doi: 10.1111/j.1600-0714.2007.00589.x

J Oral Pathol Med (2007) 36: 615–20

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Recurrent aphthous stomatitis revisited; clinical features, associations, and new association with infant feeding practices?

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BACKGROUND: Recurrent aphthous stomatitis (RAS) is a common oral mucosal disease, characterized by painful oral ulcerations whose causes is poorly understood. The aim of the present study was to assess the characteristics of patients suffering from RAS.

METHODS: Five hundred and twenty-eight consecutive patients attending an Oral Medicine Specialist Clinic in the UK were examined and 143 specific attributes recorded.

RESULTS: Patients with a clinical diagnosis of RAS were significantly more often males ($P = 0.001$) younger ($P < 0.001$). Significant correlations were observed between a diagnosis of RAS and trauma ($P = 0.044$), stress ($P = 0.006$), non-smoking ($P < 0.001$), a family history ($P < 0.001$), breast feeding ($P = 0.017$) and the site in the mouth (buccal, labial, floor of mouth) ($P < 0.007$).

CONCLUSION: This retrospective analysis of patients attending a tertiary referral center provides evidence to support some long held beliefs about RAS, such as young age of onset, sites affected, and associations with trauma, stress, familial history and hormonal changes, while raising a new and interesting decreased incidence with breast feeding.

J Oral Pathol Med (2007) **36:** 615–20

Keywords: atopy; clinical attributes; recurrent aphthous stomatitis

Introduction

Recurrent aphthous stomatitis (RAS) is considered the most common oral mucosal lesion (1) and is characterized by the periodic eruption of painful, solitary or multiple ulcerations of the oral mucosa (2). The prevalence of RAS is between 5% and 25% in the general population and has been reported to be as high as 50–60% in selected groups (3). The onset of these ulcers is usually during childhood and they tend to diminish in frequency and severity with age (4). It has been reported that both males and females are equally affected (1) and that RAS is the most common form of oral ulcerations in children with a peak age of onset between 10 and 19 years.

The cause of RAS remains poorly understood (3). Studies have shown that RAS can have many predisposing factors including stress, nutritional deficiencies, trauma, hormonal changes, and diet. Other contributors may be psychological factors, and there are occasional associations with Coeliac disease and other immunological disorders (4, 5) found that RAS appears to have an immuno-genetic background, with an association with a positive family history in some cases, weak histocompatibility locus antigen (HLA) associations (6), and the implication of heat shock proteins (7). There may also be associations with immunologically related diseases, such as immunodeficiencies, various allergies and atopic disease (8). Serum IgE concentrations are greater in RAS patients than in either control subjects or patients with other oral ulcerative conditions (9) and from 30% to 56% of atopic patients suffer from RAS (1). The possible association between atopy and RAS is of particular interest, as one factor in the development of the immune system and immunologically related diseases such as atopy may be breast-feeding. Breast-feeding appears to confer protection from atopy later (10). Exclusive breast-feeding in the first 3 months is negatively associated with atopic dermatitis (8). In contrast, cows milk exposure may pre-dispose to atopy (11), as may some constituents of breast milk (12). In infants who are unable to be completely breast-fed, there is evidence that prolonged feeding with a hydrolysed compared with a cow's milk formula reduces infant

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All authors would like to disclose that there are no conflicts-of-interest, or any financial arrangements entered into, that impact on this study.
Accepted for publication May 13, 2007

and childhood allergy (13). Breast milk has also been postulated to provide efficient protection against infections, to actively stimulate the infant's immune system by anti-idiotype antibodies, uptake of maternal lymphocytes and cytokines, as well as possibly providing protection against certain immunological diseases such as allergies and coeliac disease (14, 15).

The availability of a large, thoroughly documented dataset consisting of extensive questioning, examination and diagnosis of a cohort of consecutive patients attending an Oral Medicine Clinic allowed us to question the dogma that is present in the literature with regard to the numerous clinical associations of RAS and to explore the relationship to atopy, and the question of any role of breast-feeding. Therefore, the aim of the current study was to assess diverse clinical characteristics of patients suffering from RAS.

