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REVIEW ARTICLE

Recurrent aphthous ulceration: a review of potential causes and novel treatments

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ABSTRACT

Purpose of article: This review examines studies published between May 2012 and 2017 with a specific interest in potential recurrent aphthous ulceration (RAU) etiologies and treatment modalities/efficacy, including topical treatments, systemic regimens, vitamin repletion, and laser therapy, among others.

Materials and methods: PubMed MEDLINE and Cochrane Database of Systematic Reviews were searched using various combinations of: 'aphthous', 'ulcer', and 'treatment'. The titles and abstracts from the initial literature search were appraised to identify articles for full review and reference sections from each article were searched manually for relevant publications. Both randomized controlled trials and observational reports were included in this review, as some treatment types have not been formally examined in randomized trials. Relevant studies were reviewed, compared, and summarized.

Results: RAU can result from systemic disease and trauma, but recent studies have shown a variety of potential etiologies, ranging from vitamin deficiencies, oral microbiota derangements, hematological considerations, stress, genetic polymorphisms to oxidant-antioxidant imbalances, among others. Many modalities of therapy are available and have proven efficacious.

Conclusions: As the exact etiology of RAU is still unknown, therapy is based on symptomatic relief.

Abbreviations: ACE: Angiotensin converting enzyme; ARES: Paraoxonase-1 Arylesterase; BMI: Body Mass Index; CAT: Catalase; CCL3: cytokine CC-chemokine ligand 3; CCR1: cytokine CC-chemokine receptor-1; CCR5: cytokine CC-chemokine receptor-5; cEMT: carotid extra-medial thickness; cIMT: carotid intima-media thickness; CMP: Cow's Milk Protein; Cr: Creatinine; Fe: Iron; GPx: Glutathione Peroxidase; GSH: Glutathione; GSSG: Oxidized Glutathione; HAD: Hospital anxiety and depression scale; HDL: High Density Lipoprotein; Hgb: Hemoglobin; HgbA1c: Hemoglobin A1c; HOMA-IR: Homeostatic model assessment insulin resistance; HSV: Herpes Simplex Virus; LLLT: Low-Level Laser Therapy; MMP: Matrix Metalloproteinase; NRS: Numeric Rating Scale; OHIP-14: Oral Health Impact Profile-14; QoL: Quality of Life; RAU: Recurrent Aphthous Ulceration; ROS: Reactive Oxidant Species; RUT: Rapid Urease Test; SCMP: Specific Cow's Milk Protein; SEM: Standard Error of Mean; SOD: Superoxide Dismutase; TLR: Toll-like receptor; Total Chol: Total Cholesterol; TRFLP: Terminal-Restriction Fragment Length Polymorphisms; VEGF: Vascular Endothelial Growth Factor; VZV: Varicella Zoster Virus; Zn: Zinc

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Introduction

Worldwide, recurrent aphthous ulceration (RAU) is the most common oral ulcerative disease (1), with prevalence between 5 and 66% (2). The word 'aphthous' originates from the Greek word 'aphtha', meaning a mucosal surface ulcer. First described by Hippocrates in 400 BC (3), RAU has synonyms including 'recurrent oral ulcers' and 'recurrent aphthous stomatitis' (4). Clinically, patients present with painful round/oval oral ulcerations of varying sizes with clean edges surrounded by an erythematous border. In the center of the ulceration, the necrotic fundus is covered with a yellow-white fibrinous exudate (3) or mucus (5). The lesions are typically located on the buccal mucosa and tongue, with lesions on the heavily keratinized palatal and gingival mucosa being less common (6). The ulcers are painful secondary to exposed nerve endings due to epithelial necrosis exceeding the basement membrane (3). Characteristically, a burning sensation is present for 2–48 h prior to ulcer development (6). Overall, the RAU stages are 'premonitory' (hours-one day), 'pre-ulcerative' (1–2 d), 'ulcerative' (several days), and 'healing' (days–weeks) (7).

RAU is most common at young ages, with recurrence becoming less frequent as patient ages. The condition is uncommon above age 40 (4), although literature describing its occurrence in elderly populations is present (8). Table 1 describes the three types of RAU which have been described in the literature (minor, major, herpetiform) (5,6,9).

RAU is clinically diagnosed, with heavy reliance on medical history and physical examination findings. A thorough history can uncover possible RAU etiologies, including prior mucosal trauma, gastrointestinal disease (Celiac (10), Crohn (11)), Behcet disease (12), HIV (13) (including pediatric HIV (14)) and PFAPA syndrome (periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis) (15). Less common etiologies include coxsackievirus A16 (16), which is accompanied by the sudden appearance of a vesicular rash on the hands and feet (3), pityriasis rosea (17), and tuberculosis (18).

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Supplemental data for this article can be accessed here.

Table 1. Types of RAU and respective clinical findings (adapted from following references: 5,6,9).

