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Oral Supplemental Interventions for the Management of Recurrent Aphthous Stomatitis (RAS) – a Systematic Review and Meta-Analysis

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Declaration

I hereby declare that "Oral Supplemental Interventions for the Management of Recurrent Aphthous Stomatitis (RAS) –a Systematic Review and Meta-Analysis" is my own personal work, that it has not been submitted previously in its entirety or part for any degree or examination at any other University, and that all sources that I have used or quoted here have been properly indicated and acknowledged by a complete list of references.

Waqas Mirza

May 2022

Dedication

I dedicate this thesis to my amazing parents, who provided me with unconditional love and support, encouraged me to work harder and perform my best at whatever I put my mind to.

I also dedicated my work to my beloved siblings. They always believed in me and offered reassurance throughout the way. I could not have accomplished as much as I did without their love and support.

Lastly, I would like to thank all my friends, for their help and support. Because of them, I had a wonderful time staying and studying in South Africa.

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Abstract

Background: Recurrent aphthous stomatitis (RAS) is described as a painful, recurrent oral mucosal ulcerative condition in otherwise healthy individuals. Treatment objectives include pain relief/reduction, prevention of secondary infection, promoting healing and reducing recurrence. Conventional treatment modalities include topical or systemic corticosteroids, analgesics and antiseptic mouth rinses. Recent evidence suggests a therapeutic role for oral supplementation in the management of RAS.

Objectives: To assess the efficacy of oral supplements such as, vitamin B12, zinc sulfate and omega-3 to resolve and reduce the severity and recurrence of recurrent aphthous stomatitis.

Methods: Three databases were thoroughly searched for all studies published up to October 2021. All randomised clinical trials that evaluated the efficacy of vitamin B12, zinc sulfate and omega-3 in the management of RAS were included.

Results: The researchers meticulously screened clinical randomised control trials and eight clinical trials fulfilled the inclusion criteria. The follow up period ranged from two days to six months. Six studies displayed significantly better efficacy of oral supplements in reducing the pain, whereas four studies reported on decrease in ulcer diameter associated with RAS.

Conclusion: Oral supplementation with vitamin B12, zinc sulfate and omega-3 seem to be beneficial in the management of RAS. However, additional research is required that includes standardised methodologies to validate the efficacy of the oral supplementations.

Keywords:

Vitamin B12

Cobalamin

Zinc sulfate

Omega-3

Oral diseases

Randomized controlled trials

Clinical trials,

Quasi randomized clinical trials

RAS

Recurrent aphthous stomatitis

Systematic review

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1. Background

1.1 Description of the condition

Recurrent Aphthous Stomatitis (RAS), colloquially referred to as canker sores, is considered amongst the most prevalent ulcerative oral mucosal inflammatory conditions globally.

RAS represents a spectrum of painful oral mucosal ulcers characterised clinically as discrete, localised, painful, shallow, well-defined lesions, surrounded by an erythematous rim, with a yellow or greyish base (Porter et. al., 2000; Porter et. al., 1998; Ship et. al. 2000). Aphthous ulcers can arise anywhere within the oral cavity, such as the labial mucosa, buccal mucosa, floor of mouth, tip or ventral surface of the tongue, soft palate or tonsillar areas (Anne et. al., 2003). This painful, ulcerative, oral mucosal inflammatory condition often greatly influences every day activities such as, eating, swallowing, and speaking (Anne et. al., 2003; Miller et. al., 1977). The spectrum of RAS includes three main clinical variants - minor, major or herpetiform. Minor aphthous ulcers are the most prevalent subtype, have a diameter of 10 mm or less, are round, distinctly defined, symptomatic and heals within 10 to 14 days without scarring (Field et. al., 1992). On the contrary, major aphthous ulcers are greater than 10 mm in diameter, heal within two to three weeks or even longer, often with resultant scarring (Wray et. al., 1975). Herpetiform aphthous ulcers are less common and present as small (2-3 mm), pinpoint, multiple (1-100) irregular ulcers which often tend to coalesce. A female predominance is particularly seen in this type and the age of onset is greater (20-29 years) than in the previous two subtypes (Lehner, 1977; Scully et. al., 1989; Porter et. al., 1991).

1.1.1 Epidemiology

Epidemiological analyses suggest that RAS affects between 2% to 50% of the global population (Scully et. al., 2008). Most evaluations fall within 5% to 25%, with three-month recurrence rates shown to be up to 50% (Scully et. al., 2008; Rees et. al., 1996; Ship, 1972). Epidemiological studies revealed that prevalence statistics of RAS is affected by the population studied, diagnostic criteria employed and the influence of various environmental factors (Chavan et. al., 2012). It generally is seen in otherwise healthy individuals and the exact cause essentially remains unknown. The prevalence of RAS was reported to be as high as 39 percent in children, influenced by the manifestation of RAS in one or both parents. Children with RAS-positive parents are 90% more likely to develop RAS and children with RAS-negative parents are 20% more likely to develop the condition. Relationship between RAS and advancing age is believed to be indirectly proportional, with the peak onset between 10 and 19 years of age (Chavan et. al., 2012).

Although the exact cause remains idiopathic for true RAS (Porter et. al., 2000), RAS-like oral mucosal ulcerations can have a multifactorial aetiology (systemic diseases, psychological stress, puberty, trauma, smoking cessation, food sensitivities, genetic predisposition, immunological disorders, immunosuppressive medications, Human Immunodeficiency Virus infection, etc.) (Porter et. al., 2000; Porter et. al. 1998),

1.1.2 Description of the intervention

Management strategies for RAS is largely palliative, aimed at reduction of pain, to shorten the duration of ulcers/reduce healing time and decrease recurrence. No available therapy is known to cause complete remission, though various topical and systemic therapies have been

employed. Standard of care includes the local or systemic use of corticosteroids, which come with possible side effects, such as, oral candidiasis, weight gain, muscle weakness, and lower resistance to infection. Nutritional deficiencies or haematological conditions (i.e. iron deficiency anaemia, neutropenia) have shown a strong positive correlation in the formation/aetiology of recurrent aphthous ulcerations (Casiglia et. al., 2010; Nolan et. al., 1991). Numerous studies have found that patients receiving treatment for iron, folate, and vitamin B12, show a 71% improvement after the replacement therapy (Oslon et. al., 1982; Piskin et. al., 2002; Dholakia et. al., 2005).

