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The expression of TNF-α in recurrent aphthous stomatitis: A systematic review and meta-analysis

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ABSTRACT

Objective: The pathogenesis of recurrent aphthous stomatitis (RAS) is related to an increase of pro-inflammatory cytokine, namely tumor necrosis factor α (TNF-α). This cytokine plays an important role in the development of ulcer lesions, both in saliva, tissues and blood. This systematic review analyzed the differences of TNF-α in lesions, salivary and blood and can be used as a reliable method of diagnosis for RAS.

Methods: A comprehensive search of PubMed, Scopus databases, Web of Science, Scielo, Google Scholar and Embase with keywords. The inclusion criteria were studies that assessed the saliva, serum, and RAS lesion, with the outcome reporting the mean of saliva, serum and tissue expression of TNF-α. The risk of bias was also assessed.

Result: Healthy individuals showed significantly lower TNF-α than RAS (SMD = -1.517, 95% CI [-2.25, -0.78]). Although there is a significant difference between sample (i.e., saliva, serum) and detection type (i.e., cytometry bead array, ELISA), both methods can detect a significant difference in TNF-α between healthy individuals and RAS patients.

Conclusions: The TNF-α is a useful diagnostic marker for RAS. We encourage saliva to detect changes in TNF-α during ulceration as it provides accuracy, reliability, and non-invasive procedure compared to a blood draw.

Keywords: saliva, TNF-α, recurrent aphthous stomatitis, serum, tissue expression

INTRODUCTION

The diagnosis of recurrent aphthous stomatitis (RAS) can be given definitively if it includes four criteria: recurrence, periodic, unknown etiology, and no systemic alteration [1–4]. Research evidence shows few aggravating factors for RAS, and these are categorized as local (i.e., trauma, smoking) and systemic predisposing factors (i.e., periodic fever, stress) were related to RAS development. However, considering the multiple factors influencing the diagnosis, the enforcement of RAS diagnosis has not been determined.

In the current oral medicine practice, RAS diagnosis is only determined based on the degree of recurrence, without definite etiology, ulcer period or accompanying objective examination. Since the evidence of RAS pathogenesis is related to oral bacteria changes [5,6], polymorphism of interleukin gene [7,8], and serotonin transporter [9], observing these indicators are complex and not clinically feasible. However, studies have reported that RAS is more likely to have genetic connections and changes in the immune response, such as tumor necrosis factor-α (TNF-α). TNF-α It has become a common inflammatory marker in various mucosal abnormalities of the oral cavity, including RAS [10]. If TNF-α has an essential role in lesion development, then detecting these cytokines can be an objective reference to establish the diagnosis. Several studies have reported the expression of these cytokines in various stages of RAS, and the level is elevated in saliva [11], serums [12], and tissue lesions [13].

Several research studies reported that TNF-α is a useful marker for diagnosing RAS. However, a variation is observed in their results and methods for estimating TNF-α. Hence, to understand the available evidence on utilizing TNF-α in diagnosing RAS, the

present systematic review analyzes the differences of TNF-α in saliva and blood and can be used as a reliable method of diagnosis for RAS.

MATERIAL AND METHODS

Data sources and search strategy

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were adopted for this systematic review and meta-analysis. A comprehensive search of PubMed, Scopus databases, Google Scholar, Scielo, Web of Science and Embase was conducted in March 2022. The following keyword combinations were adopted for searching articles for recurrent aphthous stomatitis: [“recurrent aphthous stomatitis” or “recurrent aphthous ulcers” or “aphthous ulcer” or “RAS” or “RAU”] AND [“tumor necrosis factor” or “TNF” or “TNF-alpha”] AND [“cytokine”] AND [“pro-inflammatory”]. In addition, the reference lists of the eligible articles were searched manually to identify additional relevant publications.

A search strategy was performed using the PICO model (patient, intervention, comparison, outcome), taking into consideration the following aspects: population/patient (patient), diagnostic/therapeutic procedure (intervention), comparison (comparison), and outcomes.

Study selection

The inclusion criteria for studies were as follows: (i) the diagnostic criteria of RAS were based on an accepted clinical description, both active and remission phases (ii) RAS patient and control (health individuals) (iii) reported the TNF-α expression. Fundamental experimental studies such as animal or cell studies, abstracts, narrative reviews, case reports and editorials were excluded from this analysis.

