

Review Article

Comparative analysis of clinical efficacy of microbiome and topical hyaluronic acid gel combination therapy in minor aphthous ulcer management

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ABSTRACT:

Introduction: RAU is usually characterized by recurring nature of painful ulcers of the mouth that are round or sometimes ovoid having the characteristic inflammatory halos. This study was aimed to compare the clinical efficacy of the combination of microbiome therapy with 0.2% topical HA gel, HA gel as monotherapy and 0.1% triamcinolone acetonide oro-mucosal paste (control) within the management of minor aphthous ulcer. **Materials and Methodology:** A total sample size of about 60 with 20 samples included in each treatment group was selected. All patients were given appropriate dietary counselling in order to avoid intake of spicy foods and are advised to encourage a bland diet. In Group A (n = 20), participants were advised to take each capsule of pre-and probiotic capsules (Bifilac HP Capsule, Tablets Ltd) twice daily with food for a week. And in Group B (n = 20), participants were advocated to apply topical 0.2% HA gel thrice daily for a week. Participants in Group C (n = 20) were directed to apply topical 0.1% triamcinolone acetonide oro-mucosal paste (Kenocort, Abbott Healthcare Pvt. Ltd), thrice daily for a week. **Results:** No patients were reported to develop any adverse effects after the intake of microbiome capsules. No new ulcers appeared on day 7 in Group - A patients. The intragroup comparison of treatment outcome at 3rd and 7th days was observed to be highly significant (P < 0.05) at day - 3 and day - 7. Pain relief on the third day alone was statistically significant with observed P value less than 0.05, on comparing group A vs. group B (P = 0.05) and Group A vs. Group C (P = 0.01). **Conclusion:** The results obtained from this study elaborated the immediate pain control of topical HA and microbiome combination therapy in the effective management of RAS. Therefore, this could be considered as an effective alternative for topical steroids in the management of RAS.

Keywords: aphthous ulcer, hyaluronic acid, microbiome therapy

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INTRODUCTION

Based on the sources available, the recurrent aphthous ulcer (RAU) is observed to be the most common form of recurrent oral ulcers. RAU is usually characterized by recurring nature of painful ulcers of the mouth that are round or sometimes ovoid having the characteristic inflammatory halos. Individual aphthous ulcers might be classified morphologically as minor, major or herpetiform ulcers. Minor aphthous ulcers constitute about 80% of all aphthous ulcers, measuring < 5 to 10 mm and are usually located on the buccal and labial mucosa

which has the capacity to heal spontaneously in 7 to 10 days without leaving behind any scarring. Almost 10% of all the lesions are mostly identified as major aphthous ulcers, which are larger than 10 mm and are deeper. They might get healed over weeks to months and often associated with scarring in the end. The remaining 10% of aphthous ulcers are herpetiform ulcers, which are usually smaller measuring 1-3 mm, grouped or coalescent ulcers that might be present on the keratinized mucosa of the dorsal tongue and palate and might heal spontaneously almost over 1 to 4 weeks. Herpes simplex virus as its definition says it

is not found in these lesions.¹ RAU could be further classified according to the severity of the clinical disease severity as simple and complex aphthous ulcers.

Simple aphthous ulcers mostly indicate the common presentation of a few lesions which has the potential to heal in 1 to 2 weeks and may recur infrequently. Complex aphthous ulcers, on the other hand, might represent a complicated clinical picture of severe disease, numerous, large or deep lesions where new lesions mostly developing as older lesions which get healed and is associated with remarkable pain or disability and occasionally presented with genital or perianal lesions.^{1,2} Oral or genital aphthous ulcers are needed to present to diagnose the condition as Behçet's disease (BD). These aphthous ulcers are mostly similar to those observed in patients with complex aphthous ulcers. The most common sites of oral ulceration are observed to be the buccal mucosa, gums, tongue, lips and pharynx. Oral ulcers in BD are typically painful, 1 to 3 cm in diameter, shallow or deep and have a yellow fibrinous base. Patients might have a single or multiple ulcers that usually lasts between 1 to 4 weeks. Herpetiform ulcers presented with pinpoint lesions mostly occurring in coalescing clusters.³