In 2009 Volkov et al., conducted a randomised double-blind, placebo-controlled trial to favour vitamin B12 in the management of RAS. The study proposed that daily use of 1000 micrograms (mcg) vitamin B12 placed beneath the tongue could be prophylactic for aphthous ulcers after five to six months of continued use. Its success as management for patients experiencing RAS was independent of their serum vitamin B12 level (Volkov et. al., 2009). The root cause of vitamin B12 deficiency is thought to be due to food-cobalamin malabsorption as a result of gastric dysfunction. Vitamin B12 is an essential supplement required for DNA synthesis and its deficiency can lead to suppression of cell division in the bone marrow. As a consequence, red blood cells (RBCs) increase in size leading to megaloblastic anaemia. In the oral cavity, this can result in various oral manifestations, one of which is generalised recurrent oral ulcerations (Volkov et. al., 2009). Therefore, oral intake of vitamin B12 can considerably reduce the rate of recurrence and symptoms associated with RAS (Volkov et. al., 2009).

Zinc sulfate plays a fundamental role in providing nutrition for growth in humans, animals, and plants (Fickel et. al., 1986; Keen et. al., 1990; Solomons, 1979; Bates et. al. 1981). In a study by Sharquie et al., 2008, the therapeutic and preventive function of oral zinc sulfate

and dapsone were evaluated in the management of RAS. A substantial decrease in the number of aphthous ulcers was seen in both treatment arms. Zinc sulfate had a much greater, rapid and prolonged action and less harmful side effects as compared to dapsone (Sharquie et. al., 2008). Oral zinc sulfate has also proven to be beneficial in the treatment of RAS. Each living cell and bodily secretions contain zinc, the second most common trace metal in the body of all human beings (Lansdown, 1996). It has evoked considerable curiosity due to its immunoregulatory, antioxidant, and antimicrobial properties. It is a crucial component of carbonic anhydrase, DNA and RNA polymerases, reverse transcriptase, proteases, and enzymes that play a fundamental role in accelerating the wound healing process (therefore, preserving epithelial integrity). Various studies encourage its possible use in the management or prevention of recurrent oral ulcers (Lansdown, 1996).

Fish oil is loaded with omega-3 polyunsaturated fatty acids, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Current studies have examined the favourable outcomes of fish oil on chronic inflammatory processes for instance, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and chronic periodontitis (Duffy et. al., 2004; Calder, 2008; Elkhouli, 2011). Various studies agree that 1.5 g of DHA/EPA supplements daily may considerably help to reduce inflammatory processes, though some say a higher dose (3.5 g/day) may be more beneficial (Kremer et.al., 1990). In 2016, Nosratzehi et. al., examined the effects of omega-3 on RAS and the results found that the symptoms associated with RAS decreased substantially with its use. EPA and DHA are considered long chain Omega-3 fatty acids because EPA contains 20 carbon atoms and DHA contains 22 carbon atoms. Both DHA and EPA control lymphocyte proliferation by alerting the cellular function of polymorphonuclear leukocytes. They are known to regulate the movement of inflammatory cells to the sites of inflammation and block the proinflammatory cytokine production. They also improve the activity and mRNA expression of antioxidant enzymes

such as glutathione peroxidase, superoxide dismutase and catalase, which ultimately reduces inflammation and stimulates tissue regeneration at the site of the lesion (El-Gendy, 2014). Higher levels of Omega-3 have a suppressive effect on the production of Omega-6 arachidonic derived proinflammatory prostaglandins and leukotrienes, thus limiting the amount of tissue damage. Omega-3 metabolites such as pro-resolving lipid mediators, resolvins and protectins can help with resolution of inflammation and aid in wound healing via their anti-inflammatory and immunoregulatory effects (El-Gendy, 2014).

The effectiveness of the intervention is evaluated through size of the lesion and pain reduction. There are a number of ways through which pain levels can be measured, such as the Visual Analog Scale (VAS) and Numerous Rating Scale (NRS) (Bielewicz et. al., 2022). For the NRS scoring system the patient is given a 11-point scale ("0" indicates no pain and "10" indicates most severe pain), is then asked to grade their own pain intensity. Whereas, the VAS scoring system requires no scale and there's a 100 mm horizontal line (left end indicating "no pain" and right end representing "most severe pain"). The patient is then asked to mark pain intensity that he/she is going through at the time of examination (Bielewicz et. al., 2022).

1.1.3 Why is it important to do this review?

RAS is common worldwide, a considerably painful condition and significantly affects quality of life. Oral supplements such as vitamin B12, zinc sulfate and omega-3 are prescribed anecdotally in the management of recurrent aphthous stomatitis, despite inadequate clinical evidence-based literature supporting its use. No previous systematic reviews have been conducted on this question to date.

1.1.4 The review question

In persons with recurrent aphthous stomatitis, are oral supplemental interventions (Vit B12, Zinc Sulphate, Omega 3), compared with standard of care, associated with improved clinical outcomes?

2. Aim of the study

The aim of this review is to assemble evidence to support or refute the use of oral supplements such as vitamin B12, zinc sulfate, and omega-3 for the management of RAS.

3. Materials and Methods

3.1. Criteria for considering studies in this review

3.1.1 Types of studies:

All studies that concentrated on the use of oral supplements such as, vitamin B12, zinc sulfate and omega-3 in the management of recurrent aphthous stomatitis were included.

- Inclusion criteria:
    - Otherwise-healthy children and adults with RAS
    - Randomised controlled clinical trials, quasi randomised clinical trials
    - Utilization of oral vit B12, zinc sulfate or omega-3 alone or in any combination with other forms of treatment for RAS will be used
    - Studies reporting clinical cure/resolution of RAS

- Exclusion criteria:
    - Patients with RAS-like lesions with systemic conditions
    - Studies without control groups
    - Case reports
    - Case series
    - Studies with inadequate data (means, *SD*)
    - In-vitro studies and
    - Review papers

3.1.2 Types of Participants/Population:

Children and adults with RAS with no underlying systemic conditions that may contribute to lesion formation. RAS is clinically defined as being discrete, localised, painful, shallow, well-defined lesions, surrounded by an erythematous rim, with a yellow or greyish base.

