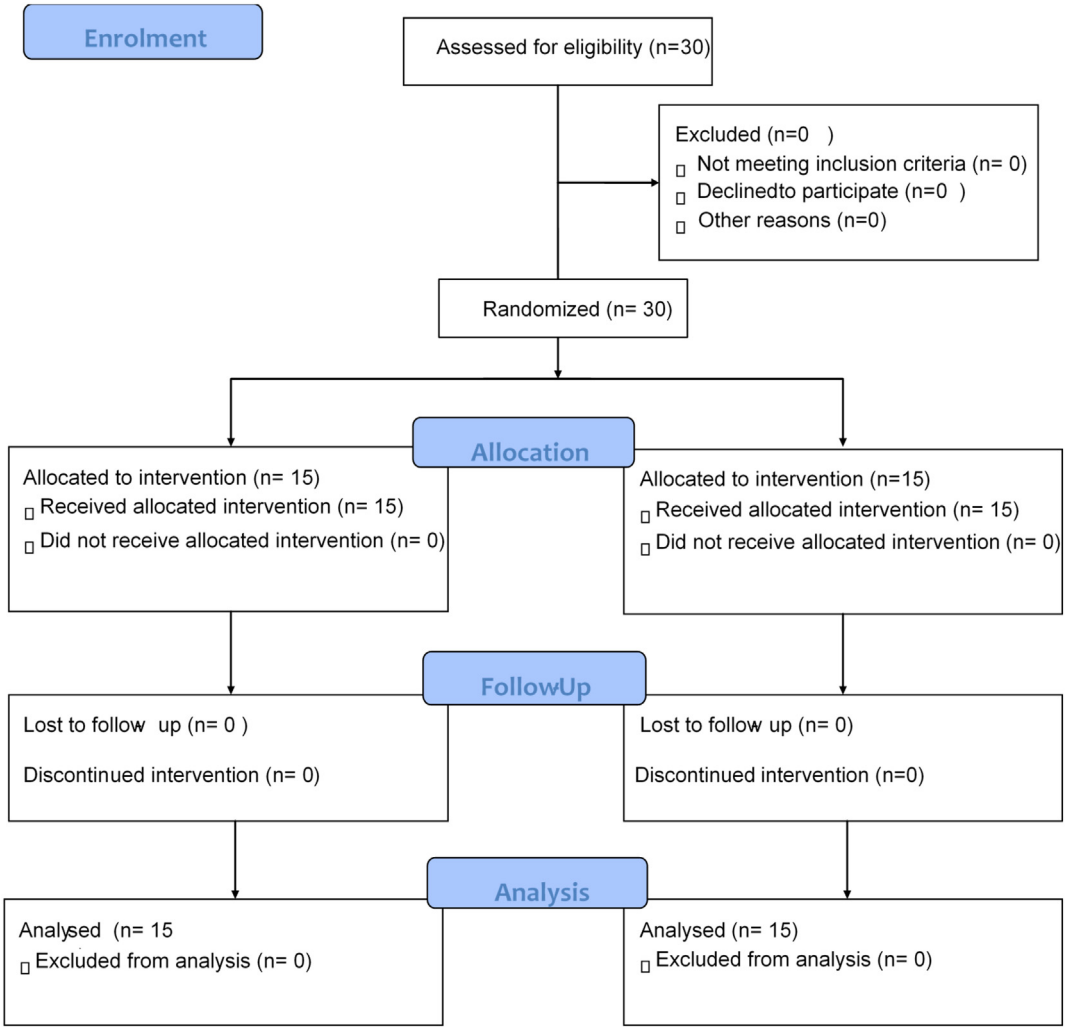

Fig. 3. CONSORT flow diagram.

Table 1
Baseline characteristics of the two studied groups.