Data extraction and quality assessment

Three authors screened each study independently (MDCS, IBPPM and PHC). MDCS is an oral medicine specialist with 3 years of experience, IBPPM is a final year residence of oral medicine, and PHC is the last year of the dentistry program. The authors first screened the title(s), abstracts, and full texts to determine whether the inclusion criteria had been met. The following information was then extracted from the studies to be included in the meta-analysis: first author's name, year of publication, age, sex, sample size, study design, RAS type, and the value of TNF-α. In case of disagreement, third investigators (DSE and AEP) will act as a referral and reach a consensus through discussion.

The Joanna Briggs Institute Critical Appraisal Tools, including the 10-item Checklist for Case-Control Studies, 10-item Checklist for Analytical Cross-Sectional Studies, and 13-item Checklist for Randomized Control Study, were used to assess the methodological quality of the included studies. Each item was scored as "yes", "no", "unclear", or "not applicable". One point was assigned to the answer "yes", and zero points were assigned to "no". The total point of each study was categorized into <50%, 51-75% and >75% for high, moderate, and low risk of bias. Furthermore, each study assessed the publication bias using ***Begg's rank*** correlation test, and a *p-value* of <0.05 indicated no publication bias.

Data synthesis and analysis

The data extracted from the included articles were entered into R *(R Foundation for Statistical Computing Version 4.0.5, Vienna, Austria)* with metafor package [14]. A random-effects model was applied to pool the value of TNF-α with corresponding 95% confidence intervals (CI). The primary size effect was analyzed with the standardized mean difference (SMD) using Cohens' D transformation, with a negative SMD value

indicating a higher amount of TNF-α in the healthy individual group and a positive SMD value indicating a higher amount of TNF-α in the RAS group. Knapp and Hartung's adjustment test were used to reduce the number of unjustified significant result from the previous transformation. Furthermore, meta-regression analysis using a mixed-effect model was done to analyze the difference between sample acquisition (i.e., saliva or serum) and the quantification process (i.e., ELISA and cytometric bead array (CBA), etc.).

RESULT

Characteristics of included studies

A literature search with the specified keywords resulted in 5113 published articles. After title screening was done, only 247 articles were chosen for the next step. Finally, 30 studies were selected in this systematic review based on abstract reading and full-text availability. The PRISMA flowchart of the study search is presented in **Figure 1**.

TNF-α expression on saliva

Seven studies reported the saliva expression of TNF-α with nine observations. Two hundred and seventy-six RAS patients and 190 health patients as control were analyzed for salivary TNF-α. Six studies analyzed the salivary TNF-α using ELISA [11,15–19], and one study analyzed using CBA methods [20]. These methods resulted in a higher salivary expression of TNF-α in RAS patients compared to the healthy individual's cohort [11,15–18,20]. In contrast, only one study showed lower salivary expression of TNF-α in RAS patients compared to healthy individuals [19] (**Table 1**).

TNF-α expression on serum

The serum TNF-α was reported by seven studies with twelve observations. A total of 283 RAS patients and 351 health patients as control were analyzed for serum expression of TNF-α. Six studies analyzed the serum expression of TNF-α using ELISA methods [19,21–24], and three studies analyzed using CBA [25–27]. The ELISA method showed a higher serum expression of TNF-α in RAS patients compared to healthy individuals. One study showed lower salivary expression of TNF-α in RAS patients compared to the healthy individuals [24] **(Table 2).**

Risk bias assessments

The risk assessments provide in **Table 3**, **Table 4** and **Table 5**. Publication bias was not detected in the current study sample ($p < 0.05$).

Meta-Analysis

Fourteen studies with 21 observations were included in the meta-analysis. The SMD value reported from the random effect model favored the healthy individual group (SMD = -1.376, 95% CI [-2.05, -0.7]). High heterogeneity was observed with a significant Q-test (I^2 = 91.68%, Tau^2 = 1.19) **(Table 6)**.