Material and methods

A total of 528 consecutive patients attending an Oral Medicine Specialist Clinic in the UK were enrolled in this study by a single clinician (CS). During the examination and history, 143 specific attributes and questions were recorded. A thorough Medical (including medications, smoking and alcohol intake) and Dental history was recorded and a complete physical examination, including a thorough head, neck, and oral examination undertaken and recorded. For the purposes of this study, further specific attributes were also recorded and are outlined in Table 1. Diagnosis of RAS was made on clinical grounds at the time of this examination using recognized criteria (4, 5).

Statistical analysis was undertaken using SPSS 13.0 for Windows Statistical Software (Release 13.0). Categorical data was submitted to chi-squared analysis and numeric data was analyzed parametrically by either two-sample *T*-tests or one-way ANOVA according to the postulates of each test. A cross tabulation of each categorical response variable by each potential explanatory variable was undertaken and the association assessed by a chi-squared test. Binary response variables were analyzed using stepwise logistic regression, using the chi-squared test to screen out explanatory variables with little evidence of association. Statistical significance was considered where $P < 0.05$.

Table 1 Specific attributes, other than routine Medical and Dental history, recorded during the examination and diagnosis of 528 consecutive patients attending an Oral Medicine Specialist Clinic

Patient information
DOB
Gender
History of recurrent oral ulceration
Past history of oral ulceration
*Ulcer history/*site
Buccal
Labial
Vestibular
Floor of mouth
Attached gingiva
Hard palate
Soft palate
Ventrum of tongue
Lateral border of tongue
Dorsum of tongue
*Ulcer history/*frequency and precipitating factors
Age of onset
Age of cessation
Number per year
Duration of episode
Average number of ulcers
Average size (mm)
Trauma dental treatment
Foods
Drugs
Drinks
Stress
Other factors known
*Birth/*infant history
Birth normal
Birth premature
Breast-fed only
Bottle-fed only
Breast- and bottle-fed
Menaecological history
Ulcers related to menstrual cycle
Dysmenorrhea
Married
Ulcers related to pregnancy
Taking contraceptive pill
Ulcers related to contraceptive pill
Age of menopause
Denture history
Source of dentures
Fit of dentures
Denture cleanliness
Dentures worn
Denture cleanser used

Results

The greatest proportion of the 528 consecutive patients attending this Oral Medicine Clinic and enrolled in this study were female (391/528, 74%; male 137/526, 26%). Females presented proportionally more often complaining of a sore tongue; dry mouth; were currently taking medication (or within the previous month); or suffered from other common medical complaints, such as anaemia, hay fever, low back pain and depression than did the male patients ($P < 0.001$; chi-squared test). Interestingly, male patients attending this clinic proportionally more often had a parent that was affected by recurrent oral ulceration (10% male, 5% female; $P = 0.028$) and were more often unmarried (93% male, 56% female; $P < 0.001$; chi-squared test). Furthermore, male patients were proportionally more often diagnosed as having RAS than female patients (51% male, 36% female; $P = 0.001$; chi-squared test) and were also significantly younger than female patients (137 males, mean age = 37.8 years; 391 females, mean age = 44.5 years; *T*-test, $P = 0.001$)

Two hundred and nine (39.6%) patients had a clinical diagnosis of RAS. One hundred and eighty-two (87.1%) of these patients were diagnosed with minor, 18 (8.6%) major and nine (4.3%) with herpetiform RAS. One patient with minor and one with major RAS were also diagnosed with Behcet's Syndrome.

Patients enrolled in this study were aged between 2 and 84 years. Stratification of the patients into six equal groups of 88 patients in each group according to their age (group 1, 2–22 years; group 2, 22–31 years; group 3, 31–43 years; group 4, 43–54 years; group 5, 54–64 years; group 6, 64–84 years) showed that RAS occurred significantly more often in the younger 2 age groups ($P < 0.001$; chi-squared test) (Fig. 1).