	HPL group (n = 15)	Clobetasol group (n = 15)	P-value*
Age (years) [mean ± SD]	42.2 ± 11	47.6 ± 11.6	0.2
RA duration (years) [mean ± SD]	3 ± 2.5	3.7 ± 2.6	0.45
MTX dose (mg) [mean ± SD]	17 ± 2.7	16.7 ± 2.3	0.74
MTX duration (years) [mean ± SD]	2.4 ± 2	3.3 ± 2.3	0.26
NRS score	10	10	
WHO score	3	3	
Oral ulcer size (cm) [range]	(0.5 × 0.5) - (1 × 1.5)	(0.5 × 0.5) - (1 × 2.2)	
Oral ulcers number [mean ± SD]	2.3 ± 1.5	1.7 ± 1.2	0.23

* Significant at $P \leq 0.05$.

2.2. Interventions

Pre-treatment measures were recording detailed history including RA and drug history; giving oral hygiene instructions (verbally and written), collecting baseline data (demographic data, outcomes measures) and obtaining signed informed consent after explaining the steps of the study with discussing the treatment plan. Patients were instructed to keep the MTX dose fixed during their participation in the clinical trial.

HPL preparation was prepared in the Rheumatology Department, Faculty of Medicine, Cairo University. A 40 mL venous blood sample was drawn by operator (EM) from antecubital vein with pain-free blood test butterfly needles (25 Gauge, Shandong Zibo Shanchuan Medical Instrument Co., Ltd, China). The sample was withdrawn in acid citrate dextrose-Adenine (ACD-A) or Ethylenediaminetetraacetic acid (EDTA) tube. Both are anti-coagulants which functions by binding calcium in the blood and keeping the blood from clotting. The sample was centrifuged at 900 rpm for 10 min, subsequently the PRP was collected and frozen at −80 °C (thermal shock), then thawed at 37 °C to induce lysis and PDGFs release. All manipulations were performed under sterile conditions (Fig. 1).

The final formulation of topical HPL was prepared by mixing 1:1 (w/w) Orabase with HPL; aliquoted in sterile disposables, 40 mL volume each; and maintained at 4 °C for a maximum of 14 days [9]. Clobetasol Propionate 0.05% ointment (Dermovate. Pharma derm. US) was mixed 1:1 with Orabase [10].

Table 2
Comparison between daily NRS score of the two studied groups.

	Mean ± SD		t	P-value	Mean difference	95% CI	
	HPL group (n = 15)	Clobetasol group (n = 15)				Lower	Upper
Day 1	5.2 ± 2.4	8.6 ± 1.2	4.9	< 0.0001*	3.4	1.97	4.8
Day 2	3.2 ± 1.9	7.8 ± 1.1	8.1	< 0.0001*	4.6	3.44	5.76
Day 3	2 ± 2.2	7.4 ± 1.2	8.3	< 0.0001*	5.4	4.06	6.7
Day 4	1.3 ± 1.9	6 ± 1.2	8.1	< 0.0001*	4.7	3.5	5.88
Day 5	0.4 ± 0.9	5.7 ± 1.4	12.3	< 0.0001*	5.3	4.42	6.18
Day 6	0.2 ± 0.6	5.3 ± 1.8	10.3	< 0.0001*	5.1	4.08	6.18

* Significant at P ≤ 0.05, CI: confidence of interval.

The orabase was prepared in a pharmacy laboratory using: 16.6% gelatin, 16.6% pectin, 16.6% sodium carboxymethyl cellulose (Zibo Hailan Chemical Co., Ltd), 100% plasti-base (emollients topical. Bristol-Myers Squibb. Canada). This muco-adhesive paste was prepared by gradual addition of sodium carboxymethyl, cellulose, pectin and ultimately, adding gelatin to the plastibase and constant stirring to obtain a homogeneous base [11].

All manipulations were carried on under sterile conditions. Tests for contaminations of aerobic and anaerobic bacteria and fungi, after orabase is mixed were performed (total microbial count test).

The interventions were applied on all ulcerated mucosal surfaces 3 times per day, after arousal, 1 h after lunch and before bed. All patients were instructed to refrain eating or drinking for 1 h after treatment application. The directions of treatment application were illustrated carefully and a print out of these instructions was delivered to all patients. The treatment was repeated until complete healing occurs. Complete healing was expected within 28 days in HPL group [9] and Clobetasol Propionate group [10]. Nystatin 100,000 IU/cm^3 in aqueous solution (Egyptian Int. Pharmaceuticals Ind Co. – Egypt) was given to the patient as a mouth wash twice daily to avoid secondary fungal infection.