	Type of RAS		
Clinical feature	Minor	Major	Herpetiform
Peak age (decade)	2nd	1st, 2nd	3rd
Number of ulcers	1–5	1–10	5–20 (up to 100)
Size of ulcers (mm)	<10	>10	1–2 (coalescing)
Duration of ulcers	7–14 d	2 weeks–3 months	7–14 d
Site	Non-keratinized mucosa, dorsum, and lateral borders of tongue	Keratinized and non-keratinized mucosa, including soft palate	Non-keratinized mucosa, particularly floor of mouth and ventral surface of tongue
Heal with scarring	No	Yes	No

A physical examination for oral ulcers should include an inspection for the number, type, and size of lesions, followed by palpation to evaluate for the consistency of the base (soft or hard) and fixation to underlying structures. One must consider the typical features of oral neoplastic lesions, including induration or rolled borders (5). Based on the history and physical examination, one can differentiate between RAU and other ulcerative oral diseases. While herpes simplex virus (HSV) may present with similar-appearing ulcers, it presents with diffuse gingival erythema and fever preceding lesion appearance. Also, HSV ulcers are preceded with vesicles and are found primarily on keratinized mucosa, including the hard palate and gingiva. Varicella zoster virus (VZV) can be differentiated based on its unilateral presentation following the trigeminal nerve path, painful prodrome, and extraoral manifestations (5). While phone screenings and photograph recognition have proven effective in RAU diagnosis, laboratory tests, and histopathology with regards to RAU are nonspecific (19).

Materials and methods

This review outlines publications which have been published since May 2012 regarding aphthous ulceration, specifically focusing on potential causes and treatments. PubMed MEDLINE (May 2012–2017) and Cochrane Database of Systematic Reviews (May 2012–2017) were searched using various combinations of: 'aphthous', 'ulcer', and 'treatment'. The titles and abstracts from the initial literature search were appraised to identify articles for full review and reference sections from each article were searched manually for relevant publications. Both randomized controlled trials and observational reports were included in this review, as some treatment types have not been formally examined in randomized trials.

Literature search findings

Mucosal barrier function

In healthy epithelium, superficial cells are desquamated at the end of their life cycles. In RAU, the mucosa reveals top-to-bottom apoptosis, resulting in the sloughing of dead cells and the formation of an ulcer (20). One proposal is that a lack of anti-inflammatory scavenger macrophages may result in apoptotic cells undergoing secondary necrosis. This releases proinflammatory signals and results in the inflammatory halo surrounding the ulcer. This hypothesis was supported with the finding of 'self-DNA-induced TNF-α synthesis' in SCC-25 keratinocytes ($p = .02$) (20). Zad et al. investigated mucin MUC7 oligosaccharides in samples from three RAU patients and three healthy controls. These oligosaccharides provide many of the salivary protective properties against microbial pathogens. A differential MUC7 glycosylation pattern was noted between the groups, suggesting functional changes in the mucin, and subsequent immunological/anti-bacterial functions (21).

Oral microbial flora

Microbial causes for RAU have been of scientific interest for over 50 years. In 1963, Barile et al. postulated that an unstable L-form of *Streptococcus sanguinis* 2A would convert to a transient, pathogenic form, potentially explaining the recurrence nature of the disease (22). Salivary microbial DNA isolation highlighted a higher frequency of isolation of *Neisseria* (88.9 vs. 25%, $p < .0001$) and *Veillonella* (22.2 vs. 0.00%, $p < .0001$) in healthy controls when compared to RAU patients. More frequent isolation of *Rothia dentocariosa* and *Streptococcus mitis* was noted from non-ulcerated RAU mucosa in comparison with ulcerated RAU mucosa ($p < .05$) (23). PCR analysis of terminal-restriction fragment length polymorphisms (TRFLP) in bacterial DNA from buccal swabs showed differences in the microbiota of non-inflamed buccal mucosa between RAU patients and healthy controls. These differences were more pronounced in those with active lesions during the sampling. In addition to 36 species being found exclusively in the RAU group, a statistically higher prevalence of peaks 60 and 489 TRFLP was found ($p = .04$ and $p = .03$, respectively), confirming the presence of differing microbiota (24). *16S rRNA* gene sequencing demonstrated decreased *Firmicutes* ($p = .034$) and increased *Proteobacteria* ($p = .032$) when comparing ulcerated RAU sites to healthy RAU sites, in addition to increased total *Bacteroidales* in RAU patients with healthy sites compared to healthy controls ($p = .04$) (25).

In a comparison of mucosal and salivary mucosa in RAU patients and healthy controls, a decreased amount of healthy core microbiota (*Streptococcus salivarius,* $p = .02$) and increased rare microbiota (*Acinetobacter johnsonii,* $p = .03$) was found. *A. johnsonii* was found to inhibit gingival epithelial cell proliferation, with greater cytotoxicity to these cells than *S. salivarius* (26). In addition, some streptococcal bacterial strains release 65-kDa heat shock proteins which cross-react with oral epithelial peptides, resulting in an autoimmune reaction leading to mucosal damage (27).

Non-bacterial organisms have also been associated with RAU, including high Epstein–Barr virus salivary load (RAU 43% vs. healthy controls 25%, $p = .0109$) (28) and the presence of candida (29) in RAU. Overall, these experiments demonstrate that idiopathic RAU patients may be suffering from oral mucosal microbiota derangements, which may then trigger the lesions (24,25). However, it is possible that the presence of RAU may alter the oral microbiota. While more investigation is warranted, future treatments may involve restoring the oral microbiota to a healthier balance (23).