3.1.3 Types of Intervention:

We included all interventions that used oral supplements including vitamin B12, zinc sulfate or omega-3 alone or in any combination with other forms of treatment.

3.1.4 Types of Comparator/Control:

Standard of care.

3.1.5 Types of outcomes measures:

Primary outcomes

The primary objective was to determine whether oral supplements were successful in causing complete resolution of signs (clinical cure), aided in the resolution of symptoms and reduction in the lesion severity (clinical response). This was accomplished by measuring pain via VAS and NRS scores and the diameter of ulcers.

Secondary outcomes

The secondary objectives included rate of effectiveness of the intervention, rate of recurrence, healing time and adverse effects associated with all of the three interventions.

3.1.6 Time Frame and Language

There were no restrictions set on time frame or language in the electronic database search during the search process. We did not find any articles that were not written in English.

3.2 Information sources and search strategy

The protocol for this systematic review was registered with PROSPERO registry of the University of York, with the registration number (CRD42020201197). It followed the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P) (Moher et. al., 2009).

A thorough literature search was carried out and completed in October, 2021. We used the following search (strategy in PubMed (Table 1), which was then altered according to the various databases being used for the searching process. The searches were merged with the medical subject headings (Mesh) and the following keywords were used “vitamin B12, cobalamin, zinc sulfate, omega-3” AND “recurrent aphthous stomatitis or RAS”, i.e., "Stomatitis, Aphthous"[Mesh] OR Aphthous[Title/Abstract] AND ORAL[Title/Abstract] AND "Vitamin B 12"[Mesh] OR "Zinc Sulfate"[Mesh] OR "Fatty Acids OR "Omega-3"[Mesh].

Table 1: Example of search strategy for oral supplements fir managament of ras

Search number	Query	Sort By	Filters	Search Details	Results
12	#11 AND #9			(("Aphthous"[Title/Abstract] AND "ORAL"[Title/Abstract]) OR "stomatitis, aphthous"[MeSH Terms]) AND ("Vitamin B 12"[MeSH Terms] OR "Zinc Sulfate"[MeSH Terms] OR "fatty acids, omega 3"[MeSH Terms])	55
11	#4 OR #1			("Aphthous"[Title/Abstract] AND "ORAL"[Title/Abstract]) OR "stomatitis, aphthous"[MeSH Terms]	4,149
9	#6 OR #7 OR #8			"Vitamin B 12"[MeSH Terms] OR "Zinc Sulfate"[MeSH Terms] OR "fatty acids, omega 3"[MeSH Terms]	49,828
8	"Fatty Acids, Omega-3"[Mesh]			"fatty acids, omega 3"[MeSH Terms]	25,691
7	"Zinc Sulfate"[Mesh]			"Zinc Sulfate"[MeSH Terms]	1,853
6	"Vitamin B 12"[Mesh]			"Vitamin B 12"[MeSH Terms]	22,379
4	#2 AND #3			"Aphthous"[Title/Abstract] AND "ORAL"[Title/Abstract]	1,473
3	ORAL[Title/Abstract]			"ORAL"[Title/Abstract]	630,512
2	Aphthous[Title/Abstract]			"Aphthous"[Title/Abstract]	3,531
1	"Stomatitis, Aphthous"[Mesh]			"stomatitis, aphthous"[MeSH Terms]	3,420

The following databases were used to conduct the search: PubMed, The Cochrane Library and Scopus. We also used google scholar (first 200 hits) to complement our search strategy. It included randomized controlled clinical trials and quasi randomized clinical studies of vitamin B12, zinc sulfate, and omega-3 supplements alone or combined/compared with other forms of treatment. Hand-searching of references of the included studies was carried out, to find any additional studies that pertained to this systematic review. The search results were fully documented, reported and compared between the various databases. The reference

manager that we used for our references was Mendeley software. We used Rayyan software to aid us in screening of the abstracts and titles and accelerate the inclusion and exclusion process (Ouzzani et. al., 2016).

3.3 Study procedure and statistical methods

3.3.1 Study selection and data extraction

All the articles were confirmed by two reviewers (WM and HH) independently, according to the eligibility of studies, and the discrepancies were resolved by consensus. Both the researchers individually performed title, abstract screening and full-text evaluation. The entire data was extracted and gathered in a standardised template with excel software. For any lacking information, we tried to communicate with the corresponding authors. Standard data extraction tables included study characteristics, eligible criteria, participants' characteristics, interventions, outcomes, follow-ups, adverse events.

To assess the quantitative data from the included studies, the researchers tabulated these results into Review Manager (RevMan) software for statistical analysis and then all the required data was gathered to perform a meta-analysis of the studies. We assessed two primary outcomes: 1) pain severity levels; and 2) the diameter of the ulcer. These studies analysed clinical change at different time intervals for the interventions. Forest plots were made for each of the interventions at various time intervals.

All data involving the continuous variables was expressed and reported using mean difference and 95% confidence interval (95% CI). The weight of every study was estimated based on the inverse of the variance. We used a random-effects model for this analysis. Where the data was lacking and incomplete, a narrative report was generated of the results.

3.3.2 Assessment of methodological quality of trials and risk of bias

Both the reviewers carried out an assessment for the quality of the study and risk of bias for all the included studies. We used the Cochrane Collaboration risk of bias tool to accomplish this. The quality of the research will be evaluated by using the Cochrane risk of bias criteria for grading of the included studies.

After Applying this approach, the evidence will be classified as high, moderate, low, very low quality based on the risk of bias, inconsistency, indirectness, imprecision, and other domains. We assumed that the quality of the evidence was the highest at first and incrementally decreased according to the deficiencies of the study.

3.3.3 Investigation of heterogeneity

To analyse heterogeneity, we used the I^2 test. If $I^2 < 50\%$, we considered that there is no heterogeneity, and a fixed effects model was performed. If $I^2 > 50\%$, we considered that there is heterogeneity, by using a random effects model in the study. Where heterogeneity was present, we tried to carry out sensitivity analysis and subgroup analysis to detect the source of heterogeneity.