Patients were instructed to report directly re-appearance of any new ulcer and not to wait in such case for the next follow up visit.

2.3. Outcomes

Pain intensity was evaluated using numerical rating scale (NRS) at baseline, daily for the first week then weekly. NRS consists of a 10-cm horizontal line between extremities with (0) indicating no pain and (10) for unbearable pain [12].

Oral ulcers were scored on WHO scale for oral mucositis at baseline, then weekly by assessor (SA). In WHO scale, ulcers are given score from 0 to 4 as follows: Grade 0 = No oral mucositis; Grade 1 = Erythema and soreness; Grade 2 = Ulcers, able to eat solids; Grade 3 = Ulcers, requires liquid diet (due to mucositis) and Grade 4 = Ulcers, alimentation not possible (due to mucositis) [13].

The size of the largest ulcer was recorded at base line, then weekly. The ulcer size was determined by measuring the distance between two opposite edges of the ulcer border, using a periodontal probe in millimeters. The surface area of the ulcer was the product maximum ulcer diameter and the measurement perpendicular to it. The total number of oral ulcers was recorded.

The time frame of this clinical trial was 8 weeks; patients were treated for 4 weeks and followed up for 4 weeks.

2.4. Sample size

Based on previous studies an effect size of approximately 0.25 was expected. Estimated from Rodrıguez et al. [14] and Del Fante et al. [9] with 80% power and 5% significance level, a total sample size of 22 patients would be sufficient to detect a medium effect size (F = 0.25). The number was increased to a total sample size of 25 to allow for losses of around 20%. Then, it was increased to a total sample size of 30 to adjust for non-parametric correction. Sample size was calculated using G*Power program, University of Düsseldorf, Düsseldorf, Germany [15].

2.5. Randomization

Enrolled patients were randomly distributed between the two groups with allocation ratio 1:1 using: http://www.Randome.org by investigator (SG). The allocation sequence was concealed using numbered, opaque, sealed envelopes. The envelopes were opened after completing all baseline assessments, when it was time to allocate the intervention.

2.6. Blinding

Both Clobetasol Propionate 0.05% and HPL were packed in identical containers and labelled with a code number (Fig. 2). Preparations were similar in color, flavor and consistency. Investigators, participants and statistician were blinded to the intervention.

2.7. Statistical methods

Statistical analysis was done using Statistical Package for Social Sciences, Version 21.0 (SPSS, IBM) for Windows. Comparisons between the two studied groups was done by Student-*t*-test. Adjustments of the

Table 3
Comparison between the two studied groups for weekly assessment.

Visit	Mean ± SD		t	P-value	Mean difference	95% CI	
	HPL group (n = 15)	Clobetasol group (n = 15)				Lower	Upper
NRS score							
Week 1	0.2 ± 0.6	5.3 ± 1.8	10.4	< 0.0001*	5.1	4.09	6.1
Week 2	0 ± 0	2.5 ± 2.3	4.2	< 0.0001*	2.5	1.2	3.7
WHO mucositis scale							
Week 1	0.4 ± 0.8	2 ± 0.8	5.47	< 0.0001*	1.6	1.002	2.19
Week 2	0 ± 0	0.9 ± 0.8	4.03	< 0.0001*	0.87	0.4	1.3
Size of the largest ulcer							
Week 1	0 ± 0	0.6 ± 0.6	15	0.006*	0.6	0.28	0.9
Week 2	0 ± 0	0.6 ± 0.6	15	0.006*	0.6	0.28	0.9
Total number of oral ulcers							
Week 1	0 ± 0	1 ± 0.7	30.6	< 0.027*	1	0.63	1.37
Week 2	0 ± 0	0.3 ± 0.5	5.4	< 0.027*	0.3	0.04	0.56

* Significant at P ≤ 0.05, CI: confidence of interval.