The mixed-effect model for meta-regression analysis found a significant difference oinSMD between saliva and serum sample acquisition, with saliva samples giving a higher SMD value (SMD = -1.618, 95% CI [-2.64, -0.59]). In the detection type, ELISA and CBA significantly different from each other, CBA gives higher SMD value (SMD = -1.881, 95% CI [-3.11, -0.84]). High heterogeneity was detected in each meta-regression model with a significant Q test **(Table 6).**

DISCUSSION

TNF-α plays a significant role in mediating acute inflammation. A similar relation between TNF-α and RAS is observed. Our current finding suggests that multiple types of research explore the quantified amount of TNF-α produced when various predisposing factors were accounted. Nevertheless, all findings agreed that TNF-α changes between healthy individuals and RAS patients. These differences in TNF-α provide evidence that a reliable and easy to enforce RAS diagnostic is through TNF-α. Unfortunately, no published literature explains the role of molecules that allow elevation of TNF-α expression in saliva and blood patients with RAS.

TNF-α becomes an important marker for the occurrence and development of RAS lesions. TNF-α was found to be consistently higher in active lesions [15,27], and recurrent [15,27], even when the lesion has healed [28]. In the formation and development of RAS lesions, trauma frequently plays a role in RAS onset. In this context, trauma is a local factor that can occur in the oral cavity due to masticatory or occluding forces or other harmful habits. In addition, immunological abnormalities (deficiencies/suppressed) can assist during traumatic episodes by triggering an immunological response to develop RAS. During this immunological response, an abnormal cytokine cascade is activated in the oral mucosal environment, which leads to a cell-mediated immune response in a focal area of the oral mucosa [29]. During the development of the lesion, the CD4+/CD8+ ratio is disrupted, recruitment lymphocytes and macrophages in the lesion — therefore, increasing the cytokine production of TNF-α [30]. The immune response occurs not only in the local region of ulcerated tissue but also triggers an increased blood flow and capillary permeability. Thus, the systemic influence of TNF-α is noted in the bloodstream. Increased TNF-α in the blood and

saliva does not cause clinical manifestation in RAS patients but represents a sign of damage to oral tissue triggered by an immunological alteration in the body.

Meanwhile, the pathogen recognition receptor (PRR) releases phagocytic-chemokines cells (i.e., macrophages, dendritic and mast cells) to secrete pro-inflammatory cytokines such as TNF-α. These inflammatory mediators cause an increase in vascular permeability expression of cell adhesion molecules (CAM) and chemokines. Hence, the epithelium becomes an inflamed form of ulceration [31].

High TNF-α levels in the blood serum in patients with active disease indicate a polarized Th1 response [32]. Therefore, Th1 will be seen in the RAS due to the release of TNF- α. This event will stimulate cytotoxic T lymphocytes and increase endothelial expression, causing inflammatory cells migration to the inflammation site, which causes ulcer development [30,33].

Several cytokines that are linked with RAS pathogenesis have also been studied, however there were no confirmatory observations on those cytokines in RAS. Clinical studies that focused on estimation of cytokine levels in RAS are interferon and Interleukins, such as IFN [34,35], IL-8 [36], IL-1β [37–40], IL-1 [35,41,42], IL-2 [34,43–46], IL-4 [44,45,47,48], IL-6 [37,42,46,49], IL-10 [39,45,50–53], IL-12 [50,51], IL-13 [35,45], IL-17 [35,54], IL-17C [55], IL-17F [56]. Unfortunately, these findings on cytokines were not able to achieve any clinically reliable application in RAS while comparing TNF-α.

One of the invasive methods that assist in analyzing TNF-α levels is obtaining tissue samples of RAS lesions. However, current evidence indicates that tissue sampling from RAS does not yield good results for estimating TNF-α levels. RAS lesions for

immunohistochemical examination [32,57], mRNA extraction [28,58], and RNA [59] showed that TNF-α is higher than in healthy individuals. However, this method is invasive and requires surgical procedures, so that it cannot efficiently provide a favorable clinical application.

Nowadays, saliva is the most helpful component of chair side diagnosis. Saliva can use to diagnose systemic illnesses, monitoring general health, understand the prognosis of a disease, or identify an oral sign of systemic disease. The serum component of saliva is derived originally from the vascularity of carotid arteries. Saliva has the same molecule found in systemic circulation [60]. From this review, we found that the elevation of TNF-α expression in saliva and serum can be detected. The increased TNF-α expression in the saliva is easier to analyze in the clinical setting of oral medicine for diagnosing RAS due to its relevance and non-invasive nature of specimen collection.

