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Clinical profile, associated risk factors and oral health-related quality of life in recurrent aphthous stomatitis: A cross-sectional study

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Abstract:
Recurrent aphthous stomatitis (RAS) is the most prevalent ulcerating condition of the mouth mucosa. Therefore, it is of interest to evaluate the clinical profile and distribution of RAS subtypes and determine risk factors which are associated with disease severity as well as its effect on oral health-related quality of life (OHRQoL). Psychological stress (adjusted OR = 3.42, 95%CI = 1.24-9.43, p = 0.018) and haematinic deficiency (adjusted OR = 2.96, 95%CI = 1.02-8.59, p = 0.046) were independently associated with moderate and severe RAS and their OHIP-14 scores showed a significant increase with increasing severity (Mild: 9.1 ± 4.2, Moderate: 16.8 ± 5.6, Severe: 24.3 ± 6.1; ANOVA $p < 0.001$). Thus, RAS in this population was mainly minor, predominantly occurred in young females with psychological stress. Moreover, haematinic deficiency and it is the independent factors predicting severity and poor OHRQoL.

Keywords: Recurrent aphthous stomatitis (RAS); aphthous ulcer; oral ulcer; cross-sectional study; risk factors; quality of life; OHIP-14; oral medicine.

Background:
Recurrent aphthous stomatitis (RAS) is the most frequent type of ulceration in the oral mucosa. It comprises recurrent painful ulcers of varying sizes, occurring mainly in the non-keratinised mucosa, which occurs in up to 5-25% of the population worldwide **[1]**. Traditionally, three subtypes of RAS are identified based on ulcer size, number, location and healing time, namely minor, major and herpetiform; the former subtype accounts for the vast majority of cases **[2]**. Despite being self-limiting, the repetitive occurrence constitutes a significant burden of the disease for the affected individuals. The multifactorial pathogenetic mechanisms of RAS involve several immunological, genetic, metabolic, psychological and nutritional processes **[3]**. In terms of the former mechanism, RAS is considered a T-cell mediated disease characterized by excessive production of pro-inflammatory cytokines (such as TNF-α, IL-6, IL-17), leading to damage to keratinocytes **[4]**. There have been numerous case reports linking RAS with systemic diseases (such as Behçet disease, inflammatory bowel disease, celiac disease, H. pylori infection) that emphasize its immunological underpinning **[5]**. In addition to psychological stress, which acts as both a triggering and exacerbating factor **[6]**, various haematologic and micronutrient deficiencies, especially of vitamin B12, iron/ferritin and folate, have also been associated with increased risk and recurrence **[7]**. Moreover, a positive family history and genetic predisposition are established as significant risk factors **[8]**, while fluctuations in hormones and food triggers, especially spicy or acidic food, are reported to be more common in female patients **[9]**. Beyond its direct clinical manifestations, RAS significantly impairs the daily routine, interfering with speech, eating and oral hygiene, while recurrent occurrences have been associated with irritability, anxiety, poor mental well-being **[10]**. The latter aspect has been increasingly assessed through oral health-related quality-of-life measurement tools (such as OHIP-14) **[11]**. In the context of diagnostic criteria, RAS is primarily diagnosed clinically by virtue of the characteristic recurrent nature of its occurrence and the ruling out of differential diagnoses **[12]**. At the same time, the current approach to RAS management is based on symptomatic measures; although topical corticosteroids remain the first-choice medications, several adjunctive therapies such as photobiomodulation and probiotics have been recommended recently **[13]**. Despite the abundant literature on the biology and management of RAS, data on region-specific clinical characteristics, relevant risk factors and quality-of-life effects of the disease in a single group of patients remain sparse, especially in outpatient settings in the Indian subcontinent **[14]**. Therefore, it is of interest to establish the clinical features and distribution of RAS clinical subtypes, to evaluate demographic and behavioral risk factors of disease severity and to assess the effect of the disease on oral health-related quality of life.

Materials and Methods:

Study design and setting:

This was a single-centre, hospital-based descriptive cross-sectional study. The subjects for the study were recruited from the outpatient department consecutively.

Duration of study:

Data collection took place over a span of 12 months, with each individual being evaluated only once, depending on whether or not he or she had an active/recurrent case of the condition under investigation at that time.

Inclusion criteria:

The inclusion criteria for the subjects in the study were patients over the age of 18 years clinically diagnosed to be suffering from recurrent aphthous stomatitis (that is, having experienced at least two distinct episodes of aphthous stomatitis in the past 12 months), giving their informed consent in writing.

