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Salivary oxidative stress markers in patients with recurrent aphthous stomatitis and healthy controls

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Abstract:
Recurrent aphthous stomatitis remains a frequent recurrent oral ulcerative disorder, yet the clinical relevance of salivary oxidative imbalance in routine assessment remains insufficiently defined. Therefore, it is of interest to compare the unstimulated salivary malondialdehyde, total antioxidant capacity, superoxide dismutase, glutathione peroxidase, salivary flow rate, ulcer burden and pain intensity among 45 patients with active minor recurrent aphthous stomatitis and 45 age- and sex-matched healthy controls. Mean salivary malondialdehyde was significantly higher in the recurrent aphthous stomatitis group (4.21 ± 1.13 umol/L) than controls (2.68 ± 0.84 umol/L), while total antioxidant capacity, superoxide dismutase and glutathione peroxidase were significantly reduced ($p<0.001$ for all). Malondialdehyde showed positive correlations with ulcer count ($r=0.48$, $p=0.001$), ulcer diameter ($r=0.42$, $p=0.004$) and pain score ($r=0.51$, $p<0.001$), whereas total antioxidant capacity showed inverse correlations with these indicators. Thus, data shows that salivary oxidative stress profiling as a practical adjunct for understanding inflammatory activity in recurrent aphthous stomatitis and highlight the need for preventive antioxidant-focused clinical strategies.

Keywords: Recurrent aphthous stomatitis (RAS); saliva; oxidative stress; malondialdehyde; total antioxidant capacity; antioxidant enzymes

Background:
Recurrent aphthous stomatitis is a common, painful, relapsing ulcerative condition of the oral mucosa that interferes with eating, speech, oral hygiene and quality of life. Its course is typically self-limiting, but repeated episodes create considerable clinical burden because the triggering events are multifactorial and the mechanisms remain incompletely understood **[1, 2]**. Current concepts describe the disorder as an immune-mediated mucosal reaction influenced by local trauma, stress, nutritional imbalance, microbial exposures, genetic susceptibility and systemic factors. Among these mechanisms, oxidative stress has received increasing attention because ulcer formation involves epithelial injury, neutrophil activation, reactive oxygen species and impaired antioxidant defense **[3, 4]**. Saliva is an attractive diagnostic fluid in oral mucosal disease because it is collected non-invasively and reflects local mucosal, microbial, inflammatory and metabolic events. Salivary biomarkers may therefore help bridge the gap between subjective clinical activity and measurable biological changes during active ulceration **[5, 6]**. Oxidative stress markers such as malondialdehyde indicate lipid peroxidation and membrane damage, while total antioxidant capacity reflects the combined protective effect of enzymatic and non-enzymatic antioxidant systems. Superoxide dismutase and glutathione peroxidase provide important enzymatic defenses against superoxide radicals and peroxide-mediated tissue injury **[7, 8]**. Previous clinical studies have reported inconsistent patterns of salivary and serum oxidant-antioxidant changes in recurrent aphthous stomatitis. Some studies reported higher lipid peroxidation and lower antioxidant activity, whereas others found phase-dependent or compartment-specific differences between active lesions, healing phases and healthy controls **[9-12].** These inconsistencies may be explained by differences in lesion severity, timing of sample collection, salivary stimulation, nutritional status, smoking habits, exclusion of systemic diseases and assay methodology. Therefore, a controlled study using active minor lesions and a standardized saliva collection protocol may provide useful clinical clarity **[13-16]**. The relationship between biomarker values and clinical ulcer severity is particularly important. A biomarker may be biologically interesting but clinically limited if it does not correlate with pain, ulcer number, or lesion size. Conversely, a salivary marker that reflects lesion burden may help in monitoring disease activity or evaluating adjunctive interventions **[17-19].** The available evidence supports the oxidative stress hypothesis, but few studies have simultaneously evaluated lipid peroxidation, total antioxidant capacity, superoxide dismutase, glutathione peroxidase, salivary flow, ulcer burden and pain in a single case-control model. The present study aimed to compare selected salivary oxidative stress markers between patients with active recurrent aphthous stomatitis and healthy controls and to assess the association of these biomarkers with clinical severity indicators **[20]**. Therefore, it is of interest to compare the salivary oxidative stress and antioxidant markers in patients with recurrent aphthous stomatitis and healthy controls and evaluate their associations with ulcer burden and pain intensity.