Patients were questioned where in the oral cavity their ulceration occurred (usually, sometimes or never). Of the 209 patients with RAS, significantly more reported that their ulceration usually occurred on the labial mucosa (39%), buccal mucosa (30%) or the vestibule (29%) ($P < 0.001$) (Fig. 2) than elsewhere in the mouth. Interestingly, over 50% of patients with RAS responded that their ulcers occurred either usually or sometimes in the vestibule (72%), labial mucosa (69%), buccal mucosa (69%) and the lateral margin of the tongue (59%) ($P < 0.001$; chi-squared test) (Fig. 2).

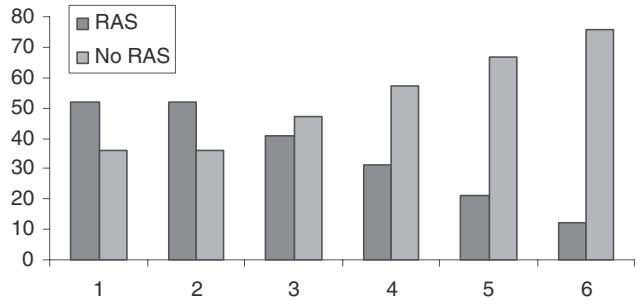

Figure 1 Occurrence of recurrent aphthous stomatitis (RAS) (presence, ■; absence □) in each of the age groups of consecutive patient attending an Oral Medicine Clinic. All patients who were diagnosed with RAS were divided into six equal numbered groups according to their age (group 1, 2–22 years; group 2, 22–31 years; group 3, 31–43 years; group 4, 43–54 years; group 5, 54–64 years; group 6, 64–84 years).

Recurrent aphthous stomatitis was significantly correlated with the presence of teeth ($P < 0.001$; chi-squared test), site in the mouth ($P = 0.007$; chi-squared test) but intriguingly not with the number ($P = 0.49$; chi-squared test) or the size ($P = 0.076$; chi-squared test) of the ulcers, although this latter attribute approached significance. Significantly more patients with a family history of oral ulceration (67.3%) were diagnosed with RAS ($P < 0.001$; chi-squared test). 27.5% (145/528) of all patients enrolled in this study smoked tobacco. Significantly more 45.9% (176/381) of the non-smokers in this cohort of patients were diagnosed with RAS ($P < 0.001$; chi-squared test) than smoker 22.8% (33/145). A significant correlation was found with trauma ($P = 0.044$; chi-squared test) and stress ($P = 0.006$; chi-squared test) but not between a diagnosis of RAS and medications, eczema, asthma, hay fever, rhinitis, food allergies, allergies or dental treatment ($P > 0.05$; chi-squared test).

Female patients who related the onset of their oral ulceration to their menstrual cycle ($P < 0.001$; chi-squared test), pregnancy ($P = 0.009$; chi-squared test) and who had dysmenorrhoea ($P = 0.002$; chi-squared test) were significantly more often diagnosed with RAS than other causes of oral ulceration.

Upon questioning 51% (269/528) of all patients knew, if they were breast or bottle-fed as an infant, with 56.1% (151/269) of these 'breast-fed only', 34.6% (93/269) 'bottle feed only' and 9.3% (25/269) both breast and bottle feed. Significantly more (53.8%, 50/93) patients who reported that they were 'bottle-fed only' suffered from RAS compared with those that reported that they were 'breast-fed only' (37.7%, 57/151) ($P = 0.017$; chi-squared test).

Logistic regression analysis

The first logistic regression model fitted included age alone and this was highly significant with an estimated