3.3.4 Sensitivity Analysis

Where heterogeneity was high sensitivity analysis was performed.

3.3.5 Subgroup Analysis

There was sufficient data to perform subgroup analysis.

3.3.6 Assessment of Reporting Bias

Funnel plot was used to detect publication bias of included studies. There was not enough data to perform the assessment of reporting bias.

4. Results

4.1 Study selection

The PRISMA flowchart was used in the study selection process (Fig. 1). We found 128 titles among the electronic databases searched. No additional articles were found via hand-searching. The titles were assembled and 27 duplicates were removed. The remaining 101 titles were analysed and 25 were excluded based on title and abstract and further six were excluded due to full-text not being available. Seventy full-text articles were assessed for eligibility, out of which 62 were excluded with reasons, as mentioned in Figure 1. There were eight studies included for the qualitative synthesis and seven were used for quantitative synthesis (meta-analysis).

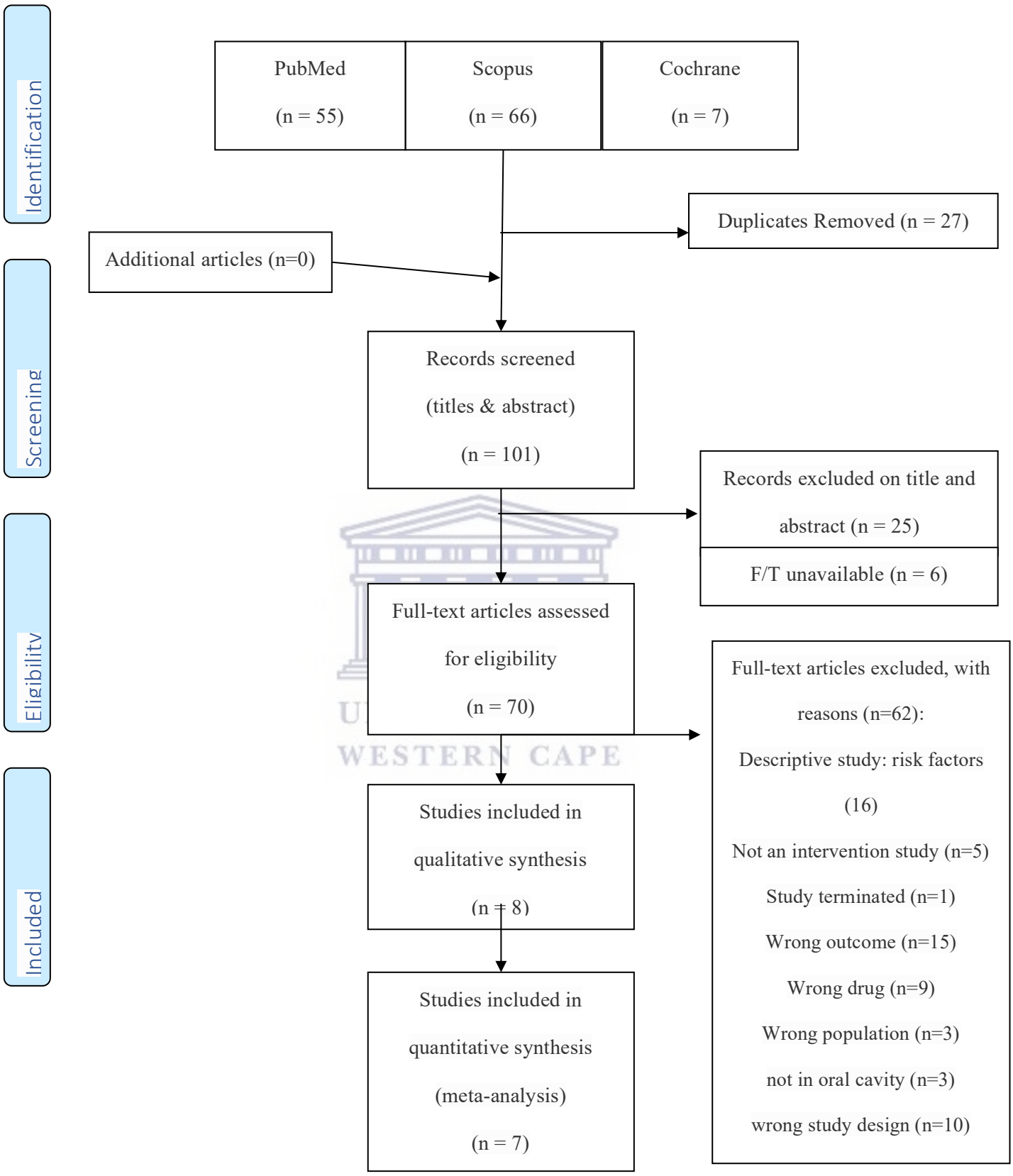

Figure 1. Schematic PRISMA flow diagram of the literature search.

4.2 Study designs

All studies that consisted of oral supplements (vitamin B12. Omega-3 and zinc sulfate) for the management of recurrent aphthous stomatitis were included, all of which were randomised control trials.

4.3 Study Participants (Table 2)

All the studies included in this review were carried out in different parts of the world. Three of the studies were conducted in Iran (Ghobani et al., 2020; Hadian et al., 2021; Nosratzehi et al., 2016), the rest of the studies were conducted in Egypt (El Khouli et al., 2014), Taiwan, (Liu et al., 2015), Turkey (Orbak et al., 2002), Iraq (Sharquie et al., 2008), and Israel (Volkov et al., 2009). All studies mentioned the mean age of the participants, except for the study conducted by Sharquie et. al., in 2008. It also did not mention the male: female ratio in all of the study groups. We tried contacting the author but unfortunately, we did not receive a response.