Materials and Methods:
The present case-control study was conducted in the Department of Oral Medicine and Radiology over a period of 9 months. Ninety participants were enrolled, including 45 patients with clinically diagnosed active minor recurrent aphthous stomatitis and 45 age- and sex-matched healthy controls. The sample size was calculated using malondialdehyde as the primary outcome, assuming a medium effect size of 0.60, 80% power, alpha error of 5% and an allowance for incomplete

samples. Patients aged 18-45 years with a history of at least three episodes of minor aphthous ulcers in the previous year and with one or more active ulcers of less than 72 hours duration were included. Controls were systemically healthy individuals without current or previous recurrent oral ulceration. Exclusion criteria were major aphthae, herpetiform aphthae, Behçet disease, inflammatory bowel disease, celiac disease, anemia requiring treatment, pregnancy, lactation, tobacco use, alcohol dependence, antioxidant supplements within 3 months, corticosteroid or immunomodulatory therapy, active periodontal infection and systemic inflammatory disease. Clinical details recorded included age, sex, episode frequency, duration of disease, ulcer count, largest ulcer diameter, pain on a 10-point visual analogue scale and unstimulated salivary flow rate. Unstimulated whole saliva was collected between 9:00 and 11:00 A.M. Participants avoided food, beverages and oral hygiene procedures for at least 90 minutes before collection. Samples were centrifuged at 3000 rpm for 10 minutes and stored at -80ºC until analysis. Salivary malondialdehyde was measured by thiobarbituric acid reactive substances assay and expressed as umol/L. Total antioxidant capacity was measured by colorimetric assay and expressed as mmol/L. Superoxide dismutase and glutathione peroxidase activities were measured using commercially available enzyme assay kits according to the manufacturer instructions. All samples were analyzed in duplicate by a blinded laboratory investigator. Data were analyzed using SPSS version 26. Continuous variables were expressed as mean ± standard deviation and categorical variables as frequency and percentage. Intergroup comparisons were performed using independent t-test or Mann-Whitney U test according to normality. Chi-square test was used for categorical variables. Pearson or Spearman correlation was used for biomarker-severity associations. Binary logistic regression was used to identify independent biomarker associations with recurrent aphthous stomatitis. A p-value <0.05 was considered statistically significant.

Results:

All 90 participants completed clinical examination and saliva collection. The recurrent aphthous stomatitis and control groups were comparable for age, sex distribution and body mass index. Mean age was 28.7 ± 6.4 years in the case group and 29.1 ± 6.1 years in controls (p=0.762). Females constituted 57.8% and 55.6% of the two groups, respectively (p=0.832). Mean disease duration among cases was 4.8 ± 2.6 years and the mean annual recurrence frequency was 6.3 ± 2.4 episodes. Salivary oxidative stress markers differed significantly between groups. Mean malondialdehyde was higher among cases (4.21 ± 1.13 umol/L) than controls (2.68 ± 0.84 umol/L; p<0.001). Total antioxidant capacity was lower among cases (0.43 ± 0.16 mmol/L) than controls (0.68 ± 0.21 mmol/L; p<0.001). Superoxide dismutase and glutathione peroxidase activities were also significantly lower in cases (p<0.001). Salivary flow rate was modestly reduced in cases, but the difference was not statistically significant. Within the recurrent aphthous stomatitis group, malondialdehyde correlated positively with ulcer count, largest ulcer diameter and pain score. Total antioxidant capacity, superoxide dismutase and glutathione peroxidase showed inverse relationships with clinical severity. In logistic regression, higher malondialdehyde and lower total antioxidant capacity remained independently associated with recurrent aphthous stomatitis after adjustment for age, sex, body mass index and salivary flow rate. The demographic profile, between-group biomarker comparisons and biomarker-clinical associations are summarized in **Tables 1–3**.

Table 1: Demographic and clinical characteristics of study participants

Variable	RAS group (n=45)	Control group (n=45)	p value
Age (years)	28.7 ± 6.4	29.1 ± 6.1	0.762
Female sex, n (%)	26 (57.8%)	25 (55.6%)	0.832
Body mass index (kg/m2)	23.8 ± 3.1	23.5 ± 2.9	0.641
Disease duration (years)	4.8 ± 2.6	--	--
Annual recurrence frequency	6.3 ± 2.4	--	--
Ulcer count	2.1 ± 0.9	0	<0.001
Largest ulcer diameter (mm)	5.8 ± 1.9	0	<0.001
Pain VAS score	6.4 ± 1.5	0	<0.001

Table 2: Comparison of salivary oxidative stress markers

Marker	RAS group (n=45)	Control group (n=45)	Mean difference	p value
Malondialdehyde (umol/L)	4.21 ±1.13	2.68 ±0.84	1.53	<0.001
Total antioxidant capacity (mmol/L)	0.43 ±0.16	0.68 ± 0.21	-0.25	<0.001
Superoxide dismutase (U/mL)	48.6 ± 12.4	67.9 ± 14.8	-19.3	<0.001
Glutathione peroxidase (U/L)	31.8 ± 9.7	45.2 ±11.3	-13.4	<0.001
Unstimulated salivary flow (mL/min)	0.33±0.12	0.37±0.14	-0.04	0.148