Further analysis of detection type indicates that CBA is significantly different in TNF- α detection compared to ELISA due to its better sensitivity. This assures clinicians, especially in RAS patients who have passed the acute phase, both in the active and remission phase, that low concentrations of TNF-α can still be detected using CBA. This finding strengthened the procedures for the enforcement of diagnosis from RAS objectively and confirmed the role of TNF-α in the pathogenesis of RAS. Despite the significant difference, both methods are acceptable forms of diagnosis regarding TNF-α detection.

CONCLUSION

In the current dental or oral medicine practice, RAS cases are diagnosed through clinical examination. However, this approach is not completely adequate for starting RAS management. Hence, estimation of TNF-α is recommended as chair side consideration. Both systematic review and meta-analysis findings of this study state that TNF-α should serve as a reliable diagnostic marker for RAS. Whilst the detection method is comparably similar, we encourage saliva to detect changes in TNF-α during ulceration as it provides accuracy, reliability, and non-invasive procedure compared to a blood draw.

CONFLICT OF INTEREST

Authors have no conflict of interest

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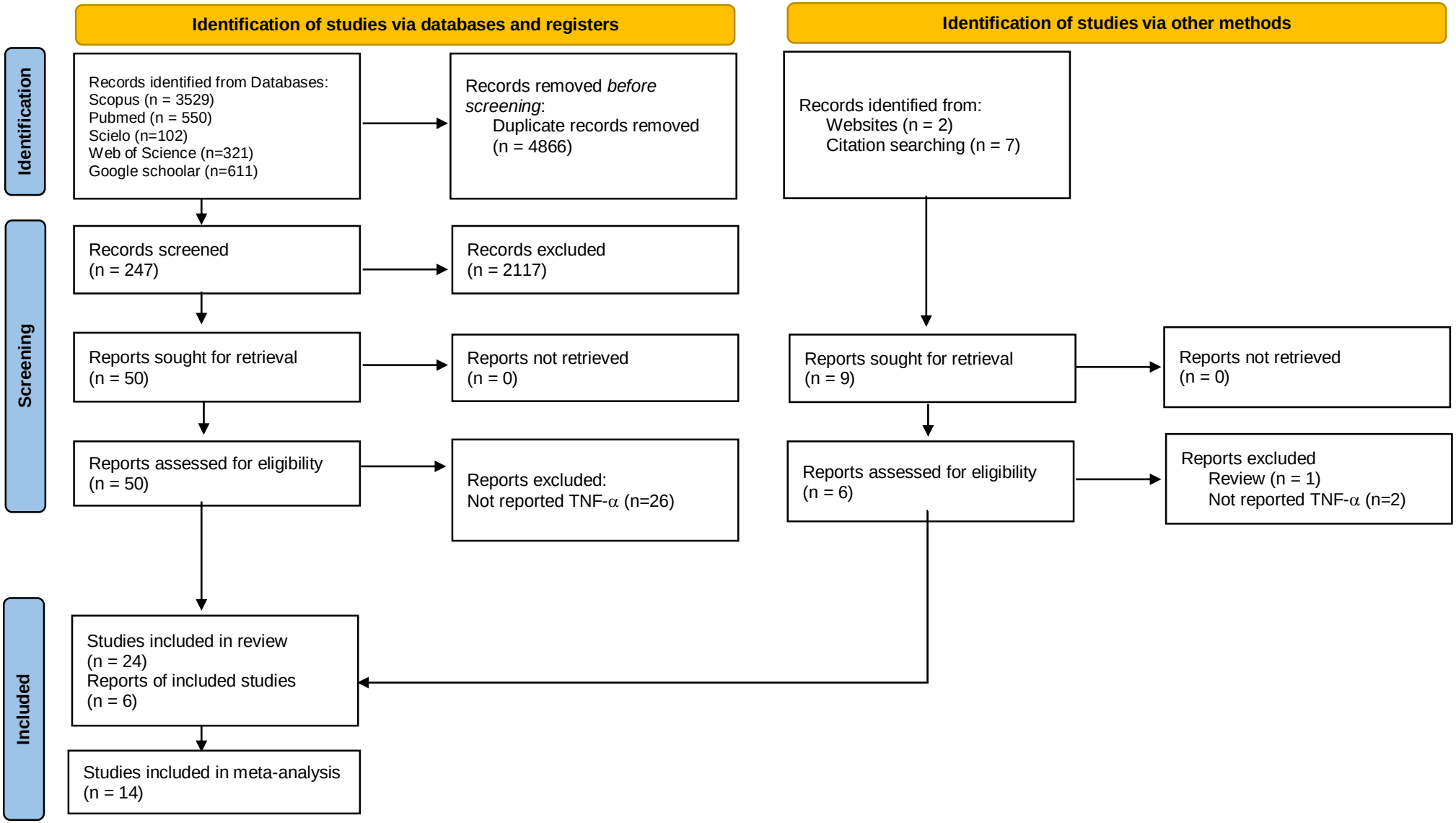