Helicobacter pylori

Helicobacter pylori is a gram-negative microaerophilic bacterium mostly known for its association with gastric ulcers has also been investigated as a potential factor in RAU. After reviewing 15 studies, Gomes et al. did not find any strong evidence of a direct cause-effect between *H. pylori* infection and RAU development. They postulate that *H. pylori* in the ulcer may reflect a passenger infection (30). Another meta-analysis of seven case-control studies (339 RAU cases, 271 healthy controls) found 100/339 (29.50%) of RAU cases to be *H. pylori* positive, compared to 54/271 (19.93%) of healthy controls ($p = .002$) (31). Dental plaque samples from 38 RAU patients and 42 healthy controls were tested for *H. pylori* utilizing the rapid urease test (RUT), which was positive in 34/38 (89.5%) RAU patients, compared to only 24/42 (57.1%) healthy controls ($p = .002$) (32). In an experiment utilizing 46 RAU patients as self-controls, the number of RAU lesions and vitamin B12 levels during the past six months was recorded (33). Utilizing endoscopic biopsy, 30/46 was found to be *H. pylori* positive and received subsequent treatment for eradication. After three months, these 30 patients underwent the urea breath test, with 18 testing negative and 12 positive. Six months post-eradication, the mean number of RAU lesions (over six months) was significantly decreased in the eradicated group ($p = .0001$) but not in the non-eradicated group ($p = .677$). Interestingly, the B12 levels were significantly increased in the eradicated group ($p = .001$), but not in the non-eradicated group ($p = .638$) (33). Thus, while Gomes et al. report that *H. pylori* likely does not directly cause RAU, a potential benefit of eradicating *H. pylori* in RAU patients may be due to an increase in B12 levels (30).

Diet, allergies, and nutrition

In a study of 50 RAU patients and 50 healthy controls, the levels of anti-SCMP (specific cow's milk protein) IgA, IgG, and IgE antibodies were significantly higher in the RAU group ($p < .005$), especially to several casein proteins (34). Similarly, two cases of chronic pediatric RAU stopped within two weeks of discontinuing consumption of cow's milk protein (CMP). These children remained RAU free while avoiding this protein, only to have a recurrence with re-ingestion, with the presence of elevated IgE to the CMP (35). Another study involved patch testing 24 RAU patients and 22 healthy controls to 23 food additives. Then, 21/24 RAU patients (87.5%) experienced positive reactions to one or more allergens, in comparison to 3/22 healthy controls (13.6%) ($p < .05$). However, the RAU in this study was only diagnosed via clinical history without analysis of patient histories of atopic disease or infectious/autoimmune causes of oral ulceration via lab testing. Finally, they did not analyze the association between the patch test outcomes and the severity of the RAU (36). One study contradicting the allergic hypothesis examined the role of contact allergies to toothpaste ingredients in RAU (37). Overall, the role of contact allergies was unclear (37).

Vitamin/mineral deficiency is also a potential cause of RAU. Aynali et al. compared vitamin B12, hemoglobin (Hgb), and folic acid in 57 RAU patients and 45 healthy controls. While a significant difference between serum Hgb and folic acid was not present between the groups, the serum B12 levels were significantly lower in the RAU group ($p < .05$) (38). Lower B12 levels in RAU were also noted in other studies of 60 active RAU patients and 60 healthy controls ($p < .001$) (39) and 355 RAU patients and 355 healthy controls ($p = .03$) (40). A meta-analysis of nine case-control studies (710 total RAU patients, 602 total healthy controls) found that the rate of deficiencies in vitamin B12 (OR: 3.75, 95% CI: 2.38–5.94) was higher in the RAU group (41), as did another study of 273 RAU patients and 273 healthy controls ($p < .001$) (42). These results correlated with Tas et al.'s concurrent finding of decreased RAU occurrences and higher vitamin B12 levels in RAU patients after *H. pylori* eradication (33). A study examining vitamin D levels in 46 RAU patients and 49 healthy controls found significantly lower vitamin D levels in the RAU group ($p = .0001$) (43), while another study comparing vitamins A, E, and C in 25 RAU patients and 24 healthy controls did not find a significant difference between the groups ($p > .05$) (44).

In a study comparing 156 RAU patients and 115 healthy controls, the mean serum Zn was significantly higher in the healthy control group. None of the healthy controls had a Zn level below the minimum normal value, while 33/156 (21.2%) of the RAU group were Zn deficient ($p < .001$) (45). In an investigation comparing 25 RAU patients to 25 healthy controls, Zn deficiency was present in 28% of the RAU group, compared to only 4% of the healthy controls, with serum Zn levels being significantly lower in the RAU group ($p = .001$) (46). When comparing 33 RAU patients and 30 healthy controls, Ozturk et al. found serum Zn and selenium levels to be significantly lower in the RAU patients, while copper was higher in the RAU patients ($p < .05$) (47).

Immunology

The oral epithelium participates in adaptive immunity via upregulating the expression of major histocompatibility class II antigens (48). The nuclear factor kappa beta (NF-κB) pathway is the main pathway of adaptive immunity and involves post-receptor activation via mucosal interactions with pathogenic bacteria/antigens (49). In a test group of 14 RAU patients, immunohistochemical staining of NF-κB activation was present in all cases. The strong staining was present specifically in areas within squamous epithelium adjacent to the ulcerated lesions, the subepithelial vascular endothelial cells, and the present inflammatory cells. In contrast, mucosal epithelium far from ulcerated areas showed some NF-κB staining only in the basal layer. Thus, modulating inappropriate NF-κB signaling may play a role in treating RAU (49). A study comparing 30 RAU patients to 30 healthy controls found higher salivary IL-2 levels in the RAU group (50). IL-2 is a molecular cytokine which is secreted by activated T helper cells and is involved in regulating both cellular and humoral chronic inflammatory responses ($p < .001$) (50).