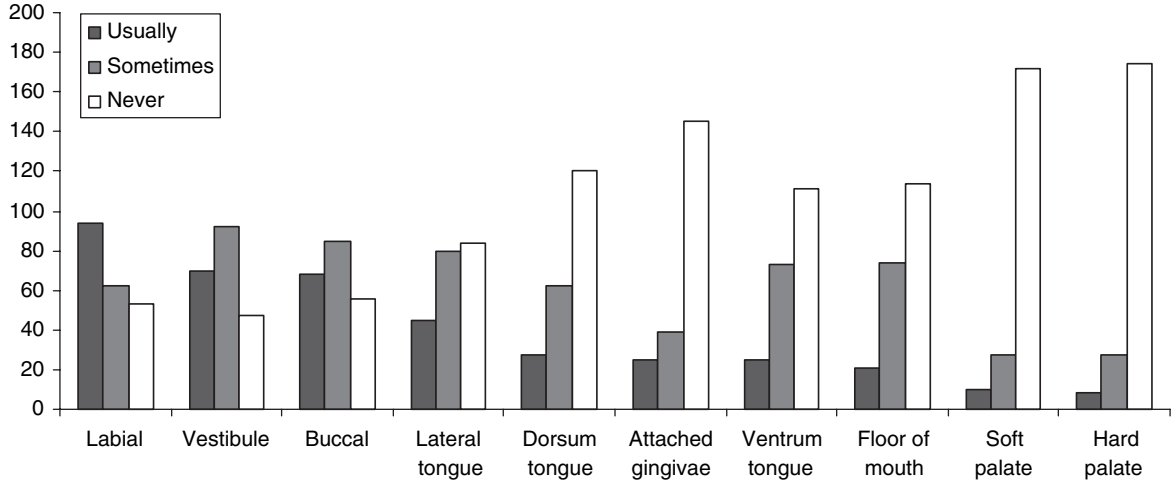

Figure 2 Reported sites of ulceration by the 209 patients who were diagnosed with recurrent aphthous stomatitis. Patients were questioned where their ulceration occurred usually ■, sometimes ■, or never □. As is obvious from the figure, significant variation occurred among these sites of ulceration ($P < 0.001$).

Odds Ratio (OR) of 0.962 (95% CI: 0.952–0.972). Thus, each year older, the odds of a positive RAS diagnosis in this patient group are multiplied by a factor of 0.962, i.e. they decrease by 3.8%. Therefore, over a 20-year period the odds that a patient will have RAS decreases by about half (0.962 to the power of 20 = 0.46). The second logistic regression model included gender alone whose effect was significant, with an OR of 1.89 (95% CI: 1.28–2.81) with males having almost twice the odds of having a RAS diagnosis as females. However, when age is included in this second model, the OR drops to 1.58, and the *P*-value is substantially larger (0.032) because of the fact that males attending this clinic were overall younger than females, and so the effects of age and sex were confounded.

All the variables mentioned above that were significantly associated with a diagnosis of RAS by chi-squared analyses were tested by logistically regression analysis and each variable was considered firstly alone, and secondly with age and sex. A cross table of the variable with age and with sex was used to assist in interpretation, particularly with regard to confounding.

The following variables are significant when corrected for age and sex: average number of ulcers, with 10 or more ulcers less likely to be diagnosed with RAS ($P < 0.001$; OR = 0.037, 95% CI: 0.009–0.158); average size of ulcers, with ulcers >10 mm less likely to be diagnosed with RAS ($P = 0.002$; OR = 0.070, 95% CI: 0.013–0.381); family history of oral ulcers more likely to have a diagnosis of RAS ($P < 0.001$, OR = 3.236, 95% CI: 1.993–5.254); patients who were smokers were less likely to be diagnosed with RAS ($P < 0.001$, OR = 0.385, 95% CI: 0.224–0.606); those who reported that there ulceration was associated with trauma were more likely to be diagnosis with RAS ($P < 0.001$, OR = 3.362, 95% CI: 2.108–5.363); and finally, patients who reported that the ulcers were related to stress were highly likely to be diagnosed with RAS ($P < 0.001$, OR = 8.710, 95% CI: 4.149–18.286), irrespective of age or gender.

The following variables were not significant when corrected for age and sex: presence of teeth and breast-feeding, even though both these factors were shown to be significant in the chi-squared analysis. For both these variables the distribution for the two possibilities was concentrated at different ends of the age distribution, and so the confounding was very strong. Thus, in regard to the causation, the effect of the presence of teeth and breast-feeding could be largely explained by the age of the patient.