Exclusion criteria:

The exclusion criteria for the study were patients with oral ulcerations caused by an established alternative aetiology (such as herpetic stomatitis, oral lichen planus, pemphigus vulgaris, trauma or malignant ulcers); Behçet's disease; inflammatory bowel disease; pregnant/lactating women; individuals using immunomodulatory therapy systemically; and subjects refusing to participate in the study.

Sample size calculation:

The sample size was estimated using the standard formula for a single population proportion in a cross-sectional study:

$$n = Z^2 \times P\,(1 - P) \,/\, d^2$$

where n is the required sample size, Z is the standard normal deviate at the 95% confidence level (1.96), P is the anticipated proportion and d is the absolute precision (margin of error). An expected proportion of P = 0.20 (20%) was adopted, corresponding to the prevalence of haematinic deficiency among RAS patients reported in previous studies, which has ranged broadly between approximately 13% and 27%; the conservative mid-range estimate of 20% was chosen because a proportion closer to 0.50 was not warranted and would have inflated the requirement unnecessarily. With an absolute precision of d = 0.09 (9%):

$$n = (1.96)^2 \times 0.20 \times 0.80 \,/\, (0.09)^2 = 0.6147 \,/\, 0.0081 \approx 76$$

This generated a minimum sample size of 76 participants. To account for any missing information from the proforma, non-response rate and potential exclusions during the data cleaning process, an increase of about 12% of participants was added to get a target of ≈ 85 participants. A final sample of 85 participants was hence recruited, which also happened to constitute the maximum number of potential participants that could be reliably assessed during the single-centre study period.

Sampling strategy:

A convenience (non-probability) sampling strategy was employed whereby each eligible patient reporting to the outpatient clinic during the study period was invited to join the study until the desired sample size was attained.

Data collection methods:

The following data were collected using a structured pre-tested questionnaire comprising four sections: (i) socio-demographic data including age, sex, place of residence, occupation and level of education; (ii) a comprehensive disease history involving age of onset, duration of disease, frequency of episodes, ulcers per episode, time to heal and precipitating factors; (iii) a clinical examination form and; (iv) the OHIP-14 questionnaire. All measurements were conducted by a calibrated observer to minimize variations, while precipitating factors were taken as binary variables (present/absent).

Clinical examination and assessment of disease severity:

Intra-oral examination using standardized illumination was conducted to establish the clinical subtype (minor, major, or herpetiform), the number and location of ulcers and the severity of pain using a 10-point Visual Analogue Scale (VAS). Disease severity was classified into mild, moderate and severe based on number and size of ulcers, frequency of episodes, time taken to heal and pain severity, following a previously described classification system for RAS.

Assessment of oral health-related quality of life:

Oral Health Related Quality of Life (OHRQoL) was evaluated using the validated OHIP-14 instrument which assesses OHRQoL using 14 questions, two questions for each of the seven conceptually identified domains namely functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap. Each question was scored on a five-point Likert scale ranging from 0 to 4, giving a range of 0 to 8 in each domain and a cumulative score range of 0 to 56, with higher scores indicating poor OHRQoL.

Haematinics evaluation:

Blood samples collected from venipuncture were assayed for serum vitamin B12, serum ferritin and serum folate. A concentration below the reference ranges for each parameter constituted haematinic deficiency, while haematinic deficiency was defined as below reference range values for one or more of these parameters. (Blood samples were analyzed in the laboratory of the institution conducting this research and no sensitive information handling was involved.)

Definitions:

Recurrence frequency was coded into ≤ 3, 4-6 and > 6 times per year. Family history was defined as the presence of RAS in at

least one first-degree relative. Psychological stress, sleep disturbance, food triggers, mechanical irritation, gastrointestinal symptoms and smoking were documented as self-reported binary variables. In the analysis of data, the moderate and severe disease groups were combined to give a new category called moderate-to-severe disease for comparison against the mild disease group.

Statistical analysis:

The data were transferred to a spreadsheet program and subsequently analyzed using the SPSS package (IBM Corp, Version 26.0). Continuous variables were summarized as mean±SD or median and IQR, while categorical variables were presented as proportions or frequencies, as appropriate. The association between categorical variables and disease severity was examined using chi-squared tests/Fisher's Exact tests where the expected frequency was <5. Analysis of variance (ANOVA) was used to compare mean OHIP-14 scores among different severity groups. Variables significantly associated with moderate-to-severe disease at the p<0.20 significance level in the bivariate analyses were selected for further binary logistic regression analyses to obtain independent predictors for moderate-to-severe disease, expressed as adjusted odds ratio (aOR). A two-tailed p-value <0.05 was taken as statistically significant.