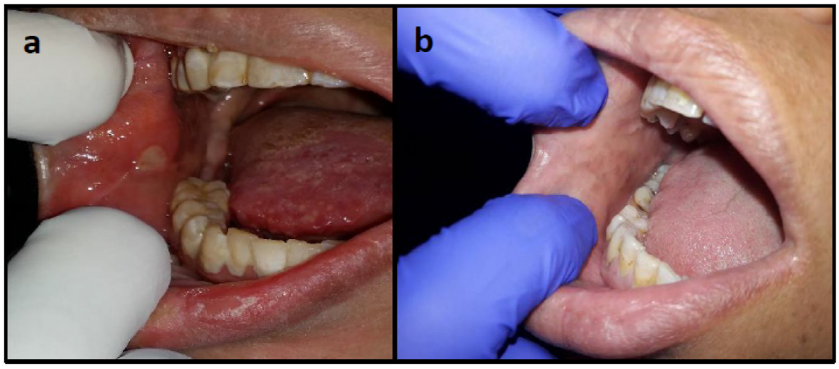

Fig. 4. Clinical photograph of a case showing the examination visit (a) and one week after HPL treatment (b).

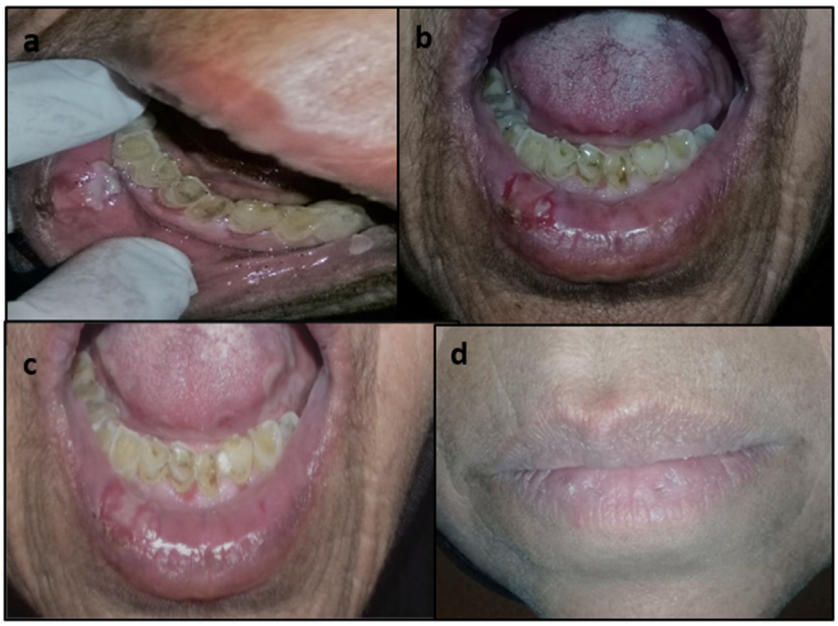

Fig. 5. Clinical photograph of a case showing the examination visit (a), one week (b), two weeks (c) and three weeks (d) after Clobetasol Propionate treatment.

P-value for multiple testing was performed using Bonferroni method. P value < 0.05 was considered significant. All tests were two tailed.

3. Results

The total sample size comprised 30 patients, 15 patients in each group. None of our patients discontinued the treatment or dropped off during the follow up periods. The collected data of all the patients were subjected to statistical analysis without exclusion (Fig. 3).

Baseline demographic and clinical characteristics for each group are revealed in Table 1. There were no significant differences among the two groups regarding demographic and clinical characteristics at baseline.

A statistically significant difference was detected between HPL and Clobetasol groups on comparing NRS scores throughout all daily and weekly visits. The NRS scores reduced considerably in HPL group with 47% pain reduction in the first day, 98% at the end of the first week and 100% pain reduction obtained for all patients by the end of the second week. In Clobetasol group, only 14% pain reduction was obtained in the first day and complete relief of pain was achieved by the end of the third week (Tables 2 and 3). All patients were followed up after complete healing till the end of the preset time frame and no pain was reported.

A statistically significant difference was detected between HPL and Clobetasol groups on comparing WHO mucositis scale, size and total number of oral ulcers throughout all weekly visits. A quick clinical improvement was observed in HPL group at the end of the first week where ulcers completely disappeared leaving erythematous areas and complete healing was observed at the end of the second week. In Clobetasol group, complete healing was noticed at the end of the third week (Table 3, Figs. 4 & 5). All patients were followed up till the end of the preset time frame and no breakouts or new lesions were observed or reported by the patients.