The hyaluronic acid (HA) is a naturally occurring polymer usually presented within the skin and has been studied since its discovery in 1934. It normally consists between 200 and 10,000 disaccharide units with a molecular weight in normal tissues exceeding 106. Based on the chemical structure, it belongs to the group of glycosaminoglycans, which have repeating disaccharide units of uronic acids and hexosamines. It can be seen associated with many tissues and body fluids of mammals, with the highest concentrations seen in connective tissue and skin.⁴ HA has been predominantly used in ophthalmology, rheumatology and dermatology because of its anti-inflammatory and anti-oedematous effects.⁵ The reported clinical applications in dermatology is not only being used as a biomaterial for bioengineering purposes or a temporary dermal filler in aesthetic dermatology but also for the enhancement of effective wound healing and can be used as a drug vehicle in topical formulations.⁶ Only a few studies reported the effects of HA in oral disease were observed. Recently, the effect of topical formulation of 0.2% HA gel on RAU was observed.⁷ Hence, this study was aimed to compare the clinical efficacy of the combination of microbiome therapy with 0.2% topical HA gel, HA gel as monotherapy and 0.1% triamcinolone acetonide oro-mucosal paste (control) within the management of minor aphthous ulcer.

MATERIALS AND METHODOLOGY

After obtaining the approval from the ethical committee, this study was conducted. A total sample size of about 60 with 20 samples included in each treatment group was selected. The inclusion criteria

of the study participants where patients belong to both genders with the age range of 18–30 years, participants who were willing to participate in the study and satisfied diagnostic criteria of RAS minor given by *Natah et al.*⁸ The exclusion criteria of the study include those patients did not show any interest to participate in the study, major herpetiform, RAS and ulcers in the inaccessible areas, RAS associated with other syndromes namely Reiter's, Behçet's, MAGIC (mouth and genital ulcers with inflamed cartilage), PFAPA (periodic fever, Aphthous stomatitis, Pharyngitis, and cervical adenitis), RAS patients already under topical or systemic management, Medically compromised patients such as Diabetes Mellitus, liver, renal disorders, polyketonuria, pregnant and lactating women, patients who are allergic to HA and pre-and probiotics, smokers.

All patients were given appropriate dietary counselling in order to avoid intake of spicy foods and are advised to encourage a bland diet. In Group A (n = 20), participants were advised to take each capsule of pre-and probiotic capsules (Bifilac HP Capsule, Tablets Ltd) twice daily with food for a week as a microbiome therapy and are educated to apply topical 0.2% HA gel (Gengigel, Milano, EU) thrice daily for a week. And in Group B (n = 20), participants were advocated to apply topical 0.2% HA gel thrice daily for a week. Participants in Group C (n = 20) were directed to apply topical 0.1% triamcinolone acetonide oro-mucosal paste (Kenocort, Abbott Healthcare Pvt. Ltd), thrice daily for a week.

MODE OF APPLICATION

Both topical 0.2% HA gel (Groups A and B) and 0.1% triamcinolone acetonide paste (Group C) were administered topically over and around 1 cm of each ulcer by the participants using a cotton applicator and are advised not to eat or drink for the next 30 mins. Each capsule of Bifilac contains prebiotics-fructooligosaccharides, inulin, and probiotics-lactobacillus sporegens (50 million spores), Bacillus mesentericus (TO-A, 1 million spores), Clostridium butyrcum (TO-A, two million spores), and Streptococcus fecalis (T-110, 30 million spores). The primary clinical outcome measure was pain relief and was recorded using ten graded Numerical Rating Scale (NRS) (0—nil, 1 to 3—mild, 4 to 6—moderate, 7 to 9—severe, >10—worst, unimaginable) and secondary outcome measures were degree of ulcer healing and occurrence of new ulcer.

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software program, version 25.0 (IBM). One-way ANOVA with post hoc Tukey test was performed among three groups and independent t- tests for comparing the degree of ulcer healing at third and seventh days. Paired t -test was performed for intragroup

comparison of outcome on day 3 and day 7. The level of statistically significant difference (α) was set at $P < 0.05$.