IgA is produced by plasma cells and is a primary protective antibody at mucosal surfaces (51). One study comparing 33 RAU patients and 33 healthy controls found that IgA levels were significantly increased during active RAU phases in comparison to remission RAU phases ($p = .01$) and in healthy controls ($p < .001$). Even during remission phases, RAU patients still had higher IgA levels compared to healthy controls ($p = .01$) (52). In contrast, another study examining salivary IgA levels in 20 RAU patients and 20 healthy controls did not find a significant difference between the groups ($p = .42$) (51). These conflicting results highlight the need for further study in the immunologic aspects of RAU. IgE is a key to the pathogenesis of many allergic diseases. In a study of 49 RAU patients, at IgE levels greater than 120 IU/mL and 150 IU/mL, there was a significant association with RAU onset at a younger age ($p = .016$, $p = .022$, respectively). In addition, IgE levels greater than 150 IU/mL were associated with the presence of a RAU episode every two weeks ($p = .017$) (53).

Hepcidins are proteins which have antimicrobial effects and have been shown to be lower in saliva from RAU patients ($n = 30$) compared to healthy controls ($n = 25$) ($p = .03$). Salivary prohepcidin was also shown to be lower in the RAU group ($p = .007$) (54).

This finding parallels the findings that oral microbiota in RAU patients may differ than those of healthy controls, possibly due to altered immune defenses. RNA for the cytokine CC-chemokine ligand 3 (CCL3) and its receptors (CCR1 and CCR5) was found to be overexpressed in RAU tissues from 29 RAU patients compared to 20 healthy controls ($p < .01$). In blood samples from the same patients, only CCR1 overexpression was present in the RAU patients ($p < .05$). As these cytokines enhance T-cell activity, stimulate macrophage function, and protease secretion, these results suggest an altered immune system response in RAU and may explain the intense inflammation present at the lesion sites (55). Finally, in a study comparing ulcerated RAU mucosa ($n = 12$) and clinically normal mucosa ($n = 6$), a significant difference in *p53* gene immunostaining was noted in the RAU lesions ($p = .01$). *p53* is a tumor suppressor gene whose activation leads to transcription of multiple apoptotic genes, including Bax, while inhibiting the production of proteins which inhibit cell death, such as bcl-2. When comparing Bax and bcl-2 immunostaining between the two groups, a significant difference was not found (56).

Stress

A questionnaire-based survey involving 1006 college students found that high frequencies of colds and bedtimes after 11 pm ('physical stress') are independent risk factors for RAU occurrences ($p < .001$, $p < .001$, respectively) (57). In another study, recent stress and anger/anxiety were assessed in patients with active RAU ($n = 26$) and in healthy controls ($n = 55$). The RAU group was more likely to have recent stress ($p < .01$) and more angry/anxious feelings ($p < .001$) (58). One study of 160 RAU patients found that stressful life events were significantly associated with RAU onset (OR, 2.72, $p < .001$), with mental stressors having a larger impact than physical stressors (OR =1.44, CI: 1.04–1.99). The RAU episodes were not found to occur with increased frequency or last longer with more severe stress (59). In a study of 53 RAU patients, the patients had significantly lower oral health impacts and quality of life during active RAU episodes compared to non-active time periods (oral health impact profile-14 (OHIP-14), $p < .0001$). Higher OHIP-14 scores were also significantly associated with higher depression indices ('Hospital anxiety and depression (HAD) Scale') ($p = .036$) and anxiety ($p = .012$) (60).

Chemical markers of stress have been shown to be elevated in RAU. In a study comparing 30 RAU patients to 30 healthy controls, the mean serum, salivary, and urine cortisol levels were significantly higher in the RAU cases ($p < .001$, $p < .001$, $p < .001$, respectively) (61). In another study of 30 RAU patients and 30 healthy controls, the mean salivary cortisol levels were higher in the RAU group ($p < .001$), as were anxiety scores (Hamilton anxiety scale) ($p < .001$). A correlation of 0.980 was found between anxiety and salivary cortisol ($p < .001$) (62).

Reactive oxidant species and antioxidant levels

RAU histology reveals immune cell infiltration and the activity of these cells increases reactive oxidant species (ROS) which damage cells via peroxidation (63). Paraoxonase-1 arylesterase (ARES) is an enzyme which protects cell low-density lipoprotein from oxidation. In a study comparing 44 RAU patients and 38 healthy controls, serum total antioxidant status, and ARES activity were significantly lower in the RAU group ($p < .001$, $p < .001$, respectively), while total oxidant status and an oxidative stress index were significantly higher ($p = .008$, $p < .001$, respectively) (64). Malondialdehyde (MDA), a stable end product of membrane lipid peroxidation by free radicals is a frequent subject of investigation as it represents increased lipid peroxidation (44). In a study comparing 28 RAU patients and 28 healthy controls, salivary MDA levels were significantly higher ($p < .001$) and total antioxidant capacity levels were significantly lower ($p < .042$) in the RAU group (65). Glutathione (GSH) and oxidized glutathione (GSSG) are markers of oxidative status in cells, with the normal GSH:GSSG ratio being 10:1–100:1. In a study comparing 28 RAU patients with active ulceration and 29 healthy controls, GSH levels were significantly lower in the RAU group, while mean MDA and GSSG levels were significantly higher ($p < .01$). In addition, antioxidant levels from nine of the 28 RAU patients were compared during active RAU flares and RAU remissions. During active episodes, GSH levels were significantly lower while MDA and GSGG levels were significantly higher ($p < .05$) (66). Glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) are enzymes which function to protect the body against oxidative stress. In a comparison of 97 RAU patients and 102 healthy controls, when comparing RAU patients with active flares to healthy controls, serum levels of GPx, SOD, and CAT were lower in the RAU group ($p < .05$). A comparison of the 97 RAU patients during a flare and a remission period revealed lower levels of all three enzymes during flares, while a comparison of the RAU remission phase to healthy controls showed no significant difference in any enzyme levels ($p > .05$) (67). Under normal settings, a balance between ROS and antioxidants is present. When ROS predominate, cell death may result. Whether oxidative stress-related cell death directly leads to RAU requires further study. Based on the above findings, however, it is evident that ROS/antioxidant levels are imbalanced in those who suffer from RAU, even when comparing individuals as self-controls during RAU flares and times of remission.