Results:

A total of 85 patients suffering from recurrent aphthous stomatitis were recruited for the study. The average age of the sample was 29.8 ± 9.4 years (range 18-58), with more females comprising 60% of the participants, while the highest percentage of the patients were within the age group of 18-29 (55.3%). Age of disease onset was 19.6 ± 6.8 years. The detailed sociodemographic and baseline clinical characteristics of the cohort are presented in (**Table 1)**. The minor variant was the dominant clinical subtype, comprising 80.0% (n = 68) of cases, with major and herpetiform variants representing 12.9% (n = 11) and 7.1% (n = 6), respectively (**Figure 1**). Self-reported precipitating and associated factors are summarised in **Figure 2**. Psychological stress was the most common cause, affecting 61.2% of people, while 48.2% had eaten spicy or sour food. Positive family history occurred in 44.7%, while sleep disturbances were seen in 36.5% of subjects. Haematinic deficiency was diagnosed in 27.1% cases (B12 16.5%, iron and ferritin 14.1%, folic acid 8.2%). Mechanical trauma occurred in 25.9%, gastrointestinal symptoms in 16.5% and current smoking in only 10.6%. Regarding the bivariate analysis (**Table 2**), the following parameters showed significant associations with moderate to severe disease (p-value <0.05): Age above 30 years; psychological stress; haematinics deficiency; sleep disturbance; and frequent recurrence (>6 episodes/year). On the other hand, no statistically significant correlation was observed between female gender, familial history, food triggers and smoking. There was progressive decline in oral health-related quality of life depending upon the severity of the disease. Average scores of the OHIP-14 total scale were 9.1 ± 4.2, 16.8 ± 5.6 and 24.3 ± 6.1 in mild, moderate and severe categories, respectively, which was statistically highly significant by one-way ANOVA (P < 0.001) **(Figure 3**). This trend was noted in all seven subdomains of the OHIP-14 scale; physical pain and psychological distress were most significantly affected subcategories **(Table 3).** The anatomical distribution of ulcers **(Figure 4)** showed the labial mucosa (62.4%) and buccal mucosa (56.5%) to be the most frequently involved sites, followed by the lateral/ventral tongue (41.2%) and the labial/buccal sulcus (30.6%), consistent with the predilection of RAS for the non-keratinised lining mucosa. On multivariable logistic regression **(Table 4),** after adjustment for all candidate covariates, psychological stress (aOR 3.42; 95% CI 1.24-9.43; p = 0.018) and haematinic deficiency (aOR 2.96; 95% CI 1.02-8.59; p = 0.046) remained the only independent predictors of moderate-to-severe RAS. The model demonstrated satisfactory calibration (Hosmer-Lemeshow χ^2 = 4.81, p = 0.78) and explained approximately 31% of the variance (Nagelkerke R^2 = 0.31).

Table 1: Sociodemographic and baseline clinical characteristics of the study participants (N = 85).

Characteristic	Value, n (%) or mean ± SD
Age, years (mean ± SD)	29.8 ± 9.4
Age group: 18-29 years	47 (55.3)
Age group: 30-39 years	24 (28.2)
Age group: 40-49 years	10 (11.8)
Age group: ≥ 50 years	4 (4.7)
Sex: Female	51 (60.0)
Sex: Male	34 (40.0)
Residence: Urban	50 (58.8)
Residence: Rural	35 (41.2)
Occupation: Student	36 (42.4)
Occupation: Employed	31 (36.5)
Occupation: Homemaker / other	18 (21.2)
Clinical subtype: Minor	68 (80.0)
Clinical subtype: Major	11 (12.9)
Clinical subtype: Herpetiform	6 (7.1)
Age at onset, years (mean ± SD)	19.6 ± 6.8
Disease duration, years (median, IQR)	6 (3-10)
Recurrence: < 4 episodes/year	36 (42.4)
Recurrence: 4-6 episodes/year	33 (38.8)
Recurrence: > 6 episodes/year	16 (18.8)
Ulcers per episode (mean ± SD)	2.7 ± 1.6
Mean healing time, days (mean ± SD)	9.4 ± 3.1
Pain, VAS 0-10 (mean ± SD)	6.2 ± 1.9
Severity: Mild	44 (51.8)
Severity: Moderate	29 (34.1)
Severity: Severe	12 (14.1)

SD, standard deviation; IQR, interquartile range; VAS, visual analogue scale.