RESULTS

A total of 60 patients were enrolled in the study and completed the treatment and follow-up. The mean age of the participants was 23.3 ± 3.9 years with the range of 18–30 years. Various differences in baseline ulcer measurements such as NRS of pain, ulcer diameter and area between the groups were statistically insignificant ($P > 0.05$) as detailed in table – 2. Patients were followed up continuously

eventually to check the treatment adherence and diet. No patients were reported to develop any adverse effects after the intake of microbiome capsules. No new ulcers appeared on day 7 in Group - A patients. Mean and standard deviation of NRS, ulcer diameter, area and degree of ulcer healing are mostly tabulated in table - 3. The intragroup comparison of treatment outcome at 3rd and 7th days was observed to be highly significant ($P < 0.05$) at day - 3 and day - 7. Pain relief on the third day alone was statistically significant with observed P value less than 0.05, on comparing group A vs. group B ($P = 0.05$) and Group A vs. Group C ($P = 0.01$).

Table - 1: One-way ANOVA for baseline parameters

Parameters	Group A	Group B	Group C	P – value
Subjects (N)	20 (M=9, F=11)	20 (M=9, F=11)	20 (M=11, F=9)	
Ulcer duration (days)	7.2 ± 1.4	7.4 ± 2.6	7.23 ± 2.3	>0.05
Mean age \pm SD (years)	22.8 ± 3.7	224.7 ± 3.75	25.6 ± 3.95	>0.05
Ulcer diameter \pm SD(mm)	7.15 ± 0.99	7.74 ± 0.73	6.76 ± 1.45	0.22
Mean ulcer areas \pm SD(mm ²)	39.22 ± 10.6	43.7 ± 8	35.92 ± 13.44	0.263
NRS \pm SD	8.8 ± 1.13	8.9 ± 1.24	8.4 ± 0.95	0.062

Table - 2: Intergroup and Intragroup comparisons with Mean \pm SD of NRS score, ulcer diameter, ulcer area, degree of ulcer healing at day 3 and 7

Variables	Time	Group A	Group B	Group C	P – value
Mean NRS \pm SD	Day 3	3.1 ± 0.9	4.65 ± 1.72	3.52 ± 1.08	<0.05
	Day 7	0.32 ± 0.45	0.72 ± 0.93	0.54 ± 0.63	>0.05
	P - value	<0.05	<0.05	<0.05	
Mean ulcer diameter \pm SD	Day 3	2.83 ± 0.72	3.55 ± 1.2	3.32 ± 1.4	>0.05
	Day 7	0.52 ± 0.79	0.65 ± 0.62	0.85 ± 1.3	>0.05
	P – value	<0.05	<0.05	<0.05	
Mean ulcer area \pm SD	Day 3	6.46 ± 3.5	10.82 ± 4.8	10.76 ± 7.5	>0.05
	Day 7	1.14 ± 2.48	0.9 ± 1.12	1.66 ± 2.43	>0.05
	P – value	<0.05	<0.05	<0.05	
Degree of healing	Day 3	59.6 ± 9.12	59.22 ± 19.4	49.68 ± 15.77	>0.05
	Day 7	93.6 ± 11.24	90.63 ± 11.91	89.12 ± 14.91	>0.05
	P – value	<0.05	<0.05	<0.05	
Recurrence of new ulcers	Day 3	N=3	N=10	N=11	
	Day 7	N=0	N=6	N=9	

Table - 3: Post hoc Tukey test from One way ANOVA for intergroup comparison of NRS score, ulcer diameter, ulcer area, and independent t test for degree of ulcer healing at third day and seventh day

Variables	NRS for pain		Ulcer diameter		Ulcer area		Degree of healing	
	3 rd day	7 th day	3 rd day	7 th day	3 rd day	7 th day	3 rd day	7 th day
A vs B	0.05	0.51	0.62	0.57	0.99	0.98	0.37	0.08
B vs C	0.38	0.38	0.1	0.82	0.17	0.88	0.46	0.27
C vs A	0.01	0.27	0.5	0.51	0.09	0.62	0.11	0.53