Figure 1. PRISMA flow chart of the literature search and study selection

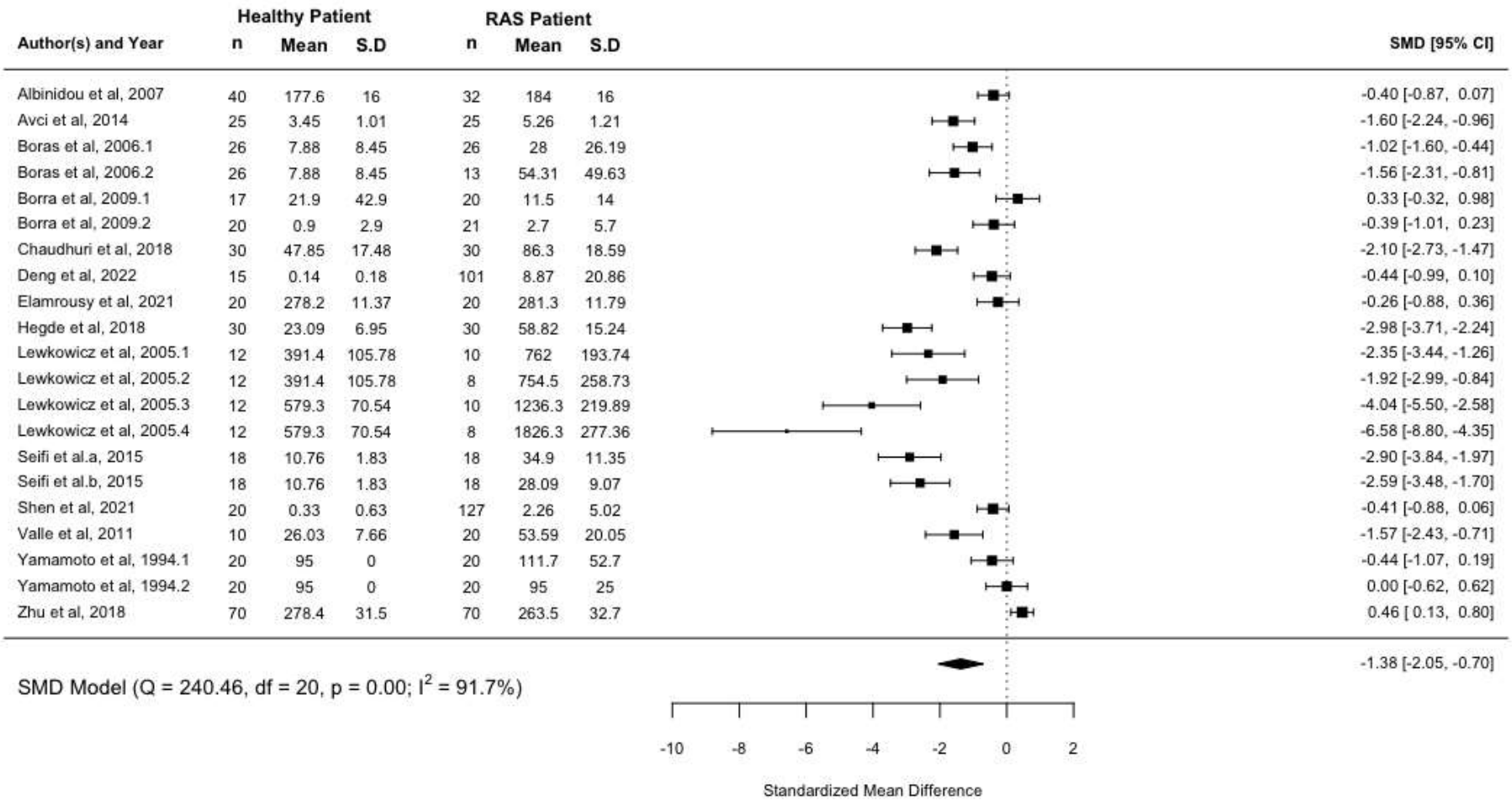

Figure 2. TNF-α expression on saliva. n: Number of patients in each group, S.D: standard deviation, SMD: Standardized Mean Difference, 95% CI: Confidence Interval, Q(df): Q test for homogeneity and degrees of freedom, I^2: Total Heterogeneity.

Table 1. TNF-α expression on saliva

Author	Reference	Type of RAS	Subject		TNF-α expression (Mean±SD)		Method of detection	Samples
			Health patient	RAS	Health patient	RAS		
Boras *et al*	[15]	MiRAS	26	26	7.88 ± 8.45	28.00 ± 26.19	ELISA	Saliva
	[15]	MiRAS - remission	26	13	7.88 ± 8.45	54.31 ± 49.63	ELISA	Saliva
Chaudhuri *et al*	[11]	RAS	30	30	47.85 ± 17.48	86.30 ± 18.59	ELISA	Saliva
Valle *et al*	[16]	RAS	10	20	26.03 ± 7.66	53.59 ± 20.05	ELISA	Saliva
Hegde *et al*	[17]	MiRAS	30	30	23.09 ± 6.95	58.82 ± 15.24	ELISA	Saliva
Seifi *et al*	[18]	MiRAS	18	18	10.76 ± 1.83	34.9 ± 11.35	ELISA	Saliva
	[18]	MiRAS	18	18	10.76 ± 1.83	28.09 ± 9.07	ELISA	Saliva
Borra *et al*	[19]	RAS	17	20	21.90 ± 42.90	11.50 ± 14.00	ELISA	Saliva
Deng *et al*	[20]	MiRAS	15	101	0.14 ± 0.18	8.87 ± 20.86	CBA	Saliva