Table 2: Association of demographic, clinical and behavioural factors with RAS severity (mild vs. moderate-to-severe), N = 85

Variable	Mild (n = 44), n (%)	Mod.-Severe (n = 41), n (%)	χ^2 / t test	p-value
Female sex	24 (54.5)	27 (65.9)	1.14	0.285
Age ≥ 30 years	14 (31.8)	24 (58.5)	6.10	**0.014***
Psychological stress	21 (47.7)	31 (75.6)	6.92	**0.009***
Positive family history	16 (36.4)	22 (53.7)	2.56	0.110
Haematinic deficiency	7 (15.9)	16 (39.0)	5.86	**0.015***
Spicy / acidic food	19 (43.2)	22 (53.7)	0.93	0.334

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Sleep disturbance	11 (25.0)	20 (48.8)	5.21	**0.022***
> 6 episodes/year	4 (9.1)	12 (29.3)	5.78	**0.016***
Current smoking	6 (13.6)	3 (7.3)	Fisher	0.486

*χ^2, chi-square test; Fisher's exact test applied where expected cell count < 5. *Statistically significant at $p < 0.05$.*

Table 3: OHIP-14 domain and total scores across RAS clinical severity groups (mean ± SD), N = 85.

OHIP-14 domain	Mild (n = 44)	Moderate (n = 29)	Severe (n = 12)	F	p-value
Functional limitation	1.2 ± 0.9	2.3 ± 1.1	3.4 ± 1.2	22.6	**<0.001**
Physical pain	2.1 ± 1.0	3.6 ± 1.2	4.8 ± 1.3	31.4	**<0.001**
Psychological discomfort	1.6 ± 1.0	2.8 ± 1.1	4.1 ± 1.4	27.8	**<0.001**
Physical disability	1.0 ± 0.8	2.0 ± 1.0	3.2 ± 1.2	24.1	**<0.001**
Psychological disability	1.3 ± 0.9	2.4 ± 1.1	3.5 ± 1.3	20.9	**<0.001**
Social disability	0.9 ± 0.7	1.8 ± 1.0	2.9 ± 1.2	23.7	**<0.001**
Handicap	0.8 ± 0.7	1.6 ± 0.9	2.6 ± 1.1	21.3	**<0.001**
Total OHIP-14	**9.1 ± 4.2**	**16.8 ± 5.6**	**24.3 ± 6.1**	**48.9**	**<0.001**

One-way ANOVA. Domain score range 0-8; total score range 0-56; higher scores indicate poorer quality of life.

Table 4: Multivariable logistic regression: independent predictors of moderate-to-severe RAS (N = 85).

Predictor	Adjusted OR	95% CI	p-value
Psychological stress (yes vs. no)	3.42	1.24-9.43	**0.018***
Haematinic deficiency (present vs. absent)	2.96	1.02-8.59	**0.046***
> 6 episodes/year (yes vs. no)	3.10	0.92-10.41	0.067
Age ≥ 30 years	2.18	0.84-5.66	0.110
Sleep disturbance	2.05	0.77-5.49	0.151
Positive family history	1.47	0.57-3.80	0.428

*OR, odds ratio; CI, confidence interval. Model adjusted for all listed covariates. Hosmer-Lemeshow $\chi^2 = 4.81$, $p = 0.78$; Nagelkerke $R^2 = 0.31$. *Statistically significant at $p < 0.05$.*

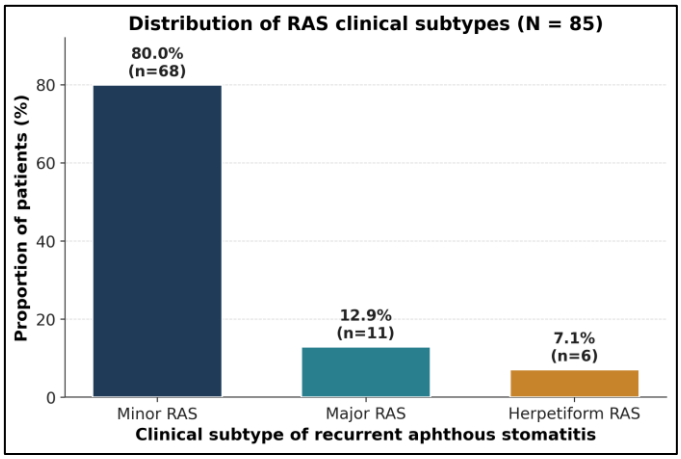

Figure 1: Distribution of recurrent aphthous stomatitis clinical subtypes among the study participants (N = 85). The minor variant predominated, followed by major and herpetiform forms.