* $P < 0.05$ is statistically significant

DISCUSSION

RAS is reported to be the most common multifactorial inflammatory disorder with an overall predominance rate of somewhere in the range of 5% and 66% in the age group of 10-40 years.⁹ In this study, the mean age of participants was 23.3 ± 3.9 years which was observed to be similar to previous studies.⁷ Ulcers that are resulted usually round or oval, regular, shallow floor covered by pseudo-

membrane with an erythematous halo surrounding it which predominantly manifest in the non-keratinized mucosa. Almost all the RAS patients in our study reported pain on the scale of mean NRS in the range of – 8.5 out of 10, which significantly decreased the patient’s quality of life, as it resulted in constant painful/burning sensation which normally interfered with daily activities like speaking, eating or even drinking as observed in the literature.¹⁰ RAS could be

classified into three different types: Minor, Major and Herpetiform ulcers.¹¹ Out of these, minor RAS is the most frequently observed one and it measures less than 1 cm in diameter which mostly heal without leaving any scarring behind.¹ Various factors like host and genetic factors, hormonal imbalance, stress, vitamin deficiency have been frequently attributed in the aetiologies of RAS. But all these factors affect the progression of the disease by influencing the composition of microbiota that normally resides on the oral mucosae, which in turn causes immunomodulation. This is called Microbial Dysbiosis in which there is a paradigm shift of pathogenic microbial community in the place of symbiotic ones.¹² Various other bacterial species have been identified in abundance in RAS patients when compared to healthy controls like L-form of *Streptococcus sanguinis* 2A,¹³ Bacteroidales,¹⁴ *Neisseria*, *Veillonella*, *Rothiadentocariosa*, *Streptococcus mitis*,¹⁵ *Acinetobacter johnsonii*¹⁶ showing the relationship between dysbiosis and RAS. Moreover, an autoimmune reaction is proposed against the oral epithelial peptides due to cross-reaction of some 65-kDa heat shock proteins produced by *Streptococcus* species, leading to mucosal damage.¹⁷ Microbiome therapy reverses the oral mucosal dysbiosis to eubiosis which is the transversion of pathogenic to symbiotic microbiota community similar to cariogenic bacteria and periodontal pathogens by enabling the host immune response.¹⁸

The recognised mechanism of actions of microbiome therapy are bacteriocin and hydrogen peroxide production; co-aggregation and growth inhibition of pathogenic bacteria; competitive antagonism on adhesion and nutrition; Immunomodulation by modulating the cytokines like IL-10, TNF- α , IL-8, and Toll-like receptors also might be a contributing factor.¹⁹ Its metabolites might activate the aryl hydrocarbon receptor which thereby enhance the synthesis of the tight junction proteins and increases the production of ZO-1 to effectively strengthen the epithelial barrier.⁴ This present study inferred a significant pain relief at the end of the third day in Group A patients who are receiving both microbiome therapy and topical HA gel, which could be effectively attributed to their epithelial barrier formation. Therefore, recent studies by *Dugourd et al*¹⁹ and *Pederson et al*²⁰ referred no statistically significant results on the comparison between probiotics over placebo in the treatment of RAS. Based on the results obtained from *Cheng et al*²¹ the combination of probiotics and steroids or anaesthetic antiseptic gel is more efficacious than its monotherapy in the management of RAS patients. In this study it was attempted to utilize the epithelial barrier-inducing property of both probiotics and HA and studied its efficacy when comparing it with monotherapy using conventional steroids.

HA proposes to induce re-epithelization through basal keratinocyte proliferation.²²

In our study, Group B patients who were treated with HA alone enlisted a statistically significant pain relief and ulcer healing when comparing the baseline and post-treatment values and only 8 patients developed new ulcers during the course of treatment. The same results were obtained by *Nolan et al*²³ in which only a few ulcers were observed on the day 5 of the investigation than those treated with placebo. *Dalassendri et al*²⁴ compared the two different formulations of the HA gel and mouth rinse in the treatment of RAS and inferred both the barrier formulations were effective in reducing the size of ulcer and pain intensity on the third day. But HA-based oral gel determined better results both in terms of the number of oral lesions and in terms of lesion sizes when compared with chlorhexidine mouth rinses.²⁵

CONCLUSION

The results obtained from this study elaborated the immediate pain control of topical HA and microbiome combination therapy in the effective management of RAS. Therefore, this could be considered as an effective alternative for topical steroids in the management of RAS.

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