The final logistic regression model included three variables relating to sites in the mouth; these variables were defined as: group A site = 'classic' sites for RAS on non-keratinized mucosal surfaces; buccal, labial, and vestibular. Group B sites = keratinized surfaces; dorsum of tongue, attached gingivae and hard palate. Group C sites = non-keratinized and unusual sites for RAS; floor of mouth, ventrum of tongue, soft palate and lateral border of tongue. For each group, a single categorical variable was defined with three levels, as follows: level 1 = 'never on all sites'; level 2 = 'sometimes or usually on one site only'; level 3 = 'sometimes or usually on more than one site'.

A stepwise logistic regression (with a forward selection procedure) was undertaken with all three site variables, together with all the variables found in the previous analysis to be significant [average number of ulcers; average size of ulcers; family history of oral ulcers; smoking; trauma; stress; presence of teeth; breast-fed only vs. bottle-fed only (even though theses last two variables were not significant when corrected for age and sex)]. The variables which entered the model were: average number of ulcers ($P = 0.003$; OR = 0.022); age ($P = 0.001$; OR = 0.965); group A sites ($P < 0.001$; OR = 0.040) and group B sites ($P < 0.001$; OR = 21.3). In other words, the logistic regression analysis found that younger patients with <10 ulcers in their mouth occurring on the buccal, labial, and vestibular mucosa, and not on keratinized surfaces were statistically significantly more likely to be diagnosed with RAS. Thus, using all the available information gathered during this study, the use of these four variables would correctly predict a diagnosis of RAS in 86% of patients.

Discussion

This cohort consisted of consecutive patients attending an Oral Medicine Clinic, not necessarily for recurrent oral ulceration and the female patients in this cohort more often presented complaining of a dry mouth, sore tongue and were more often taking medication. It is well proven that there is an increased incidence of xerostomia related to medication and that Sjogren's Syndrome occurs more often in females (16). This is born out in the fact that more males in this cohort presented with recurrent ulceration and were more often diagnosed with RAS (51%) than were the female patients (36%). Further, male patients were significantly younger and unmarried. What is intriguing is that, for those patients who were diagnosed with RAS, more male patients reported a family history of recurrent oral ulceration. It is thus obvious that the aetiology and associations of causality of RAS differ significantly between the genders within this population. Significantly more patients in the present study with a family history of oral ulceration were diagnosed with RAS, as suggested by previous studies (6, 17, 18). A more recent study found that inheritance of specific gene polymorphisms for TNF-α, TNF-β or VDR does not appear to be a significant factor in determining susceptibility to RAS, but this does not preclude from other genetic factors being involved in RAS (19).

In the present study we found that patients with RAS were more often younger and reported that their ulcers were related to periods of stress. Ship et al., (1966) (20) followed 230 medical and dental students who had a history of RAS and also found a strong correlation between emotional factors and RAS. They suggested that anxiety exerted an influence on disease activity, particularly in relation to the severity of the disease. Further support for these findings was revealed in a

12-year retrospective investigation of 651 subjects with RAS and found that the student years represent the highest levels of RAS (21). It can be concluded that stress plays a role in the aetiology of RAS and that these periods of stress occur more often in younger patients.

Our finding that patients with RAS more often reported that their ulcers were associated with trauma supports previous studies where 38% of 105 professional students felt that RAS lesions were brought on by trauma (22). Wray et al. (1981) (18) showed that between 40% and 100% of patients with history of RAS developed lesions in the same site of deliberately induced injuries, while this did not occur in any of their control subjects.

Our observations that female patients with RAS relate the onset of their oral ulceration to their menstrual cycle, pregnancy and dysmenorrhoea supports previous findings. Over the past 4 decades a relationship between hormones, the menstrual cycle and RAS has been postulated. Ship *et al.*, 1961 reported that, although it may be unlikely that a specific endocrine cause for RAS existed, they postulated that secondary influences of the menstrual cycle upon the development of RAS probably exert an effect. It has been reported that the pattern of ulcerations usually changed (improved) during pregnancy (23), and other work suggested that RAS pattern may be affected by the sex steroids (24).