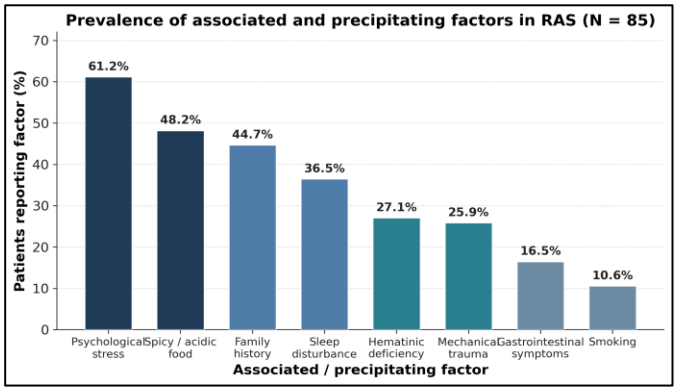

Figure 2: Prevalence of associated and precipitating factors reported by the study participants (N = 85). Values are expressed as the percentage of patients reporting each factor; categories were not mutually exclusive.

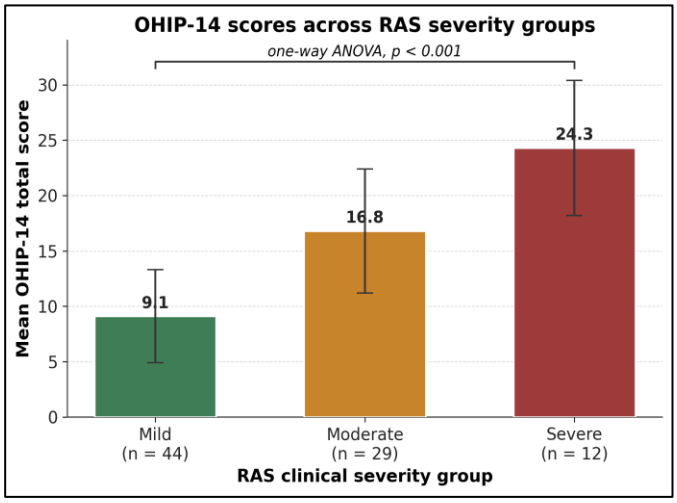

Figure 3: Mean OHIP-14 total scores across RAS severity groups (error bars represent standard deviation). Higher scores indicate poorer oral health-related quality of life; the difference across groups was significant on one-way ANOVA ($p < 0.001$).

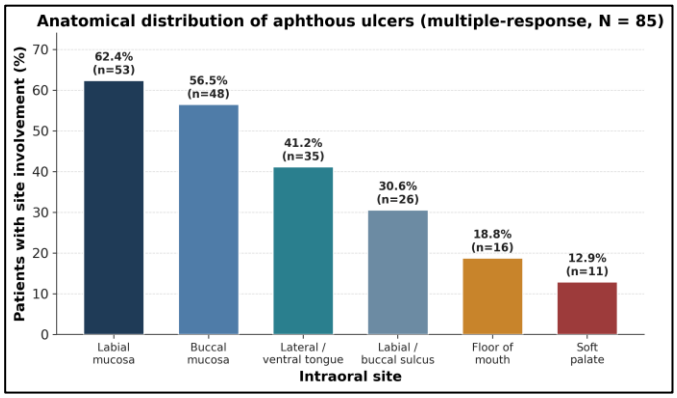

Figure 4: Anatomical distribution of aphthous ulcers among the study participants (multiple-response, N = 85). The non-

keratinised labial and buccal mucosae were most frequently affected.