RAS: Recurrent aphthous stomatitis; MiRAS: Minor recurrent aphthous stomatitis; CBA: Cytometric bead array

Table 2. TNF-α expression on serum

Author	Reference	Type of RAS	Subject		TNF-α expression (Mean±SD)		Method of detection	Samples
			Health patient	RAS	Health patient	RAS		
Borra *et al*	[19]	RAS	20	21	0.90 ± 2.90	2.70 ± 5.70	ELISA	Serum
Albinidou *et al*	[21]	MiRAS	40	32	177.6 ± 16.00	184 ± 16.00	ELISA	Serum
Avci *et al*	[22]	MiRAS	25	25	3.45 ± 1.01	5.26 ± 1.21	ELISA	Serum
Yamamoto *et al*	[23]	RAS-active	20	20	95.0 ± 0.00	111.7 ± 52.7	ELISA	Serum
	[23]	RAS-remission	20	20	95.0 ± 0.00	95.0 ± 25.00	ELISA	Serum
Zhu *et al*	[24]	RAS	70	70	278.4 ± 31.50	263.5 ± 32.70	ELISA	Serum
Elamrousy *et al*	[25]	MiRAS	20	20	278.20 ± 11.37	281.30 ± 11.79	CBA	Serum
Shen *et al*	[26]	MiRAS	20	127	0.33 ± 0.63	2.26 ± 5.02	CBA	Serum
Lewkowicz *et al*	[27]	RAS-active	12	10	391.4 ± 105.78	762.0 ± 193.74	CBA	Serum
	[27]	RAS-remission	12	8	391.4 ± 105.78	754.5 ± 258.73	CBA	Serum
	[27]	RAS-active	12	10	579.3 ± 70.54	1236.3 ± 219.89	CBA	Serum
	[27]	RAS-remission	12	8	579.3 ± 70.54	1826.3 ± 277.36	CBA	Serum

RAS: Recurrent aphthous stomatitis; MiRAS: Minor recurrent aphthous stomatitis; CBA: Cytometric bead array