Design and Demographics of included studies (Table 2)

			Intervention group			Control group		Type of interventior
Author and year (country)	**Study design**	**Nature of participants**	**Participants No.**		**Mean Age (years)**	**Participants No.**	**Mean Age (years)**	
El Khouli et al., 2014 (Egypt)	RCT	Outpatient clinic of the Oral Medicine and Periodontology Department, Faculty of Dentistry, October 6 University	25		33.68	N: 25	Age: 32.52	omega-3
Ghorbani et al., 2020 (Iran)	RCT	Referred to Mazandaran Dentistry Clinic	23		38.66	N: 23	Age: 41.28	Zinc Sulfate
Hadian et al., 2021 (Iran)	RCT	Referred to Babol Faculty of Dentistry	20		37.7	N: 20	Age: 37.75	Omega-3
Liu et al., 2015 (Taiwan)	RCT	Referred by primary physician	22		49.36	N: 20	Age: 56.30	vitamin B12
Nosratzehi et al., 2016 (Iran)	RCT	Outpatient clinic of the Oral Medicine Department, Faculty of Dentistry at Zahedan University of Medical Sciences	25		37.13	N: 25	Age: 31.6	Omega-3
Orbak et. Al., 2002 (Turkey)	RCT	Admitted to the Periodontology Department of Ataturk University Faculty of Dentist	20		26.9	N: 20	Age: 29.25	Zinc Sulfate
Sharquie et al., 2008 (Iraq)	RCT 3-arm	Outpatient clinic of the Oral Medicine Department, Faculty of Dentistry at Zahedan University of Medical Sciences	Group A: Zinc Sulphate 15	Group B: Dapsone 15		Group C: N: 15		Zinc sulfate
Volkov et al., 2009 (Isreal)	RCT	Referred by primary physician	31		33.1	N: 27	Age: 29.15	Vitamin B12

4.4 Treatment Parameters (Table 3)

4.4.1. Nature of the intervention

All of the included studies used one of the three interventions (vitamin B12, zinc sulfate or omega-3). Two studies used vitamin B12 as an intervention to see the effectiveness of it in treating RAS (Liu et al., 2015; Volkov et al., 2009). Three studies used zinc sulfate to evaluate the effectiveness of it in the improvement of RAS (Ghobani et al., 2020; Sharquie et. al., 2008; Orbak et al., 2002). Lastly, three studies evaluated the treatment of RAS by analysing the effects of omega-3 fatty acids and improvement of quality of life (El Khouli et al., 2014; Hadian et al., 2021; Nosratzehi et al., 2016).

4.4.2. Intervention arms and treatment regimen

Three studies used omega 3 capsules (1000 mg) three times daily for six months (El Khouli et al., 2014; Hadian et al., 2021; Nosratzehi et al., 2016). Three studies used zinc sulfate as the treatment; one used 5 mg mucoadhesive tablet three times a day for 7 days (Ghobani et al., 2020), the second study used 220 mg once a day for one month (Orbak et al., 2002), and the last study used 150 mg capsule 2 times a day for 3 months (Sharquie et. al., 2008). Two studies used vitamin B12 as an intervention, one used 500 µg oral ointment four times a day for 2 days (Liu et al., 2015), while the other one used a single dose of 1000 mcg sublingual tablet for six months (Volkov et al., 2009).

Table 3- Type, Dosage and Frequency of interventions in selected RCTs

Author and year (country)	Treatment arms	Intervention parameters	Condition treated	Method of clinical assessment
El Khouli et al., 2014 (Egypt)	**Intervention**: 1 g omega-3 capsule **Control**: placebo soft gelatin capsule with the same prescription.	Omega-3 (1 g, 3 times daily) for 6 months.	RAS	VAS
Ghorbani et al., 2020 (Iran)	**Intervention**: Zinc sulfate (5 mg) mucoadhesive tablets. **Control**: tablet same in appearance and content as zinc sulfate mucoadhesive tablets, except for the active ingredient of zinc sulfate. 3 times a day for 7 days.	Zinc sulfate (5 mg) as zinc sulfate (8H2O), carbopol 940, sodium alginate, starch and sucrose in mucoadhesive tablets, 3 times a day for 7 days.	Minor aphthous ulcers	VAS, Ulcer size
Hadian et al., 2021 (Iran)	**Intervention**: omega-3 capsules (1000 mg) **Control**: placebo capsules similar to the omega-3 capsules which contained flour.	Each omega-3 capsules (1000 mg) prepared by 120 g of DHA and 180 g of EPA for 6 months	RAS	VAS, ulcer size
Liu et al., 2015 (Taiwan)	**Intervention**: 500 mg vitamin B12 oral ointment **Control**: placebo oral ointment (containing the same ingredients except for the vitamin B12).	Each oral ointment box contained 500 μg of vitamin B12, triamcinolone acetonide 0.1%, and natural cherry flavor; the weight of each box of ointment was 2 mg. Given for one week	Aphthous ulcers	VAS
Nosratzehi et al., 2016 (Iran)	**Intervention**: 1000 mg omega-3 capsule **Control**: Placebo capsules with the same prescription	1000 mg omega-3 capsules, 3 times aday for six months.	RAS	VAS, ulcer size
Orbak et. Al., 2002 (Turkey)	**Intervention**: zinc sulfate (220 mg) **Control**: placebo (saccharose, once per day before a meal)	In the first group (group I), the individuals were administered zinc sulfate (220mg, once per day before a meal) for one month.	RAS	Saliva ALP, saliva ALP, Serum ALB, Serum zinc levels
Sharquie et al., 2008 (Iraq)	**Group A**: Zinc Sulfate 150 mg capsule **Group B**: Dapsone 50 mg capsule **Group C**: Glucose 250 mg capsule as placebo, 2 times daily after meals for 3 months.	Group A: Zinc Sulfate 150 mg capsule, 2 times daily after meals for 3 months. Group B: Dapsone 50 mg capsule, 2 times daily after meals for 3 months.	Oral apthous ulcers	Ulcer size
Volkov et al., 2009 (Isreal)	**Intervention**: 1000 mcg tablet of vit B12 **Control**: placebo tablets (containing the same ingredients except for the vitamin B12)	A single sublingual dose of 1000 mcg vitamin B12 tablet was used for 6 months. Each tablet contained 1000 mcg of vitamin B12, mannitol, stearic acid, magnesium stearat, and natural cherry flavor; the weight of each tablet was 100 mg.	RAS	NRS

4.4.3 Method of clinical assessment

Five studies used Visual Analog Scale (VAS) to assess the average level of pain during the follow up period (El Khouli et al., 2014; Hadian et al., 2021; Nosratzehi et al., 2016; Ghobani et al., 2020; Liu et al., 2015). One study by Volkov et. al., employed a different method by using Numerous Rating Scale (NRS) to examine the pain level.