In the present study, we found no correlation between a diagnosis of RAS and medications, food, or drinks which would indicate that allergens tend not to have a role in the aetiology of RAS. Previous reports have been conflicting with evidence showing that 30–56% of atopic patients (1) suffered from RAS and that serum IgE and IgD concentrations were greater in RAS patients than in either control subjects or patients with other oral ulcerative conditions (9).

We found a decreased incidence of RAS in patients who smoked. The present study, based on a self reporting approach, reinforces that fact which when large variation exists between study populations, it is not necessary to undertake physical measurement of the amount of smoking as has been undertaken in recent studies that utilized the measurement of cotinine in plasma, the active metabolite of nicotine (25). It has been postulated that mucosal hyperkeratinization caused by smoking has a protective effect, but studies of smokeless tobacco use have postulated that a component of tobacco that is systemically absorbed (such as nicotine) might be responsible for protecting against RAS (26).

It has been previously reported that the presence cell mediated immunity against adult human oral mucosa antigen(s) and streptococcal antigens are features characteristic of RAS (27). Further, the cytotoxic function of CD4+ lymphocytes have been identified in the pathogenesis of RAS lesions (28) and a review of the relationship between RAS and immune responses concluded that T-cell mediated hypersensitivity is involved in aetio-pathogenises of RAS (29). These previous studies highlight the potential role of immune sensitization and tolerance in RAS and when coupled with the results of the present study of the decreased incidence of the occurrence of RAS in patient's who were breast-fed as children, raises some interesting correlations.

Breast milk has been postulated to provide efficient protection against infections, to actively stimulate the infant's immune system by anti-idiotype antibodies, uptake of maternal lymphocytes and cytokines, as well as possibly providing long-lasting protection against certain immunological diseases, such as allergies and coeliac disease (15). An individuals immunological balance between tolerance and sensitization has been shown to be dependent on several factors including genetic background; nature and dose of antigen; frequency of antigenic contact; age at first antigen exposure; immunological status of the host; as well as antigen transmission via breast milk (30). It has been shown that human milk contains immunologically active substances potentially capable of altering infant immune response. In a study assessing the role of breast milk in sensitization analyzed maternal allergic status and allergic status of the child via skin-prick tests for seven common allergens in 702 6-year-old children and their mothers. This study showed that specific sensitization in the mother was associated with specific sensitization in the child, only if the child was breast-fed (31).

The present study of a large clinical cohort of Oral Medicine patients, although based on anamnestic data, reinforces many long held beliefs about RAS patient characteristics, such as younger age, site, trauma, stress, familial association and hormonal changes, while raising the interesting decreased incidence with breast-feeding. This latter association was strongly confounded by age in the patients in the present study as reflected by the lack of significance of breast-feeding in the logistic regression analysis. Thus, further research assessing the presence of RAS in age- and sex-matched patients who only differ in respect to being either breast-feed or bottle-feed as infants is necessary to establish a definitive link between RAS and breast feeding. However, this possibility raises the interesting hypothesis that the development of tolerance to common oral mucosal antigens occurs early in life, is more thorough in individuals who are breast-fed, and is intimately involved in the pathogenesis of RAS. In those patients whose immunological tolerance to these common oral antigens is not fully developed, when further challenged in latter life by these antigens, an aberrant immunological reaction occurs at the site of contact and appears clinically as RAS. This challenge could occur during the completion of the secondary dentition and the development of a more mature oral microbiological flora, as well as the widening of individuals' close social and sexual contacts, during the second decade of life. Further, over the ensuing decade or so, tolerance to these antigenic stimuli develops and the mucosal ulcerations occur less frequently, eventually ceasing. It could further be postulated that factors, such as smoking and hormonal changes related to pregnancy and menstruation influence the immune system in such a way as to render a hypersensitive individual more tolerant to common oral mucosal antigenic stimuli and thus modulating the local mucosal immune reaction and

the appearance of RAS. Although such a theory encompasses our current knowledge of RAS, as well as that presented in the current study, it does not aid our knowledge of either the exact stimuli, or the immunological reaction that occurs in RAS patients, such knowledge necessarily to allow for better intervention. However, utilization of explanations such as that above allow for our patients to better come to terms with this annoyingly recurrent, painful, mucosal condition.

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