Original Article

Low Frequency of Anti-Endomysial Antibodies in Recurrent Aphthous Stomatitis

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Abstract

Introduction: The aetiology of recurrent aphthous stomatitis (RAS) remains unknown. An association between RAS and coeliac diseases (CD) has previously been suggested, but the supporting evidence remains equivocal. The aim of the present study was to determine the likely frequency of CD in a large cohort of patients with well-defined RAS, by detailed haematological and serological analysis, including the detection of anti-endomysium and anti-reticulin antibodies. **Materials and Methods:** The study comprised 87 patients with minor RAS. Full blood counts and the presence of IgA anti-endomysial and IgA anti-reticulin antibodies were estimated in all patients. **Results:** The frequency of anti-endomysium and anti-reticulin antibodies was not elevated in patients with RAS and no patients with RAS had other serological features of CD. **Conclusions:** It is concluded that there is little significant aetiological link between RAS and CD, and that screening RAS patients for key serological markers of CD is of little clinical value.

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Key words: Anti-endomysial antibodies, Coeliac disease, Oral ulceration, Tissue transglutaminase

Introduction

Recurrent aphthous stomatitis (RAS) is a common condition of the oral mucosa of unknown aetiology.[1] Several predisposing factors have been suggested including haematinic deficiencies,[1-6] reactions to foodstuffs[7-9] and local trauma.[10] Aphthous-like oral ulceration can be a feature of various systemic disorders including Behçet's disease,[11,12] MAGIC syndrome,[13] inflammatory bowel disease,[14,15] primary[16-20] and secondary immuno-deficiencies[21,22] and drug therapy (e.g., non-steroidal anti-inflammatory agents[23]). An association between RAS and gluten-sensitive enteropathy/coeliac disease (CD) has been proposed for the last 20 years as some RAS patients showed evidence of small bowel changes suggestive of CD; nevertheless, there is still considerable dispute concerning the actual prevalence of CD in RAS patients, CD being still present in 4% to 25% of examined RAS patients.[2,24-29]

This association was suggested in 1976 when Ferguson and co-workers[24] found 8 (24%) of 33 English patients with RAS to show histological evidence of CD on jejunal biopsy (Table 1). None of these 8 patients had significant gastrointestinal symptoms. Haemoglobin indices and serum folate levels were significantly lower in the RAS patients with CD than those without. The 8 patients with RAS and CD had complete clinical and haematological remission when given a gluten-free diet. In contrast, only 1 of 26 Scottish patients with RAS was found to show histopathological evidence of jejunal CD, and did not have any notable resolution of oral ulcers with a gluten-free diet.[25]

A study of 50 Scottish patients with RAS[26] reported 2 females with CD. These 2 patients presented with typical features of minor RAS and were folate-deficient. A gluten-free diet produced a marked reduction in oral ulceration. Likewise, 6.2% of 97 English patients with RAS were

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Table 1.   Prevalence of Coeliac Disease in Patients with Recurrent Aphthous Stomatitis

References	No. of RAS patients	No. of RAS patients with CD	Improvement of RAS on a gluten-free diet
Wray et al[2]	130	5 (3.8%)	Not recorded
Ferguson et al[24]	33	8 (24%)	Yes
Rose et al[25]	26	1 (3.8%)	No
Ferguson et al[26]	50	2 (4%)	Yes
Tyldesley[27]	97	6 (6.2%)	Yes
Merchant et al[28]	100	3 (3%)	Not recorded
Veloso and Saleiro[29]	24	4 (16%)	Yes

CD: coeliac disease; RAS: recurrent aphthous stomatitis

found to have CD.[27] The affected patients had no signs of systemic illness except for a lowered serum folate, but did have an improvement in oral ulceration when given a gluten-free diet.