Four studies performed a visual assessment by measuring the decrease in the average diameter of the ulcer in millimeters, during the follow up period (Hadian et al., 2021; Ghobani et al., 2020; Nosratzehi et al., 2016; Sharquie et. al., 2008). The study by Orbak et. al., 2002, did not measure neither the pain level, nor the ulcer size, therefore it was excluded from the review.

4.5 Outcomes: Clinical improvement:pain

4.5.1. Pain scores (Any system) (6 studies, n=280)

Administration of supplemental interventions showed significant clincical improvement, as compared to the control group, thus demonstrating clincially effectiveness (mean difference (MD) -1.21 [95% CI, -2.24 to -0.18];I_2=93%) (Fig. 2).

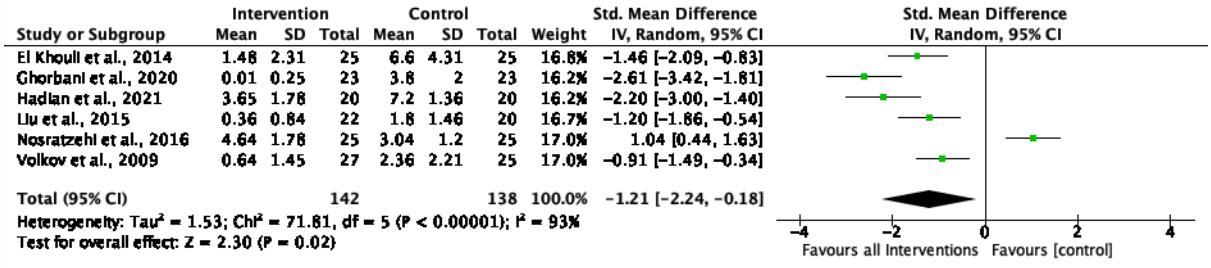

Figure 2: Forest plot of average pain level based on VAS and NRS (all interventions)

4.5.2. VAS-based outcomes (5 studies, n=228)

When using VAS to assess average pain level, supplemental interventions showed significant clincical improvement, as compared to the control group, thus being consistent with clincial effectiveness (mean difference (MD) -2.40 [95% CI, -4.59 to -0.20]; I^2 =96%) (Fig. S2).

4.5.3. Effectiveness of Omega-3 (VAS-based) (2 studies, n=90)

When analysing omega-3 on its own at the third month, the finding remained consistent with regard to clincal efficacy as assesed using VAS (Fig. 3) (mean difference (MD) -1.36 [95% CI, -2.33 to -0.40]; I_2=76%).

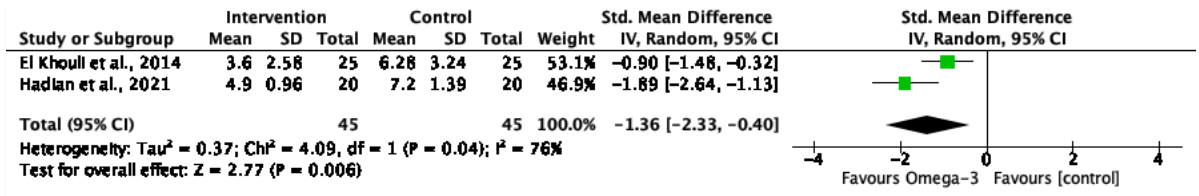

Figure 3: Forest plot of average pain level based on VAS at 3 month (omega-3)

4.5.4. Effectiveness of Omega-3 (VAS-based) (3 studies, n=140)

When analysing omega-3 on its own at sixth month, the finding remained consistent with regard to clinical efficacy as assessed using VAS (Fig. 4) (mean difference (MD) -2.30 [95% CI, -6.46 to 1.85]; $I^2 = 98\%$).

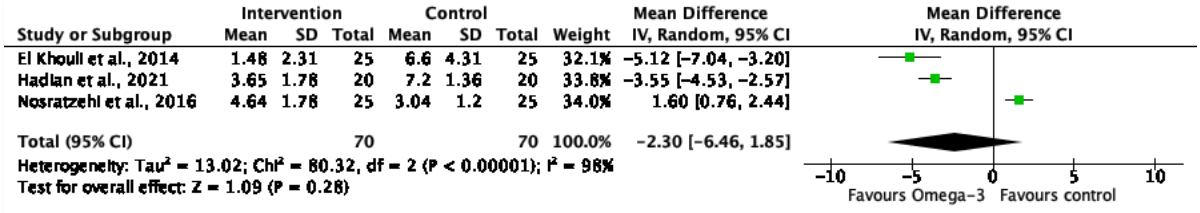

Figure 4: Forest plot of average pain level based on VAS at 6 month (omega-3)

4.5.5. Effectiveness of vitamin B12 (VAS;NRS-based) (2 studies, n=94)

When analysing vitamin B12 on its own, the findings were statistically significant in regard to clinical efficacy as assessed using both VAS and NRS (Fig. 5) (mean difference (MD) -1.04 [95% CI, -1.47 to -0.60]; $I^2 = 0\%$).