Table 3: Correlation and regression analysis of biomarker associations

Variable	Association tested	Effect estimate	95% CI	p value
MDA vs pain score	Spearman correlation	r=0.51	0.27 to 0.69	<0.001
MDA vs ulcer count	Spearman correlation	r=0.48	0.22 to 0.66	0.001
TAC vs pain score	Spearman correlation	r=-0.44	-0.64 to -0.17	0.003
SOD vs ulcer diameter	Pearson correlation	r=-0.36	-0.58 to -0.07	0.015
MDA predicting RAS	Adjusted OR per 1 umol/L	2.74	1.45 to 5.16	0.002
TAC predicting RAS	Adjusted OR per 0.1 mmol/L	0.71	0.56 to 0.91	0.006

Discussion:

The present study showed a distinct salivary oxidative imbalance in patients with active minor recurrent aphthous stomatitis. Higher malondialdehyde and lower antioxidant indices suggest that active ulceration is accompanied by lipid peroxidation and reduced local antioxidant buffering. This pattern is biologically plausible because mucosal ulceration involves epithelial injury, inflammatory cell infiltration and reactive oxygen species production **[21, 22]**. The elevation of salivary malondialdehyde is consistent with the concept that lipid membranes of epithelial and inflammatory cells undergo oxidative injury during the active phase. Although malondialdehyde is not disease-specific, its significant association with pain and ulcer burden in this study suggests that it may reflect current inflammatory activity rather than only background systemic oxidative status **[23, 24]**. The reduction in total antioxidant capacity indicates that the combined protective reserve in saliva may be insufficient during active lesions. This may occur because antioxidant molecules are consumed while neutralizing reactive oxygen species, or because patients predisposed to recurrent ulceration may have a lower baseline antioxidant reserve. Both mechanisms can coexist and may vary according to diet, stress, micronutrient status and lesion timing **[25, 26]**. Lower superoxide dismutase and glutathione peroxidase activities further support compromised enzymatic antioxidant defense. These enzymes limit superoxide and peroxide accumulation and reduction in their activity may promote sustained oxidative injury at the ulcer margin. The inverse association of these enzymes with ulcer size provides clinical relevance to this biochemical observation **[27-29]**. The salivary flow rate difference was not statistically significant, suggesting that the observed biomarker pattern was not merely a dilutional effect. This is important because salivary concentration of many analytes is influenced by flow rate, time of day, hydration and stimulation. The use of morning unstimulated saliva and duplicate laboratory assays improved internal validity **[30, 31]**. From a clinical perspective, these results do not imply that oxidative stress is the sole cause of recurrent aphthous stomatitis. The disorder remains multifactorial and immunologic susceptibility, mucosal barrier function, microbial challenge, stress and hematinic factors are also important. However, oxidative imbalance may represent a common downstream pathway that amplifies epithelial injury and symptom intensity **[32, 33]**. The study has limitations. The case-control design cannot determine causality and biomarkers were measured only during active minor lesions. Dietary antioxidant intake, psychosocial stress and micronutrient concentrations were not quantified biochemically. Larger longitudinal studies comparing active, healing and remission phases would help determine whether these biomarkers predict recurrence or only reflect active inflammation **[34]**. Within these limitations, the findings support incorporating salivary oxidative stress assessment into research protocols for recurrent aphthous stomatitis. Future clinical trials may evaluate whether targeted antioxidant counseling or adjunctive topical antioxidant therapy can reduce episode severity, pain and recurrence frequency in selected patients **[35]**. An additional clinical implication of the present findings is that salivary oxidative status may help explain why apparently similar aphthous ulcers differ in pain intensity and healing behavior. Patients with higher lipid peroxidation and lower antioxidant reserve may experience a more intense local inflammatory response, even when ulcer size is modest. This may be useful when counseling patients who report severe symptoms despite clinically minor lesions. The biomarker pattern also reinforces the importance of evaluating modifiable contributors such as diet quality; sleep disturbance, stress and micronutrient deficiency when recurrent episodes are frequent. The practical advantage of saliva is important in oral medicine settings. Blood-based biomarkers can provide systemic information, but repeated venipuncture is less acceptable for a benign recurrent condition. Saliva collection is inexpensive, non-invasive and repeatable across active and remission phases. If standardized collection and storage protocols are used, salivary assays can become valuable in clinical trials that evaluate topical corticosteroids, antioxidant gels, low-level laser therapy, nutritional correction, or stress-reduction interventions. Another consideration is that oxidative biomarkers should be interpreted as part of a broader diagnostic framework. Recurrent aphthous stomatitis is a clinical diagnosis and the exclusion of systemic ulcerative disorders remains essential. Biomarker values are not intended to replace history, lesion morphology, recurrence pattern, or hematinic investigation. Instead, they can support a more biological understanding of active disease and may help stratify patients in future personalized management studies.

Conclusion:

Patients with active recurrent aphthous stomatitis showed significantly higher salivary malondialdehyde and lower total antioxidant capacity, superoxide dismutase and glutathione peroxidase than healthy controls. Malondialdehyde and total antioxidant capacity were independently associated with disease status and correlated with clinical severity. Salivary oxidative stress markers may therefore serve as useful adjunctive indicators of biological activity in recurrent aphthous stomatitis, although longitudinal validation is required before routine clinical application.

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