4. Discussion

Platelet concentrates are blood extracts that can improve healing and control inflammation. Being a rich source of growth factors, they are known to trigger or even be involved in angiogenesis and tissue regeneration. TGF-β, PDGF, VEGF, EGF and IGF are most famed. Release of GFs into a wound bed can have a chemotactic effect on monocytes, neutrophils, fibroblasts, mesenchymal stem cells, and osteoblasts. Their autologous nature, simple collection, easy bedside preparation, and clinical application without the risks associated with allogenic products allowed platelet concentrates to gain attention of researchers from various clinical fields as a promising potential therapeutic modality in chronic wounds, ulcers, tendons and bone defects [16–20].

HPL is one of the available platelets concentrates preparations. The technique has been developed in 2011 [20]. HPL has many advantages,

they provide a high concentration of many growth factors without the platelet cell wall; can be stored frozen for long time and can be prepared from donated blood without the fear of cross-infection, blood group matching or rejection [19].

HPL has been efficient when applied on chronic wounds, eye ulcers and oral ulcers associated with GVHD and has revealed potential to enhance wound healing processes by in vitro studies. These results together with minimal adverse effects have given HPL a chance to be one of the promising modalities in management of chronic lesions and ulcers including drug-induced oral ulcers [9,19,20].

There are very few studies of HPL prepared particularly for clinical use that urge conduction of new controlled clinical trials to assess the efficacy of HPL [21].

As far as we know, the present study is the first randomized clinical trial to assess the efficacy of HPL in management of Methotrexate-induced oral ulcers in RA patients. This is also the first trial of HPL in management of drug-induced oral ulcers, in general.

RA is the best target population to study Methotrexate-induced oral ulcers because oral ulcers haven't been reported as a manifestation of the disease itself so it's much easier to monitor the drug-induced oral ulcer [22]. All participants were instructed to avoid increasing their Methotrexate dose during the study because this could affect its adverse effects including oral ulcers [23].

All enrolled patients were receiving 5 mg folic acid as a regular regimen assigned by the physician to minimize adverse effects including Methotrexate-induced oral ulcers. However, we noticed that the prophylactic folic acid couldn't inhibit the occurrence of oral ulcers or reduce its severity. This was in accordance with Whittle and Hughes who documented in their review that prophylactic dose of 5 mg folic acid hasn't proven to reduce oral ulcers caused by Methotrexate [24]. Moreover, a Cochrane review concluded that although there is a trend towards reduction in oral ulceration with folic acid regimen; but this reduction is not statistically significant [25].

In the current trial, HPL effect on pain was prominent starting from the first day compared to Clobetasol. Regarding HPL group, oral ulcers completely disappeared at the end of the first week and complete pain relief as well as clinical improvement was obtained by the end of the second week. On the other hand, Clobetasol resulted in complete pain relief and clinical improvement at the end of the third week.

Regarding assessment of corticosteroids in management of Methotrexate-induced oral ulcers, the previous trials are case reports. Their results are heterogenous; time of healing varied from 1 week, 2 weeks and 1 month [7].

Concerning HPL, the result of the present trial is in accordance with the results of HPL in management of chronic oral ulcers of GVHD that are refractory to other therapies including corticosteroids. A case series reported the response of six patients out of the included seven patients to HPL. Two patients showed complete improvement; three showed 50% response and two patients showed 25% response with no adverse effects [9]. Another case series reported 57.1% pain reduction and 43.2% clinical improvement in the five included patients at the end of the study with no side effects [26].

5. Conclusions

The results of the current trial revealed that both HPL and Clobetasol have been effective in management of Methotrexate-induced oral ulcers in patients with RA. However, HPL has shown a noticeable quick pain relief and clinical improvement than Clobetasol. In conclusion, HPL has superior effect when compared to one of the most potent topical corticosteroids, Clobetasol Propionate, in reducing pain and clinical signs of Methotrexate-induced oral ulcers in patients with RA. We recommend conducting more RCTs with large sample size and long follow-up period to continue evaluating HPL in management of drug-induced oral ulcers.

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