Merchant and co-workers[28] in Glasgow found 3 of 100 dental patients with RAS to have CD. Three patients were found to have IgA reticulin antibodies; only 1 of whom had histopathological evidence of CD. Four of a group of 24 RAS patients (16%) were found to have subtotal jejunal villous atrophy and significantly higher intra-epithelial lymphocyte counts than healthy control subjects. Furthermore, a significantly higher intra-epithelial lymphocyte count was reported in the remaining 20 patients with normal intestinal morphology when compared with healthy controls.[29]

Liability to oral ulceration in CD may be immunogenetically based as the frequencies of HLA DRW10 and DQW1 may be significantly higher in some groups of CD patients with oral ulceration than those without oral ulcers.[30]

Identification of RAS patients with underlying CD is important for effective management as CD is eminently treatable with a gluten-free diet. In relation to this, 25% of a group of RAS patients known to have no jejunal disease had resolution of RAS with a gluten-free diet.[31] Similarly, in another study,[32] 10 patients with RAS and a normal jejunal biopsy were divided into 2 groups based on their anti-gliadin antibody levels. Four patients had raised levels of the antibody while the remaining 6 had normal levels. Three of the 4 patients with elevated anti-gliadin antibodies responded to a gluten-free diet with resolution of oral ulcers and had relapse with gluten challenge. None of the 6 anti-gliadin antibody-negative patients had remission of the ulceration during the period of gluten withdrawal.

However, a gluten-free diet is not always effective in the management of RAS. Hunter and co-workers[33] conducted a well-designed double-blind study of a gluten-free diet in patients with RAS but who had no detectable gluten enteropathy. Out of 23 patients with RAS who completed

the trial, 11 were given a gluten-free diet; the other 12 served as controls and received a gluten-free diet supplemented by gluten given blind. Four out of the 11 patients on the gluten-free diet and 7 out of the 12 on the control diet reported significant benefit in terms of the RAS, but there were no significant statistical differences between the responses.

Thus, there is equivocal evidence of an association between RAS and CD. This may in part reflect low numbers of study patients or possibly the lack of a genuine association. In the present study, we have specifically examined the frequency of anti-reticular and anti-endomysium antibodies in patients with RAS. While perioral intestinal biopsy together with clinical improvement on a gluten-free diet remains the "gold standard" for the diagnosis of CD,[34,35] the detection of anti-gliadin, anti-reticulin and anti-endomysium antibodies are more acceptable in the screening and subsequent progress of CD.[36,37]

Tests for anti-gliadin antibodies have moderate sensitivity but poor specificity and positive predictive value for CD. IgA anti-gliadin antibodies are present in up to 90% of untreated coeliac patients while the IgG class is seen in about 82% of cases and up to 3.4% of healthy controls. However, nearly 25% of patients with Crohn's disease and 10% of patients with ulcerative colitis can have anti-gliadin antibodies.[37-41] The presence of anti-reticulin antibodies is both more sensitive and specific for untreated CD.[42,43] IgA anti-reticulin antibodies have been reported in 97% of untreated CD patients and in 2% of control subjects,[44] although 2 large studies failed to detect the antibodies in healthy subjects.[38,40] The detection of IgG anti-reticulin antibodies is not as useful as that for IgA antibodies as one-third of patients with coeliac disease may not have IgG anti-reticulin antibodies.[44]

The presence of IgA anti-endomysial antibodies is currently considered a better serological marker for untreated CD.[36,45] Sensitivity and specificity, as well as positive predictive values of the antibody have approached almost 100% in several studies.[38,39,46,47] Indeed, the remarkable reliability of endomysial antibodies has even led one group to question the need for small bowel biopsy in adults with suspected CD and IgA endomysial antibodies.[48]

The aforementioned 3 serological markers have been used in various combinations to improve detection of CD. In particular, the detection of IgA anti-reticulin antibodies combined with IgA anti-endomysial antibodies is useful for the screening of silent or latent forms of CD, such that some workers believe that regardless of clinical circumstances, the presence of serum IgA anti-reticulin antibodies, particularly when IgA anti-endomysial antibodies are also detected, is an absolute indication of

jejunal biopsy to confirm CD.[49]

Tissue transglutaminase has recently been identified as the target antigen recognised by anti-endomysial antibodies.[50] A reliable enzyme-linked immunosorbent assay (ELISA) has been developed to detect IgA tissue transglutaminase antibodies.[51] The sensitivity and specificity of this test has been shown to correlate highly with anti-endomysial antibodies in the detection of CD.[52-54] In future, the detection of antibodies to tissue transglutaminase may prove to be the superior serological marker for screening CD.[55]

Thus, in view of the controversies surrounding the purported association of RAS and CD and the availability of a reliable non-invasive screening test for CD, the present study was undertaken to more precisely determine the frequency and association of gluten-sensitive enteropathy in RAS using relevant haematological and serological abnormalities of CD, in particular anti-endomysial antibodies, in a large cohort of patients with well-defined RAS.