Table 3. Study quality of analytical cross-sectional study

Author	Reference	Type of study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Total
Chaudhuri *et al*	[11]	Analytical cross-sectional study	√	√	√	√	X	X	√	√	75%
Borra *et al*	[19]	Analytical cross-sectional study	√	√	√	√	X	X	√	√	75%
Deng *et al*	[20]	Analytical cross-sectional study	√	√	√	√	X	X	√	√	75%
Avci *et al*	[22]	Analytical cross-sectional study	√	√	√	√	X	X	√	√	75%
Zhu *et al*	[24]	Analytical cross-sectional study	√	√	√	√	√	√	√	√	100%
Shen *et al*	[26]	Analytical cross-sectional study	√	√	√	√	X	X	√	√	75%

Q1: Were the criteria for inclusion in the sample clearly defined?
Q2: Were the study subjects and the setting described in detail?
Q3: Was the exposure measured in a valid and reliable way?
Q4: Were objective, standard criteria used for measurement of the condition?
Q5: Were confounding factors identified?
Q6: Were strategies to deal with confounding factors stated?
Q7: Were the outcomes measured in a valid and reliable way?
Q8: Was appropriate statistical analysis used?

Table 4. Study quality of case control study

Author	Reference	Type of study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Total
Boras *et al*	[15]	Case control study	√	√	√	NA	NA	X	X	√	NA	√	50%
Valle *et al*	[16]	Case control study	√	√	√	NA	NA	X	X	√	NA	√	50%
Hegde *et al*	[17]	Case control study	√	√	√	NA	NA	X	X	√	NA	√	50%
Seifi *et al*	[18]	Case control study	√	√	√	NA	NA	X	X	√	NA	√	50%
Albinidou *et al*	[21]	Case control study	√	√	√	NA	NA	X	X	√	NA	√	50%
Yamamoto *et al*	[23]	Case control study	√	√	√	NA	NA	X	X	√	NA	√	50%
Lewkowicz *et al*	[27]	Case control study	√	√	√	NA	NA	X	X	√	NA	√	50%

Q1: Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?
Q2: Were cases and controls matched appropriately?
Q3: Were the same criteria used for identification of cases and controls?
Q4: Was exposure measured in a standard, valid and reliable way?
Q5: Was exposure measured in the same way for cases and controls?
Q6: Were confounding factors identified?
Q7: Were strategies to deal with confounding factors stated?
Q8: Were outcomes assessed in a standard, valid and reliable way for cases and controls?
Q9: Was the exposure period of interest long enough to be meaningful?
Q10: Was appropriate statistical analysis used?

Table 5. Study quality of randomized control study

Author	Reference	Type of study	1	2	3	4	5	6	7	8	9	10	11	12	13	Total
Elamrousy *et al*	[25]	RCT	√	√	√	√	√	√	√	√	√	√	√	√	√	100%

Q1: Was true randomization used for assignment of participants to treatment groups?
Q2: Was allocation to treatment groups concealed?
Q3: Were treatment groups similar at the baseline?
Q4: Were participants blind to treatment assignment?
Q5: Were those delivering treatment blind to treatment assignment?
Q6: Were outcomes assessors blind to treatment assignment?
Q7: Were treatment groups treated identically other than the intervention of interest?
Q8: Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?
Q9: Were participants analyzed in the groups to which they were randomized?
Q10: Were outcomes measured in the same way for treatment groups?
Q11: Were outcomes measured in a reliable way?
Q12: Was appropriate statistical analysis used?
Q13: Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?

Table 6. Meta-Regression Models

	n	SMD	95% CI		Q(df)	Tau^2	I^2
Sample Type							
Saliva	9	-1.618	-2.64	-0.59	194.46(19)	1.02	90.23%
Serum	12	-1.151	-2.05	-0.25			
Detection Type							
CBA	7	-1.881	-3.11	-0.65	239.92(19)	1.29	92.08%
ELISA	14	-1.165	-1.98	-0.35			

SMD: Standardized Mean Difference, 95% CI: Confidence Interval, Q(df): Q test for homogeneity and degrees of freedom. Tau^2: estimated amount of total heterogeneity, I^2: Total Heterogeneity; n: number of observations.