Hematologic factors

In a study comparing 44 RAU patients and 38 healthy controls, high-density lipoprotein (HDL) levels were higher in healthy controls ($p < .001$) (64), while another study of 81 RAU patients and 61 healthy controls found total cholesterol (TC) ($p = .008$) and HDL ($p = .002$) to be higher in the RAU group (68). In a comparison of 60 active RAU patients and 60 healthy controls, lower serum ferritin, hematocrit, and folic acid levels were found in the RAU group ($p = .001$, $p < .001$, $p < .001$, respectively) (39). In another comparison of 355 RAU patients and 355 healthy controls, lower mean Hgb ($p < .001$), iron (Fe) ($p < .001$), and folic acid ($p = .014$ if with concurrent atrophic glossitis, $p < .001$ if without atrophic glossitis) were found in the RAU group. Homocysteine was found to be higher in RAU patients ($p < .001$ if with atrophic glossitis, $p = .001$ if without atrophic glossitis), with the authors postulating that this may increase the frequency of thrombosis in arterioles which feed the oral epithelial cells (40). Another study of 273 RAU patients and 273 healthy controls confirms these findings, with Hgb, Fe, and folic acid deficiencies being significantly more common in the RAU group ($p < .001$, $p < .001$, $p = .022$, respectively), while homocysteine was found to be more frequently abnormally elevated ($p < .001$) (42). A meta-analysis of nine case-control studies (701 total RAU patients, 602 total healthy controls) found that the rate of hematinic deficiencies was significantly higher in the RAU group, specifically folic acid (OR: 7.55, 95% CI: 3.91–14.60), ferritin (OR: 2.62, 95% CI: 1.69–4.06), and Hgb (OR: 1.77, 95% CI: 1.12–2.80) (41).

Erythrocyte sedimentation rate (ESR) and mean platelet volume (MPV) were examined in a study of 60 RAU patients and 60 healthy controls. The ESR, a marker of inflammation, and MPV

level, an indicator of platelet activation and sign of chronic inflammation, were significantly higher in the RAU group ($p < .001$) (69). Another study of 80 RAU patients and 80 healthy controls did not find a significant difference in MPV levels, lymphocyte count, platelet count, or platelet-to-lymphocyte ratio. However, a significantly higher level of white blood cells, neutrophils, and neutrophil-to-lymphocyte ratios was found in the RAU group ($p < .05$), further supporting the involvement of inflammation in RAU (70).

Other associations

Many genes may have a potential impact on RAU pathogenesis, including genes in the vascular endothelial growth factor (VEGF) pathway (71), polymorphisms in angiotensin converting enzyme (ACE) genes (72), and a matrix metalloproteinase gene polymorphism (MMP-9) (73). Polymorphisms involved in inflammation have also been discovered in RAU patients, including IL-6 (74,75), IL-10 (76), Toll-like receptor (TLR4) (77), and inflammasome-related *NLRP3* genes (78).

Prescription medications have been reported to result in RAU. Samimi et al. report two cases of RAU occurring after Tocilizumab (anti IL-6R monoclonal antibody) treatment. Once the Tocilizumab was discontinued, the lesions resolved within 6–7 weeks. Later, re-administration led to RAU recurrence in both patients within 10 d (79). Aphthous ulceration has also been associated with orlistat use (80).

An investigation comparing tobacco usage in 102 RAU patients and 108 controls without RAU showed that tobacco users have significantly lower RAU outcomes (odds ratio: 0.41, $p = .02$) (52). RAU has also been reported as an adverse effect of illicit drug use. A case of 15-d persistent aphthous ulceration has been reported to occur in the same timeframe with cocaine smoking and rubbing. Fifteen days after discontinuing the cocaine use, the patient experienced full regression of the lesions (81). One possible explanation is cocaine's vasoconstrictive properties secondary to endothelin-1 release and inhibition of nitric oxide production (82), which would result in hypoperfusion of the mucosa.

Sereflican et al. compared carotid extra-medial thickness (cEMT) and carotid intima-media thickness (cIMT) in 32 RAU patients and 30 healthy controls. Both cEMT and cIMT were higher in the RAU group ($p = .002$, $p = .013$, respectively), providing evidence of subclinical atherosclerosis (83). This could also represent a limit to mucosal perfusion as a cause of RAU.