Materials and Methods

The study group comprised 87 patients (32 males; median age, 24 years; range, 9 to 80 years) consecutively referred to the Department of Oral Medicine of the Eastman Dental Institute for Oral Health Care Sciences, London, UK for the management of recurrent mouth ulcers. Patients' consent was obtained for oral examination and for taking venous blood samples. A general medical history and clinical examination was undertaken for each patient. All 87 patients were ultimately diagnosed with minor RAS (MiRAS) based on accepted criteria.[1] The control group comprised 87 age- and gender-matched healthy patients with a range of other ulcerative and non-ulcerative disorders of the oral mucosa.

A range of relevant haematological and serological investigations, including those that identify the effects of malabsorption were carried out.[56] A full blood cell count was performed on all RAS and control patients. This included red, total and differential white and platelet counts, red cell indices (e.g., mean cell volume and mean cell haemoglobin concentration), and total haemoglobin concentration. Red blood cell (RBC) folate and serum levels of vitamin B_{12} and ferritin were also estimated in all patients. The following additional investigations, which may be abnormal in CD, were undertaken:[56] serum levels of hepatic enzymes [alanine transaminase, alkaline phosphatase and gamma-glutamyl transpeptidase, bilirubin and total protein (48 patients)]; total and corrected serum calcium and phosphate levels (30 patients) and serum levels of IgG, IgA and IgM (34 patients), IgG antibodies to mitochondria, smooth muscle, reticulin and gastric parietal cells were estimated in 73 RAS patients.

Serum levels of IgA anti-reticulin and IgA anti-endomysial antibodies were estimated in all patients with RAS. IgA anti-reticulin and IgA anti-endomysial antibodies were detected by indirect immunofluorescence using rat kidney and monkey oesophagus as substrates respectively. Patients' sera were diluted at 1:2:5 and incubated on composite slides of rat kidney and monkeys oesophagus and the IgA antibodies concerned detected by fluorescein-labelled anti-human IgA. Positive results were reported at dilutions of 1:2:5 or greater.

Data were analysed using the SPSS version 11 programme. The Pearson Chi-square test was used to determine if there were any statistical difference between the data collected in the control and test groups. P values of more than 0.05 were considered not significant.

Results (Table 2)

Upper gastrointestinal symptoms, such as dyspepsia, were noted in 7 patients with RAS, 5 were hypertensive and 14 had asthma or allergic rhinitis. Two females reported having irregular or heavy periods and 11 patients had a variety of minor skin complaints (none had a history of ocular or genital disease).

Mild normocytic normochromic anaemia (haemoglobin concentration of less than 13 g/dL for males and less than 11.5 g/dL for females) was detected in 3 (3.5%) female patients with RAS. Six control patients (4 females, 2 males) also had a slight normocytic normochromic anaemia. There were no overt abnormalities in red, total and differential white blood cell and platelet counts in both groups of patients.

Of the 87 RAS patients, 16 patients (18%) were low in ferritin (including 2 females with anaemia), 19 (21%) had low RBC folate levels, including 1 patient with anaemia, and 13 patients with RAS (15%) had reduced serum levels

Table 2. Relevant Haematological and Serological Investigations in Patients with RAS and Control Subjects

Parameter	Patient group				P
	RAS (87)		Control (87)		
	n	%	n	%	
Anaemia	3	(3%)	6	(7%)	>0.05
Reduced serum ferritin	16	(18%)	20	(23%)	>0.05
Reduced vitamin B_{12}	13	(15%)	11	(12%)	>0.05
Reduced red cell folate	19	(21%)	16	(18%)	>0.05
Reduced total and corrected serum calcium	0	(0%)	NT		
IgG anti-reticulin antibodies	3	(3.4%)	NT		
IgA anti-reticulin antibodies	1	(1.1%)	NT		
IgA anti-endomysium antibodies	1	(1.1%)	NT		

NT: not tested; RAS: recurrent aphthous stomatitis

of vitamin B_{12}. In the control group of 87 patients, 20 patients (23%) had low ferritin levels (4 were anaemic), 16 (18%) had low RBC folate (1 patient with anaemia) and 11 (12%) were deficient in serum vitamin B_{12} (none had anaemia). The differences observed between the 2 groups were not statistically significant ($P >$0.05).