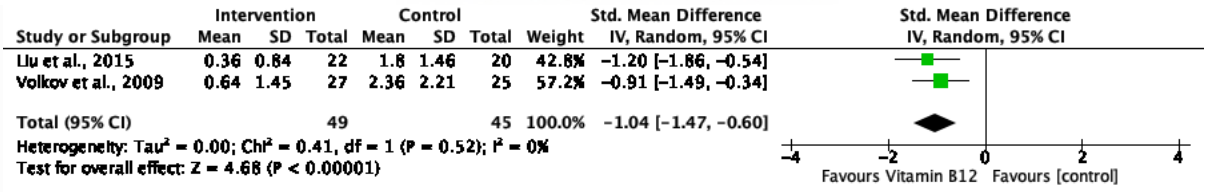

Figure 5: Forest plot of average pain level based on VAS and NRS (vitamin B12)

4.5.6. Effectiveness of zinc sulfate and omega-3 at third month (2 studies)

When analysing both omega-3 and zinc sulfate respectively, both were statistically significant as assesed by decrease in ulcer size (Fig. 6)

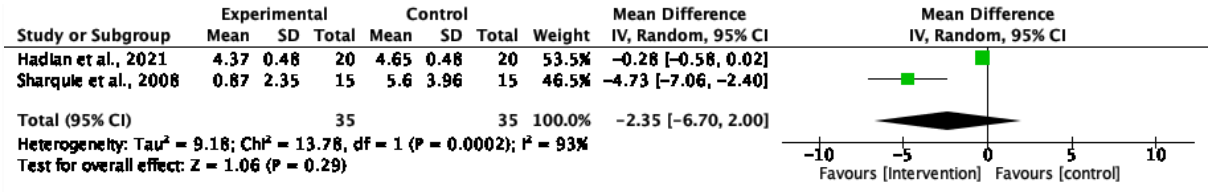

Figure 6: Forest plot of decrease in ulcer diameter in millimetres at 3 months

4.5.7. Effectiveness of omega-3 at sixth month (2 studies, n=90)

When analysing omega-3 on its own at six month interval, no statistically significant difference was found with regard to clinical efficacy, as assessed by decrease in ulcer size (Fig. 7) (mean difference (MD) 0.01 [95% CI, -2.23 to 2.34]; $I^2 = 94\%$).

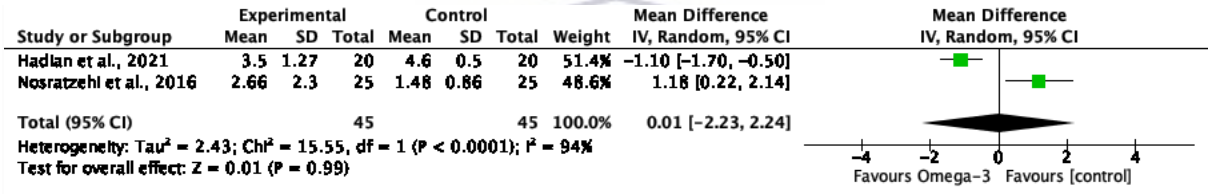

Figure 7: Forest plot of decrease in ulcer diameter in millimetres at 6 months (omega-3)

4.5.8. Effectiveness of zinc sulfate at sixth month (2 studies, n=76)

When analysing zinc sulfate on its own at six month interval, the findings were statistically significant with regard to clinical efficacy as assessed by decrease in ulcer size (Fig. 8) (mean difference (MD) -3.25 [95% CI, -5.57 to -0.92]; $I^2 = 74\%$).

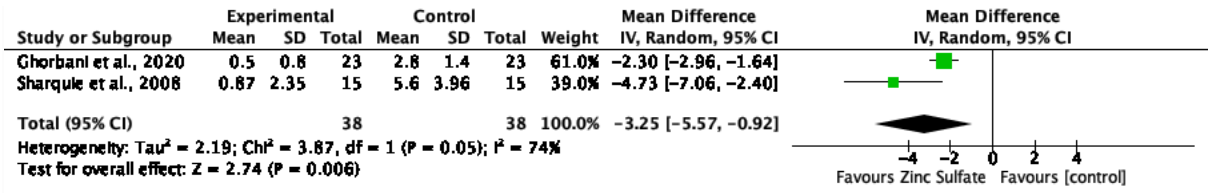

Fig 8: Forest plot of decrease in ulcer diameter in millimetres at 6 months (zinc sulfate)

4.6. Publication bias

The researchers were unable to conduct an assessment of publication bias due to the sparsity of studies.

4.7. Quality assessment

We used the Cochrane risk of bias criteria for grading of the included studies (Cochrane handbook). Overall, studies were evaluated where data was available and were considered to be of low risk of bias (Figure 12).

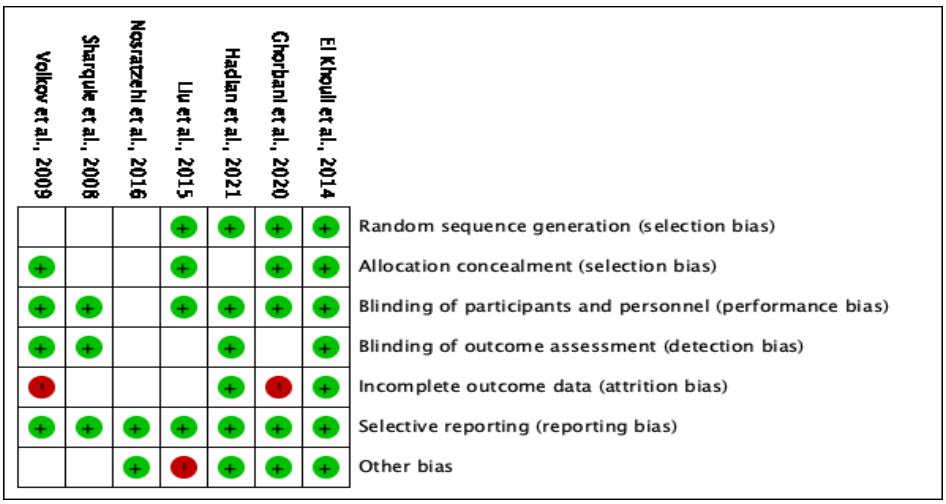

Figure 9: Risk of bias of included studies

(green and red circles, low and high risk of bias, respectively; blank space, unclear risk of bias based on study data provided)

5. Discussion

This systematic review and meta-analysis found evidence for the effectiveness of vitamin B12, zinc sulfate and omega-3 in decreasing the pain level and ulcer size, with regard to the management of RAS.