As periodontal infection was found to be associated with insulin resistance in diabetes-free adults (84) and insulin resistance was found to be higher in Behcet disease patients (85), a possible relationship between RAU and prediabetes was proposed in 2013 (86). In 2015, Takci et al. compared blood glucose, insulin, C-peptide, hemoglobin A1c (HbA1c), and the homeostatic model assessment insulin resistance (HOMA-IR) in 81 RAU patients and 61 healthy controls. In the RAU group, c-peptide, insulin, and HOMA-IR levels were significantly higher than in healthy control levels ($p = .015$, $p < .0001$, $p < .0001$, respectively). Of the 81 RAU patients, 42 had active ulceration. In this active group, the levels of HbA1c ($p = .02$), blood glucose ($p = .045$), and HOMA-IR (0.022) were higher than those in RAU passive stage. This demonstrates a higher level of insulin resistance in RAU patients, which in turn worsens with active RAU (68). While Takci et al. propose that elevated inflammatory signals may interfere with insulin signaling, further study is needed to investigate the cause-effect relationship.

Finally, two cases of chronic aphthous ulceration have been reported in patients with sympathetic nervous system (SNS) hypofunction. After treatment with dextroamphetamine sulfate to alleviate the SNS dysfunction, the aphthous ulcerations (and vasomotor symptoms) did not return (87).

Treatments

Studies investigating RAU treatment regimens have accumulated rapidly in recent years. Due to the magnitude of studies available, those with the largest sample sizes and most complete study methodologies were analyzed.

Topical treatments

A randomized, double-blind, placebo-controlled, multi-center clinical trial investigating the efficacy of dexamethasone ointment ($n = 120$) vs. a placebo group ($n = 120$) showed that ulcer size ($p = .001$) and pain values ($p = .001$) were significantly decreased in the study group by day 6 (88). In a double-blind, randomized, multi-center trial comparing topical 2% lidocaine ($n = 31$) and a topical placebo cream ($n = 33$), a significant decrease in lesion pain was present 3 min after lidocaine application compared to placebo application ($p = .025$) (89). The comparison of one-time use topical crushed doxycycline ($n = 25$) and a topical placebo ($n = 25$) revealed significantly less pain ($p < .001$) and decreased mean time of healing in the treatment group (3.7vs. 5.3 d, $p < .001$) (90). This correlates to a possible microbial cause in RAU. A randomized study comparing chemical cauterization with silver nitrate sticks ($n = 35$) and placebo sticks ($n = 30$) reported significantly decreased pain scores as soon as 1 d post-$AgNO_3$ application ($p < .01$), increased proportions of study subjects with complete re-epithelialization by day 7 ($AgNO_3$: 60% vs. placebo: 33.3%, $p < .01$), and decreased mean healing time in the study group (2.7 vs. 5.5 d, $p < .01$) (91). A randomized, double-blind trial comparing topical honey ($n = 34$ patients, 64 ulcers), triamcinolone acetonide ($n = 57$ ulcers, 30 patients), and placebo paste ($n = 56$ ulcers, 30 patients) revealed that honey treatment significantly decreased ulcer size ($p = .001$), increased pain relief ($p = .001$), and decreased healing time ($p = .001$) compared to the triamcinolone and placebo groups (92). This further supports the potential use of honey for wound healing (93). The use of curcumin gel ($n = 30$), an Indian spice pigment with strong anti-inflammatory and analgesic properties were compared to a triamcinolone acetonide gel ($n = 30$). Independent analysis of each group revealed a statistically significant decrease in ulcer pain, size, number, and duration from days 0–7 ($p < .05$ for all findings for each group). Intergroup analyses, however, did not reveal any significant differences between the two groups, leading to the conclusion that curcumin may be a suitable alternative to topical steroids in RAU treatment (94). The effect of covering choline salicylate with a flexible mucoadhesive patch revealed more rapid pain reduction and ulcer healing in the group with the presence of the patch ($p < .001$, $p < .001$, respectively) (95). A case of treatment-resistant aphthous ulceration since childhood in a 39-year-old lifetime non-smoker improved within 4 d of starting nicotine lozenges, with complete control within 14 d. The patient remained ulcer free for six months post-treatment initiation (96). One possible mechanism is nicotine's ability to modulate immune responses via the inhibition of pro-inflammatory cytokine production (97). Another proposed mechanism is nicotine's role in increased oral mucosal keratinization (98). This study also correlates with the finding that smokers had a lower incidence of RAU (52). Topical amlexanox, an anti-allergy

compound which inhibits the formation/release of histamine and leukotrienes from mast cells, neutrophils, and mononuclear cells has been shown to accelerate RAU healing (99). Topical herbal products have also been a recent subject of investigation. Iralvex, an anti-aphthous herbal agent containing 170 mg of dried rhubarb extract and 10 mg of salicylic acid has been shown to decrease pain and healing time in RAU. This product contains tannins which interact with mucosal epithelial cells in astringency, the process of strengthening mucosal adhesion and decreasing cell permeability, resulting in increased protection from toxins and microbes (100). Myrrh, the dried resin of *Commiphora* plant species, has been used as an anti-inflammatory product and for its wound healing properties. It can induce WBC maturation, differentiation, and activation, as well as increase the mRNA expression of type III collagen, resulting in healing. Topical myrrh has been shown to improve RAU healing when compared to a placebo (101). The use of diosmectite and basic fibroblast growth factor in RAU has also been studied. Diosmectite is a natural aluminosilicate clay which has previously been shown to prevent toxins and bacteria from binding to intestinal membranes and can also act as a drug carrier by binding to organic compounds. Basic fibroblast growth factor is a direct mitogen for vascular endothelial cells, fibroblasts, and epithelial cells, thus playing a significant role in wound healing (102). In combination, diosmectite and basic fibroblast growth factor resulted in a statistically significant decrease in ulcer pain and mean ulcer size in comparison to the use of each compound separately and when compared to a placebo treatment (102). Studies investigating the topical use of aloe vera (101,103), vitamin B12 (104), ozone gas (105), and triester glycerol oxide/triamcinolone acetonide (106) are also described in detail in Table 2 (see online supplementary materials).