Liver function tests were performed on 48 RAS patients. None had any significant serological evidence of hepatic disease. Three patients had mild abnormalities; 1 patient had mildly elevated serum levels of gamma-glutamyl transpeptidase (γ-GT) and alanine transferase (ALT), another had increased serum levels of total protein and one had raised levels of serum bilirubin. All 3 patients did not have anti-smooth muscle or anti-mitochondrial antibodies. These mild hepatic abnormalities are unlikely to reflect significant liver disease.

All 30 tested RAS patients had normal serum levels of total and corrected calcium phosphate and bone alkaline phosphatase. Three patients with RAS had mildly raised serum levels of immunoglobulins [combined IgA and IgG (1 patient), IgA (1) and IgM (1)]. Autoantibody screen was performed on 73 study patients. Twenty-two (30.1%) patients had one or more autoantibodies but all were present in low concentrations and did not manifest clinically.

One (1.1%) of 87 RAS patients had IgA and IgG antibodies to reticulin and IgA antibodies to endomysium. Two patients (2.3%) had IgG but not IgA anti-reticulin antibodies or IgA endomysial antibodies.

The only patient with anti-reticulin and anti-endomysial antibodies was a 70-year-old Caucasian lady not known to have CD. Detailed review of systems did not reveal any notable gastrointestinal disease. She had had MiRAS for the past 4 years and thus had a rather atypical history of RAS. She was not anaemic, had a low RBC folate level of 88 µg/L (normal range: 186 to 596 µg/L), normal serum levels of calcium and phosphate, hepatic enzymes and normal serum levels of IgG, IgA and IgM. Further detailed relevant investigations by a gastroenterologist did not detect intestinal features of CD.

Discussion

The results of the present study indicate that while patients with RAS may show evidence of haematinic deficiency, and even anaemia of unknown cause, few patients have any of the relevant serological features of CD. Three of the present group of patients with RAS had IgG anti-reticulin antibodies, 1 of whom also had IgA anti-reticulin and IgA anti-endomysial antibodies. Although she had a history of atypical RAS, this latter patient had no other clinical or biochemical or haematological features of CD. She only developed recurrent bouts of ulceration later in life.

Thus, the present results confirm previous studies findings

that there is little association between RAS and CD. Indeed, a recent US study in 2002, evaluating the presence of RAS in CD patients compared to healthy controls found no significant differences between the 2 study groups.[57] Of course, oral ulcers similar to RAS in clinical appearance can occur in undiagnosed or untreated CD, but this is the likely consequence of haematinic deficiency states due to the accompanying malabsorption state.

It is interesting to note the very high frequency of haematinic deficiency in both the RAS patients and control subjects of the present study. The reasons for these results are unclear. All of the patients were generally otherwise well aside from their oral disease and minor systemic ailments. The latter are unlikely to be responsible for the observed findings and for causing RAS.[1] Both the RAS patients and control subjects attended the same Oral Medicine clinic, were of similar socioeconomic backgrounds and all were Caucasian. While the causes of the haematinic deficiencies in patients are not known, it does suggest that the frequency of haematinic deficiencies in patients with RAS may not be different from that of some control groups and thus perhaps not of such aetiological significance as previously suggested.[58] Certainly the present data suggest that despite RAS patients having haematinic deficiencies, these are unlikely to be due to underlying CD.

The present serological study confirms previous studies suggesting there is little association between RAS and CD. It is concluded that detailed screening of patients with clinical features and a history typical of RAS for CD is unlikely to be of clinical value.

Acknowledgments

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