Despite substantial investigation, there is no conclusive therapy for the treatment of RAS. Several studies have attempted oral supplementation with vitamin B12, zinc sulfate and omega-3 in individuals suffering from RAS with mixed outcomes. As a result, the goal of this systematic review was to analyse the current and most recent data in order to answer the following primary research question: "Is oral supplemental interventions (vit B12, zinc sulphate, omega 3), compared with standard of care, associated with improved clinical outcomes for RAS management? This is the first systematic review and meta-analysis performed to compare the efficacy of these three oral supplementations.

Mainly, the findings of this systematic review revealed that oral vitamin B12, zinc sulfate and omega-3 have favourable effects in RAS patients with no or minor negative side effects. Oral supplementation with each individual supplement, was found to be more effective than the control group in the majority of the research considered.

There were two primary outcomes that were assessed through meta-analysis. One was the average level of ulcer pain using VAS or NRS and the other one was a decrease in the size of the diameter of the ulcer. There was a considerable improvement in pain level after the administration of these supplemental oral interventions (Fig. 2). The heterogeneity was quite high though. This could have been attributed to the study by Nosratzehi et al., 2016, which was a possible outlier, as evidenced by the decrease in heterogeneity to 73%, after removal from the meta-analysis. It was unclear as to why this happened, as the sample size and all the intervention parameters were similar to the study done on omega-3 previously (El Khouli et al., 2014). The effect of unidentified risk factors within their patient population may have contributed to this.

Omega-3 proved beneficial in pain reduction, when assessed on its own using VAS, at the third and sixth month interval, respectively (El Khouli et al., 2014; Hadian et al., 2021; Nosratzehi et al., 2016) (Fig. 3 and Fig. 4). However, at the sixth month interval the heterogeneity was high and after omitting the outlier study by Nosratzehi et al., 2016, the heterogeneity substantially decreased (51%). Again, we were unable to find the reason behind this study being so heterogenous.

When vitamin B12 was assessed on its own using VAS and NRS, the results showed that vitamin B12 significantly decreases the pain level associated with RAS (El Khouli et al.,

2014; Hadian et al., 2021; Nosratzehi et al., 2016) and thus, proved to helpful in the treatment of RAS (Fig. 5).

Decrease in ulcer size/diameter was another primary outcome evaluated in the review. Only omega-3 and zinc sulfate studies measured this outcome and proved effective in decreasing the size of the ulcer. Their effectiveness was notable after three months of use. The heterogeneity was high which could be due to the fact that both studies used different interventions. In addition, the frequency of the intervention differed and the trial duration was higher in the study employing omega-3 supplementation (Hadian et. al. 2021) (Fig. 6).

At six months, supplementation with omega-3 did not provide any further or additional decrease in the diameter of the ulcer, clinically. The heterogeneity of these two studies was high, which could be attributed to the fact that the total number randomised in the study by Hadian et. al., were less as compared to the study by Nosratzehi et. al. (Fig. 7).

However, zinc sulfate did provide a further decrease in ulcer size at 6 months. The factors that might have attributed to high heterogeneity could have been the varying sample size and study duration of both the studies; Ghorbani et al. sample size was more than the study by Sharquie et al. Study duration in the study by Ghorbani et. al. was significantly less than the other study. Another notable difference was in the dosage of the zinc sulfate formulation. Sharquie et. al. had a higher dose which was in a mucoadhesive tablet form, whereas, Ghorbani et al. had a lower dose in a capsule form (Fig. 8).

This reduction in pain after oral intake of zinc, highlights the fact that the trace element zinc functions as a cofactor and is essential for cell differentiation, development,

regeneration, and wound repair. Zinc has immune system regulating properties, such as limiting T helper-17 cell activity, suppressing inflammatory cytokine production, decreasing neutrophil chemotaxis, and downregulating the expression of Toll-like receptor-2 in keratinocytes (Dhaliwal et. al., 2020; Prasad, 2008). Through their anti-inflammatory and immunoregulatory properties, omega-3 metabolites such as pro-resolving lipid mediators, resolvins, and protectins can aid in wound repair and resolution of inflammation (El-Gendy, 2014).

While conducting this study, comprehensive search was undertaken and appraisal was done with a peer-reviewed tool. Robust methods were used in synthesising the data. Nevertheless, there are several factors that could pose as limitations. Firstly, the limited amount of data available for analysis represents a major limitation. Secondly, there was no standardisation of methods; various studies used different methodology and the methodologies werent rigorous enough. The interventions of oral supplements also vary from one study to another. Thirdly, there was variability of participants and assessment, imparting great heterogeneity to the included studies in terms of setting, assay methods, participant age, gender, and ethnicity was a major restriction, making it challenging to make definite conclusions.

RAS is a mild and self-limiting condition, which has a great influence on everyday life of people ascribed to the excessive recurrence rate. The goal of this systematic review and meta-analysis was to gauge the efficacy of oral supplements such as, vitamin B12, zinc sulfate and omega-3 to resolve pain or reduce the pain severity of recurrent aphthous stomatitis. This is the first systematic review and meta-analysis to evaluate the efficacy of oral supplements such as, vitamin B12, zinc sulfate and omega-3 to resolve or reduce the

pain severity and size of recurrent aphthous stomatitis. We hope that our research was beneficial to add to the current evidence supporting the use of oral supplements in the management of RAS.

6. Conclusions

6.1. Implications for Practice:

The findings of this systematic review and meta-analysis imply that vitamin B12, omega-3 and zinc supplementation appears to be effective in decreasing the size of RAS, managing the associated discomfort and pain, thus speeding up the healing process. It is a cost-effective way to manage RAS, especially in public settings and is readily available without a prescription, thus improving the quality of life. Patients suffering from RAS should be prescribed these oral supplementations and encouraged to use it frequently,as it has considerably less associated systemic side effects than standard of care.

6.2. Implications for Research

Recurrent Aphthous Stomatitis is a common condition which can tremendously affect the quality of life of the affected individual. More robust clinical trials employing these oral supplements should be carried out, in addition to including risk factors asessment in

population studied. In addition, studies correlating ulcer size and pain, needs to be investigated. Also, if both the zinc and omega-3 supplements are combined together and analysed in a clinical trial, it could possibly be a new favourable treatment regime. Also, population risk factors should be taken into account, as they could greatly influence the study outocme.

7. Conflict of interest

None to declare.

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