Mouthwash treatments

A randomized, double-blind, crossover study comparing 0.5% ($n = 14$) and 0.2% ($n = 14$) minocycline mouthwash revealed a significant reduction in ulcer pain with the higher dosing at days two and 10 of treatment ($p = .032$, $p = .027$, respectively). In addition, the ulcers healed quicker in the 0.5% group (5.64 vs. 6.85 d, $p = .011$) (107). A double-blind clinical trial investigating triamcinolone ointment with Zn mouthwash ($n = 20$) and triamcinolone ointment with a placebo mouthwash ($n = 20$) revealed that reductions in lesion size and pain severity in the Zn mouthwash group were not statistically significant ($p = .322$, $p = 1.00$, respectively) (108). Thus, despite evidence of more prevalent Zn deficiency in RAU patients, it does not appear that a Zn-based mouthwash improves lesion healing. When comparing a sucralfate suspension group ($n = 35$) and a chlorhexidine oral rinse ($n = 35$), decreased pain at days one, three, and seven post-treatment was noted in the sucralfate group ($p = .0001$, $p = .0001$, $p = .023$, respectively), as was mean healing time (1.97 vs. 2.80 d, $p < .05$) (109). Sucralfate is typically used for providing a protective barrier for ulcers in the gastrointestinal tract, which supports the finding that physical coating an aphthous ulceration with a barrier improves healing (95). Finally, when comparing chamomilla tincture, an herbal medication with anti-inflammatory and analgesic properties, to a placebo mouthwash, the chamomilla tincture group ($n = 21$) experienced a decrease in mean number of aphthous lesions compared to the placebo group ($n = 15$) ($p = .025$, $p < .001$, $p < .001$ at days two, four, and six, respectively). Ulcer pain and size were significantly less in the chamomilla group starting at day two ($p = .001$, $p = .03$, respectively) (110).

Vitamin supplementation

A randomized, double-blind, placebo-controlled trial examined the effects of multivitamin intake on new RAU episodes. Comparisons of the test ($n = 83$) and placebo groups ($n = 77$) revealed no significant differences in the duration and number of new RAU episodes ($p = .60$, $p = .69$, respectively). One study limitation was that vitamin circulatory levels were not measured at the study end (111). This finding was supported by another randomized, double-blind, controlled trial studying multivitamin tablets. No significant differences in the number and duration ($p = .60$) of new RAU episodes were seen after one year of multivitamin consumption (112). In contrast, a randomized, double-blind, placebo-controlled study examining the effectiveness of 1000 mg of omega-3 in RAU treatment found that the omega-3 group ($n = 25$) had a lower number of monthly ulcers compared to the placebo group ($n = 25$) after three months of treatment ($p < .05$). Additionally, ulcer duration decreased and pain scores began improving after three months ($p < .05$, $p < .01$, respectively) (113). Similar outcomes were found in another randomized, double-blind, placebo-controlled study comparing 1000 mg omega-3 ($n = 25$) to a placebo ($n = 25$). After six months of consuming omega-3, ulcer size, and pain scores (VAS) were reduced significantly ($p = .010$, $p < .001$, respectively). The number of monthly ulcers also declined, beginning at four months ($p = .045$). Ulcer recurrence was decreased after five and six months ($p = .038$, $p < .001$, respectively) (114). The benefit of omega-3 fatty acids may be due to their ability to mediate cellular functions of polymorphonuclear leukocytes (113,114), modulate lymphocyte proliferation, and increase mRNA expression of host antioxidant enzymes including GPx, CAT, and SOD, among other anti-inflammatory properties (113).

Systemic medical therapy

While localized therapy is preferred due to a lower risk of adverse effects, some reports describe efficacy with systemic medical therapy. A phase 0 clinical trial investigating the use of 3 mg subcutaneous enoxaparin injections in 30 RAU patients completed weekly for eight weeks total found a significant decrease in mean number of RAU episodes, ulcer size, ulcer duration, recurrence intervals, and pain scores ($p = .001$ for all outcomes). The authors postulate that low dose enoxaparin prevents T-lymphocyte migration and in turn inhibits delayed hypersensitivity 115. A phase 0 clinical trial is the first clinical trial done involving human patients and is designed to evaluate if an agent will work as intended in humans. This process involves giving less than 1% of the therapeutic dose of an investigational drug (116). A randomized, single-blind trial contained three study groups: levamisole 50 mg ($n = 20$), levamisole 50 mg and low-dose prednisolone 5 mg (combo treatment) ($n = 20$), and placebo ($n = 10$). Within the levamisole and combo treatment groups, ulcer pain ($p < .001$, $p < .001$, respectively), number of ulcers per RAU episode ($p = .03$, $p < .001$, respectively), ulcer duration ($p < .001$ for both groups), and frequency of RAU episodes per month ($p < .001$ for both groups) were decreased. When compared to the placebo group, both the levamisole and combo treatment groups had decreased pain scores ($p < .01$, $p = .01$, respectively), number of ulcers per episode ($p = .03$, $p = .04$, respectively), ulcer duration ($p = .05$, $p = .02$, respectively), and frequency of RAU episodes per month ($p < .001$ for both groups). No significant differences were found when comparing the levamisole and combo treatment groups (117). While the mechanism of levamisole's actions in RAU is still not fully understood, one possible mechanism involves the augmentation of