Discussion:

This cross-sectional study described the clinical characteristics, independent predictors and quality-of-life burden associated with the development of recurrent aphthous stomatitis in the outpatient setting of oral medicine clinic. Three main observations were made in this study: high prevalence of minor RAS, presence of psychological stress and haematinic deficiency as independent factors associated with disease severity and rapid deterioration of oral health-related quality of life in patients with increasing severity. Prevalence of female gender and a predominance of young adulthood among the participants were consistent with previous RAS studies that were conducted on student and community samples. Kamat *et al.* **[14]** studied the risk factors of RAS among healthcare professional students and noted the importance of lifestyle-associated determinants such as bedtimes. Meanwhile, Ziaei *et al.* **[15]** evaluated the impact of RAS on general health and oral health-related quality of life among dental students. Also, the identification of high rates of minor RAS with the involvement of non-keratinized oral mucosa in the current sample was a common characteristic of the disease's clinical presentation, as stated in the study by Collado Pérez *et al.* **[16]**. Haematinic deficiencies were detected in 27.1% of subjects and correlated with higher disease severity in the study under consideration. This result made sense, given the previously established relationship between RAS and micronutrient and haematinic abnormalities. Thus, Bahramian *et al.* **[17]** found alterations in serum and salivary concentrations of vitamin B12 among patients with iron-deficiency anaemia and RAS. In addition, a systematic review and meta-analysis conducted by Torabinia *et al.* **[18]** proved the association of iron and zinc deficiencies with aphthous stomatitis. These results supported the clinical utility of checking for any potential correctable haematinic or micronutrient abnormalities among patients with severe or recurring RAS. The correlation between worsening OHRQoL and higher disease severity was another valuable finding of the current research. Namely, the scores on OHIP-14 questionnaire increased with increasing disease severity in the current sample. It is similar to the study by Ziaei *et al.* **[15]** evaluating the relationships between RAS, general health and OHRQoL and the study by Weng *et al.* **[19]**, which concluded that mental health and disease-related determinants influenced the OHRQoL of subjects with RAS. Therefore, the burden of RAS in this study appears to include pain, disability, psychologic distress and impaired functioning of the oral cavity. Despite being observational, the study in question could inform the treatment approach since the associations discussed above had practical implications. Namely, the link between severity and the abovementioned risk factors justified the stratified approach with evaluation of systemic and behavioral contributors to disease in patients with severe or frequent RAS. For example, Samiraninezhad *et al.* **[20]** revealed a favorable effect of Lactobacillus reuteri-derived probiotic nano-formulation on the clinical outcomes of RAS. Also, there is evidence regarding other non-systemic or locally acting treatments such as laser therapy, photobiomodulation and probiotics. The latest findings are presented in the umbrella review of Al-Zainal *et al.* **[21]**, the systematic review by Ma *et al.* **[22]** and the pediatric clinical study by Bardellini *et al.* **[24]**. Recently, a randomized controlled trial by AlHerafi *et al.* **[29]** confirmed the benefits of photobiomodulation in RAS. Systemic therapy was still appropriate for those patients with frequently or constantly occurring or moderate-to-severe RAS. Thus, Oh *et al.* **[23]** demonstrated the real-world effectiveness and safety of colchicine in RAS, which proved the drug's effectiveness for certain patients. More detailed guidelines for treatment were offered by two systematic reviews on RAS **[27, 28]** that advised individualization of treatment plans based on severity, recurrence pattern, triggers of RAS and patient history of treatment for the disease. An umbrella review by Al-Aizari *et al.* **[25]** also advocated the choice of evidence-based therapeutic interventions rather than standard treatments for all patients with RAS. It was also interesting that the current study corroborated the recurrence pattern of the condition. Namely, the finding of about one-fifth of patients having more than 6 recurrences annually confirmed that RAS is a recurrent disease of the oral mucosa requiring long-term monitoring and individual follow-up **[26-28]**. Overall, the current findings imply that optimal treatment and prevention of RAS should include disease severity evaluation, screening of haematinic and lifestyle factors, assessment of OHRQoL and stepwise selection of treatments involving topicals, photobiomodulation, probiotics, or systemic drugs **[20-25 and 27-29]**. The major strengths of this study include the application of a standardized and calibrated examiner, the confirmation of presence of haematinics using objective methods, the application of an effective OHRQoL assessment tool and determination of an accurate sample size. However, a number of limitations must be pointed out. The study was conducted in a single centre and involved hospital participants and therefore may have suffered from referral bias and lack of external validity. The use of the cross-sectional research design means that causation cannot be inferred from the results, as well as any possible time sequences among the variables involved. A number of the precipitating factors used were reported by the participants and might have been affected by recall bias.

Conclusion:

Patients suffering from recurrent aphthous stomatitis had mainly the minor variant, which was predominantly found among young females. Stress-related issues and haematinic deficiencies were found as the independent risk factors for having a moderately severe course of RAS. Data warrant the inclusion of psychological issues and haematinics in the diagnostic process of RAS and the use of an individualized treatment approach based on severity levels.

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