suppressor T-cells which has been shown to occur *in-vitro*, with the overall result of immunosuppression (117). Lenalidomide, a second-generation immunomodulatory drug which has prominent uses in multiple myeloma (118) has also been utilized in the treatment of RAU. A case of severe pediatric aphthous ulceration resolved within several days of treatment initiation with lenalidomide after multiple preferred options failed either due to poor response or adverse effects. No adverse effects were noted after three years of treatment (119). Given the overall weak nature of evidence, more structured randomized case-control studies investigating these findings are necessary. One Cochrane systematic review involving 25 separate trials and 21 different interventions found that only one study had a 'low risk of bias'. They concluded that no single treatment was effective, likely reflecting the 'poor methodological rigor of (the) trials'. These limitations included, but were not limited to, unclear inclusion criteria, the risk for selection bias, and the potential of detection bias due to the subjective nature of the outcomes (120). Given the lack of strong evidence of these systemic therapies, they are often reserved for RAU which is recalcitrant to topical or local therapies (120,121).

Laser therapy

A prospective randomized trial was conducted in 30 patients acting as self-controls, as each had two discrete oral aphthous ulcers. Low-level laser therapy (LLLT) was applied to the test ulcers ($n = 30$), with the same technique being applied to the control ulcers ($n = 30$) without turning on the laser. In the test ulcer group, pain scores were reduced immediately post-treatment, with reduction continuing for three follow-up days ($p < .001$ for all time periods). Lesion size was significantly reduced in the treatment group starting at 1 d post-procedure ($p < .05$), while pain scores and lesion size were not significantly altered in the control group ($p > .05$). Overall complete healing time was significantly decreased in the laser group (3.05 vs. 8.90 d, $p < .001$) (122). Another study investigated the use of CO_2 laser in 25 patients serving as self-controls, as each had two oral ulcers. Within the first 24 h post-treatment, pain scores dropped significantly ($p \leq .001$). Mean healing time was decreased in the laser-treated ulcers (4.08 vs. 7.84 d, $p < .001$) (123). A split-mouth study in 40 patients with two ulcers each found that pain scores dropped significantly ($p < .05$) after 20 s of using an Er,Cr:YSGG laser. In addition, the mean healing scores of the laser-treated ulcers were significantly improved ($p < .05$ at days one, three, and seven) (124). Systematic reviews have also concluded that laser treatments significantly decrease pain and improve healing compared to respective placebo treatments. Han et al. evaluated the efficacy of six different lasers: CO_2, AMD, GaA1As, Nd:YAG, InGaA1P diode, and SIX Laser TS diode ($n = 382$, 10 studies included) (125). Najeeb et al. also performed a systematic review on four types of lasers: CO_2, diode, Nd:YAG, and GaA1As ($n = 321$, nine studied included). They found that the lasers were successful in providing patients with immediate pain relief. More specifically, Najeeb et al. found that CO_2 lasers were the most advantageous because they required a shorter exposure time (5–10 s) (126). Given the lack of rigorous methodologically designed studies, more clinical trials which compare laser treatment to the currently available medical therapy in RAU are still necessary (125). After conducting their own systematic review of 11 studies, Suter et al. highlight the need for further studies to ascertain which specific laser type and settings (wavelength, power) provide superior treatment (127). Additional studies investigating the use of a gallium-aluminum-arsenic laser (128), a non-thermal, non-ablative CO_2 laser (129), a diode laser (130), and LLLT (131) are described in detail in Table 2 (see online supplementary materials).

Ongoing and proposed clinical trials

Many clinical trials investigating potential RAU treatments are currently ongoing. A University of Copenhagen study is currently recruiting participants for a double-blind, randomized, placebo-controlled study examining the salivary and fecal microbiota of RAU patients before and after probiotics treatment (132). The University of Tokushima is currently recruiting participants for a study investigating whether night guard use suppresses RAU development. The patient's oral condition will be recorded for 60 d prior and 60 d post-night guard intervention. In addition, patient saliva will be analyzed for inflammatory cytokines/oxidative stress (133). One future study which is not yet recruiting participants is Cairo University's double-blind, randomized comparison of topical antioxidant coenzyme Q10 and carpabol gel in aphthous ulcer management (134). Another future study that is not yet recruiting participants is the Centre Hospitalier Universitaire de Nice's investigation of the efficacy and safety of probiotic *Lactobacillus rhamnosus* in RAU treatment. This study will be randomized, placebo-controlled and double-blind (135).

Conclusions

In a patient presenting with RAU, the first step is to complete a thorough history and physical examination to rule out systemic disorders and ulcerative conditions which have more definitive treatments. In those without a viable alternate cause, one can screen for dietary findings which suggest allergies or possible vitamin/mineral deficiencies. In addition, counseling patients on managing stress/decreasing stressors may prove helpful. Helpful labs included a CBC, Fe, and folic acid levels, in addition to screening for potential medication causes and tobacco dependence in long-term smokers. As RAU etiology is still not fully understood, all current treatment is aimed at symptomatic relief. One can replace antioxidants, potentially via fruit consumption which would assist with vitamin replenishment. Topical therapies, including mouthwashes, should be first-line therapy due to their lower risk of adverse effects. In non-responsive disease with/without severe symptoms, one can consider the addition of a systemic therapy to the current topical regimen (136). Finally, while the evidence is in its infancy, laser therapy may be an option for improving pain control and healing in recalcitrant lesions.

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